COMPARATIVE SWEAT GLAND ULTRASTRUCTURE OF CATTLE,

SHEEP, GOATS, HORSES AND HUMANS BEFORE AND DURING THERMAL

STIMULATION.

by

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SUMMARY.

The sweat glands of the cow, sheep, goat, horse and
man were examined ultrastructurally at different stages of
activity in an attempt to explain the different patterns
of sweat output obtained from the skin.

The studies illustrated that sweat is formed in the
fundus from the products of secretion and cell death.
Secretion involved fluid transport, exocytosis and in man
and the horse a possible subsidiary micro-apocrine
process. The appearance of the duct was similar in all
species and activity produced little change other than
dilatation of the intercellular spaces except for the
coiled duct of man. This unique portion of the duct
demonstrated secretion and seems to be more than an
absorptive region. The difference in the patterns of
evaporative loss could not be explained on the basis of
the morphological changes during secretion which were
basically similar in all the species examined.

As a prelude to the study of functional aspects of
sweat production, the site of Na,K-ATPase, an enzyme which
plays a central role in fluid transport, was localized in
unstimulated and active glands. This enzyme was not
consistently localized in the glands of cow, sheep, goat
and horse although it was always found on the lateral and
basolateral membranes of the secretory cells in the rat
footpad sweat gland, which was used as a control tissue.
When present in the other species, the enzyme was
localized on the lateral and basolateral membranes of the
secretory cells in the sheep and horse and after thermal
stimulation also on the luminal membranes of the cow,

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sheep and horse. These distributions, however, could not be unequivocally established even after detailed experimental studies. The reaction product was not representative of alkaline phosphatase which was localized on the myoepithelial membranes of the cow, sheep and goat glands and on the luminal microvilli of the horse.

The results demonstrate that the traditional classification of sweat glands as eccrine and apocrine is not valid. It is suggested that these terms are replaced by the anatomical designations atrichial and epitrichial respectively, until more detailed information on the method of sweat production is available.

CHAPTER 1.

INTRODUCTION.

The profuse sweating response of man and the horse to thermal stimulation and exercise has led to the long accepted conclusion that sweat glands are principally thermoregulatory organs (Stitt & Hardy, 1971). This belief is supported by evidence from those homeothermic mammals which do not have sweat glands and avoid temperature extremes; e.g. rodents by burrowing (Schmidt-Nielsen, 1964), cetacea by diving and the hippopotamus by wallowing (Luck & Wright, 1964). However, species with sweat glands vary in their response to thermal stimulation. The pig does not sweat in response to heat (Ingram, 1967) and sweating in sheep and the red deer has only a very limited influence on body temperature (Bligh, 1967; Johnson, Maloiy & Bligh, 1972). The former and some other ungulates depend more on panting than sweating for temperature regulation (Finch, 1972; Jenkinson, 1972). From this and regional differences in glandular action Jenkinson (1973) concluded that sweat glands are not important thermoregulatory organs except in primates, equidae and some bovidae. Other roles such as the excretion of waste products, defence against invading organisms and, in localised regions specialised functions eg. lubrication for the eyelids and improving the grip for footpads and hands, have also been attributed to sweat glands. It appears likely that they have more than one prime function which probably differs amongst species and even body regions of the same species.

Total evaporative loss from the skin has been measured using ventilated capsules (McLean, 1963) which operate as shown in Fig.1.1. In those species which respond to thermal stimulation three general patterns of evaporative loss have been demonstrated (Fig.1.2) which at high temperatures are largely due to sweating. For an explanation of these patterns an understanding of the anatomy of sweat glands is required. By definition sweat glands are tubular exocrine skin glands which open onto the body surface. Their structure (Fig.1.3) is usually

Fig.1.1. A ventilated capsule on the skin of a cow. Equivalent metered airflows pass through the capsule and room, and the humidity of the air in the two tubes is measured by wet bulbs (WB). The difference in humidity between the two airways gives a measure of evaporative loss from the animal. At high environmental temperatures the contribution of water from transport through the skin is relatively low and total evaporative loss approximates to sweat output. For the Key to this and all subsequent figures see Appendix 3.

Fig.1.2. Traces showing cutaneous evaporative loss against time for different species at high environmental temperature.

Fig.1.3. This generalised sweat gland illustrates the fundus which is composed of a secretory epithelium surrounded by a myoepithelium and a fibrocyte sheath. The duct, usually described as being composed of two cell layers and surrounding fibrocyte sheath, leads from the fundus to the skin surface.

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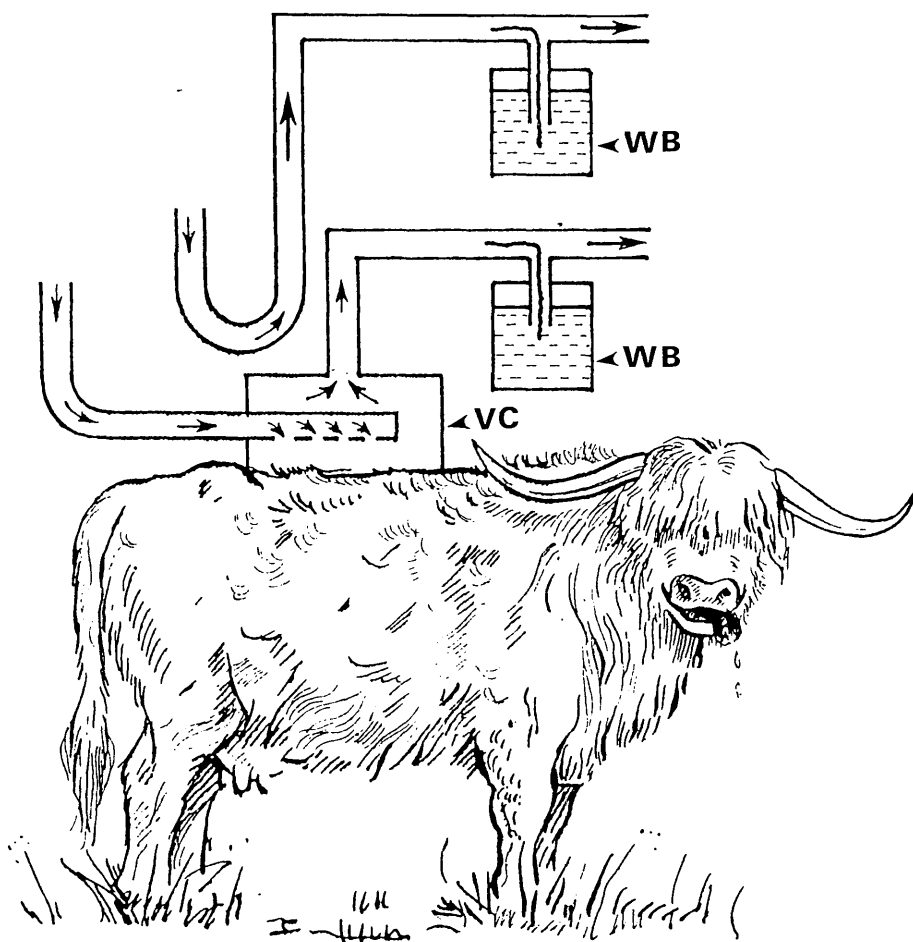
Patterns of Sweating

D.B. 40.0°C
W.B. 26.0°C

Cutaneous Evaporative Loss (Δ°C)

Cow
Jenkinson 1969

Sheep
Robertshaw 1968

Goat
Jenkinson &
Robertshaw 1971

Horse
Montgomery
Jenkinson &
Elder 1982

Human
Montgomery
Jenkinson &
Elder 1984

Time of Exposure (min)

DUCT

F

FUNDUS

MyoE

F

SE
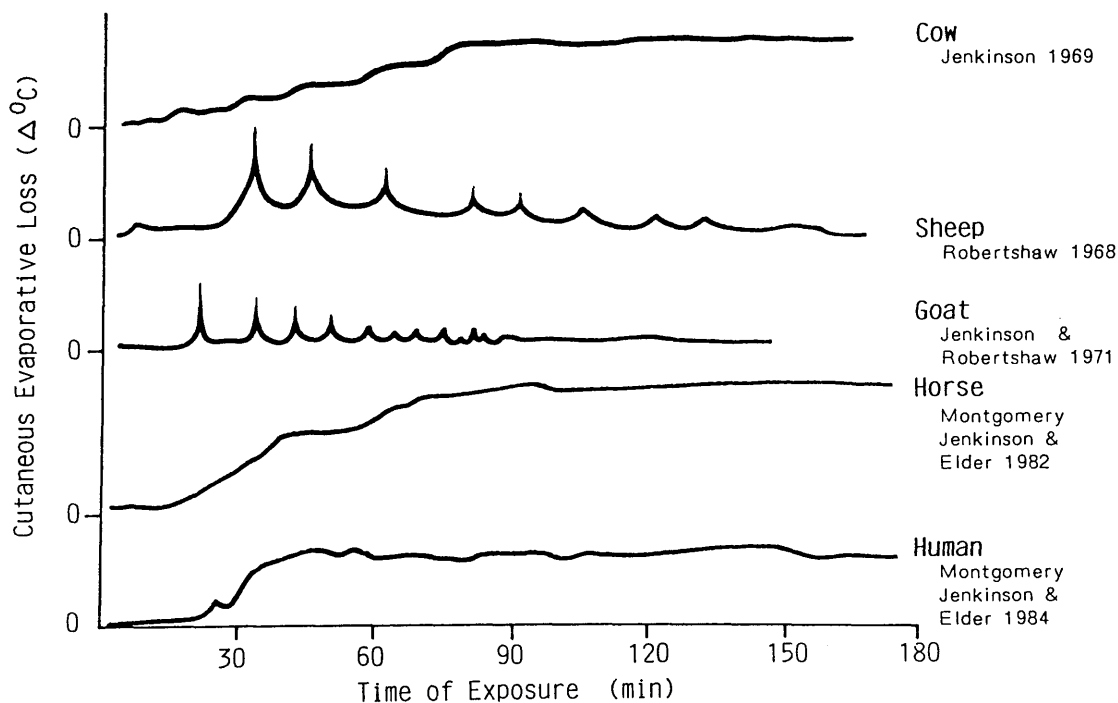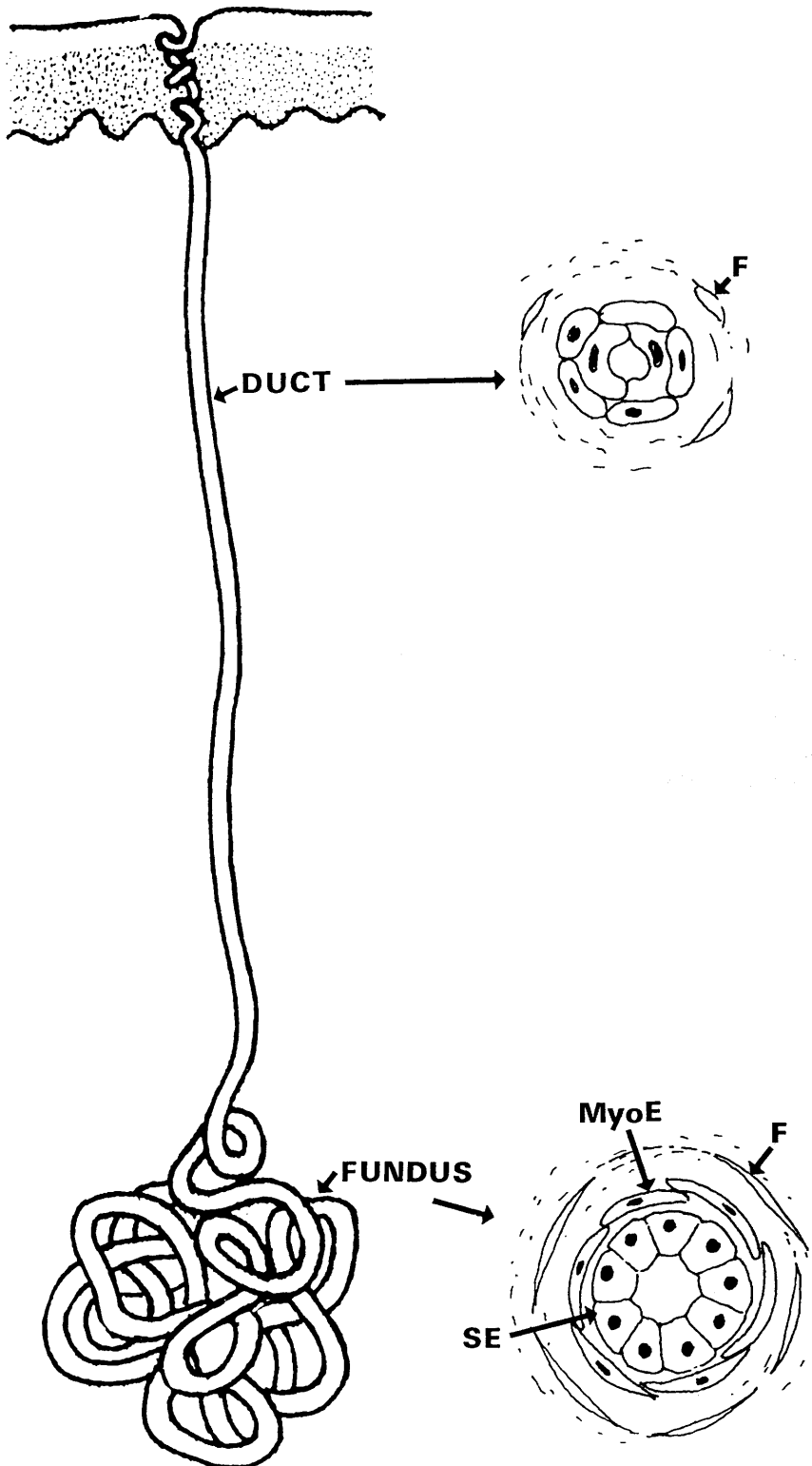
described as a fundus, composed of a secretory epithelium surrounded by a myoepithelium, and a duct, again with a wall of two cell layers, leading from the fundus to the body surface. The fundus is often illustrated as a coil but can range in shape within species and between species from the tight coil of the horse and man to the simple sac of some cattle.

Combining sweat gland anatomy and physiological data Bligh (1967) proposed a hypothesis to explain the different patterns of sweating obtained after thermal stimulation. The cow, an example of the first type, shows a stepwise increase to a plateau level which can be maintained for more than five hours. This pattern was interpreted by Bligh (1967) as being due to increasing secretory activity superimposed on myoepithelial contractions. In the second type, exhibited by the sheep and goat, there is an intermittent and decremental pattern to fatigue which is reached after 90 to 120 minutes. This is apparently due to the rate of sweat expulsion exceeding that of production (Johnson, 1973) and not to fewer glands firing (Jenkinson & Robertshaw, 1971). The third type of pattern has a smooth onset to plateau which is then sustained, although after four hours there is often a decline in activity. Man and the horse are examples of this type which is believed to result from secretory activity with little or no myoepithelial action. Output seems to be largely dependent on secretory cell activity (Takagi & Tagawa, 1959; Allen & Bligh, 1969). On exposure to heat, equidae, show a gradual increase in the rate of cutaneous water loss over a period of about one hour,

rising to a maximum which can be maintained for several hours (Allen & Bligh, 1969; Robertshaw & Taylor, 1969; Kerr, Munro & Snow, 1980).

Testing Bligh's hypothesis, which was based on physiological observations but with little evidence on secretory activity, requires an understanding of sweat gland function. Thus knowledge of changes in the ultrastructure of the glands in more than one species and in particular in those representative of the different physiological responses at various stages of secretion is necessary. Comparative ultrastructural studies would, in addition to illustrating the secretory mechanisms, provide evidence on myoepithelial activity in the fundus. The sweat glands from the general body surface of cattle, sheep, goats, horses and man were therefore selected for study as these species display the different sweat patterns which occur in response to thermal stimulation. Intimately involved in the passage of water and ions across transporting epithelia, such as the secretory epithelium of sweat glands, is the enzyme Na,K-ATPase. Cytochemical localization of this enzyme will demonstrate the position of ion pumps on secretory cell plasma membranes and will provide further evidence on the mechanisms of membrane transport in sweat glands which in view of the different patterns may vary between species.

A comparative study of glandular ultrastructure during activity should also provide evidence on the discrepancies in the classification of sweat glands and their mode of secretion which are largely based on light microscopic studies. Electron microscopic evidence is

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limited to unstimulated glands and there is a need for a more detailed electron microscopic study of active sweat glands. Schiefferdecker (1917), using light microscopy, proposed the still used Eccrine, Apocrine classification of sweat glands using three points of difference. Eccrine sweat glands (Fig.1.4) develop directly from the epidermis, are never found in association with hair follicles, and release their secretion through the luminal membrane of the secretory cell without its rupture and the loss of cytoplasm. Apocrine sweat glands (Fig.1.5) which develop from epidermal hair follicle buds are always found in association with hair follicles. Such glands exhibit both eccrine secretion and necrobiotic discharge involving the breaking away of the distal portion of the secretory cell and hence apical cytoplasmic loss. Using the classification of Schiefferdecker (1917) the sweat glands of cattle, sheep, goats and the horse which develop from the hair analage and are closely associated with hair follicles are considered to be apocrine. The glands from the general body surface of man which develop from the epidermis have been classified as eccrine.

Fig.1.4. Eccrine sweat glands develop from the epidermis and release their secretion through the membrane of the secretory epithelium.

Fig.1.5. Apocrine sweat glands are always associated with hair follicles. They exhibit both eccrine secretion and necrobiotic discharge involving the whole apex of the secretory cell.
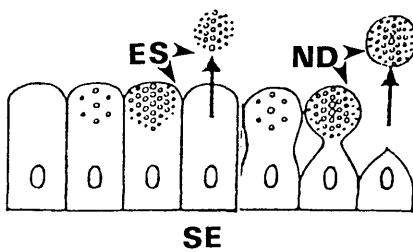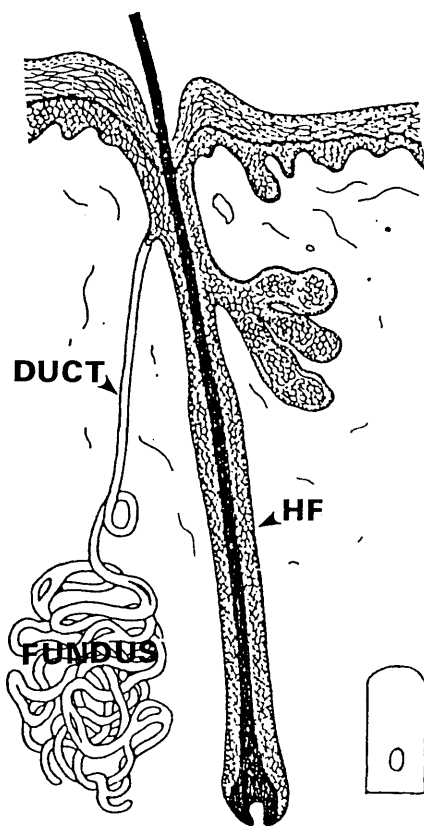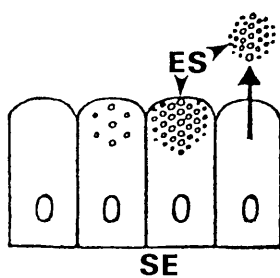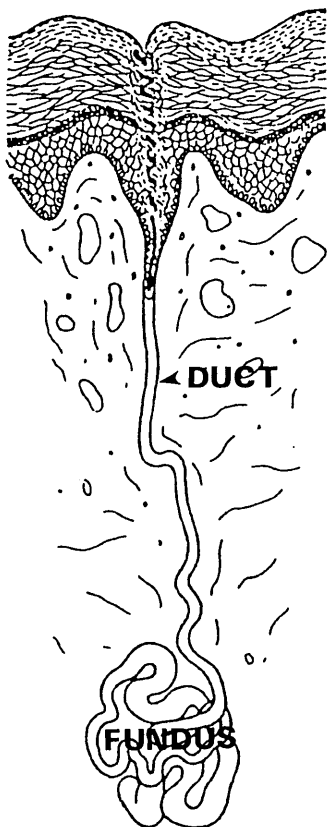
Takagi and Tagawa (1959) from light microscopic
evidence concluded that the equine sweat gland is not
apocrine. Talukdar, Calhoun and Stinson (1970) using light
microscopy and Kurosumi,Matsuzawa and Saito (1963),
Sorensen and Prasad (1973) using electron microscopy on
the other hand found cytoplasmic protrusions suggestive of
apocrine secretion. However, they failed to detect
pinched-off cytoplasm in the lumen and noted that apical
swelling of the cell could be induced by the fixative. The
presence of flatter, more vacuolated, sometimes completely
ruptured secretory cells with fragmented nuclei in the
glands within skin regions rendered anhidrotic by repeated
intradermal injections of adrenaline (Lovatt Evans, Nisbet
& Ross, 1957) suggests that cell death may occur during
activity. However, the possibility that this appearance is
unphysiological and induced by excessive stimulation
cannot be eliminated as the doses of adrenaline used were
not given. There is therefore some doubt as to the mode of
secretion and classification of equine sweat glands.

Light microscopic studies of bovine sweat glands
failed to reveal necrobiosis in the secretory epithelium
or changes in the myoepithelium during sweating (Findlay &
Jenkinson 1964). The ultrastructure of the unstimulated
bovine sweat gland did not reveal necrobiosis (Prasad,
1973) but the effects of thermal stimulation have not been
examined. These glands are therefore unlikely to be
apocrine. Light microscopy of the goat sweat gland during
sweating shows a decrease in glandular volume accompanied
by a thickening of the glandular wall (Jenkinson &
Robertshaw, 1971) which supports the view of sweat

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expulsion by myoepithelial action and not apocrine secretion. There are however no published reports on the ultrastructure of the sweat glands from sheep and goat before or during stimulation.

Information on the ultrastructure of the human eccrine sweat gland is limited to studies of the unstimulated gland (Ellis, 1962, 1965; Montagna & Parakkal, 1974; Sato, 1977; Hashimoto, 1978a; Kurosumi, Kurosumi & Tosaka, 1982). Light microscopical studies of the human gland (Dobson, Formisano, Lobitz & Brophy, 1958) during activity have demonstrated that a number of the cells in the secretory coil are vacuolated with glycogen deposits while others exhibit particle and glycogen depletion. Some of the cells are damaged to the extent that they are unable to function normally. Although the apocrine/eccrine terms have been retained to describe the human sweat glands their use has been questioned (Dobson & Lobitz, 1958; Jenkinson, 1967, 1973) as they describe the glands at rest and not during activity.

In view of the absence of data in support of Bligh's thesis (1967) and doubts on the classification of sweat glands a study of comparative ultrastructure of active glands was therefore undertaken. This design will also provide a basis for X-ray microprobe studies of sweat gland ionic transport mechanisms.

CHAPTER 2.

MATERIALS AND METHODS.

Cattle, sheep, goats, horses and humans were all
exposed to various ambient conditions of temperature and
humidity in the climatic chamber of Findlay, McLean and
Bennet (1959). The experimental animals were all fed a
standard complete diet with water ad libitum and between
experiments were housed in thermoneutral or cool
environments. The four humans were also given water or
soft drinks ad libitum during the experimental period.

The environmental conditions used were defined by Dry
bulb (DB) and Wet bulb (WB) temperatures. Relative
humidity can, if desired, be obtained from the difference
between Wet and Dry bulb temperatures using standard
tables. Total evaporative loss, which at warm temperatures
and outwith high humidities is a reflection of sweating
activity, was continuously monitored throughout the
exposure period using the ventilated capsule technique of
McLean (1963). Since the patterns of secretion
characteristic of each of the five species occur
synchronously on both sides of the thorax (Findlay &
Robertshaw, 1965; Allen & Bligh, 1969), skin samples were
removed, without anaesthesia, by biopsy at various
selected time intervals from the equivalent site on the
opposite side of each animal. Using the high speed punch
technique of Findlay and Jenkinson (1964) the samples from
cattle, sheep and goats were taken from an area of shaved
skin overlying the eighth and ninth ribs on the upper
aspect of the body towards the thoracic spinous process.

Samples from the horse were taken from the thoracolumbar
region on the back. The samples from the humans were taken
with a 3 m.m. biopsy punch (Stiefel Laboratories Ltd.)
from the loin immediately after local injection of 0.1ml
of 2% Xylocaine.

The skin specimens for transmission electron
microscopy were prepared using the techniques detailed in
Appendix 1 and 2. The stained ultrathin sections, from all
preparative techniques, were examined in an A.E.I. EM6B or
J.E.O.L. 100C electron microscope.

CHAPTER 3.

THE BOVINE SWEAT GLAND.

INTRODUCTION.

As described earlier the stepwise increase in the
cattle sweat pattern has been attributed to the effect of
myoepithelial contractions superimposed upon a rapidly
rising secretory rate. The concept of an active secretory
process in cattle is supported by evidence from studies of
the ionic composition of bovine sweat (Johnson, 1970;
Jenkinson & Mabon, 1973). Light microscopy of cattle sweat
glands indicates that a decrease in glandular volume
occurs during sweating, and supports the view of sweat
expulsion by myoepithelial action. So in an attempt to
resolve the question as to the method of production and
expulsion of sweat in cattle the ultrastructural
appearance of the sweat glands was studied, before, and at
intervals after the initiation of sweating in a controlled
warm environment.

PROCEDURE.

Four 11 month old Ayrshire oxen were placed in an
environment of 20°C Dry Bulb (DB)/16°C Wet Bulb (WB) for a
period of 6-7 hours with cutaneous evaporative loss
monitored throughout. A skin sample was taken from each
animal immediately upon entry and at the end of the
experimental period before removal from the climatic
chamber. The following day the experiment was repeated in
an environment of 40°C DB/26°C WB with skin sampling at
the times shown in Fig.3.1. All samples were fixed and

processed for transmission electron microscopy as described
in appendix 1.

RESULTS.

Patterns of evaporative loss.

The patterns of cutaneous evaporative loss from the
four cattle were essentially similar (Fig.3.1). However
variations in the magnitude of response were obtained.
Sweating did not occur when the same animals were
subjected to a cool environment.

Sweat gland structure.

The bovine sweat gland (Fig.3.2) consists of a simple
sac-like fundus with a duct which passes through the
dermis alongside the hair follicle between the lobes of
the sebaceous gland. The duct normally approached the hair
follicle in a parallel direction and penetrated the cell
layers of the follicle close to the surface before opening
into the follicular orifice. The duct as shown in Fig.3.2
can be divided into two regions.
1.) The intrafollicular region, i.e. the duct within the
hair follicle. This region, together with the adjoining
perifollicular zone had a length of $107\pm21\mu m$.

Fig.3.1. The patterns of cutaneous evaporative loss from
four oxen in an environment of $40^{o}C$ DB/$26^{o}C$ WB Skin
samples were taken from the contralateral side at the
times shown (numbered arrows).

Fig.3.2. A diagram illustrating the fundus and different
zones of the duct of the bovine sweat gland and their
situations with respect to the hair follicle.

40 °C DB/26°C WB

72 A   72 B   72 C   72 D   72 E   72 F   197·3 gm^{-2}h^{-1}

77 A   77 B   77 C   77 D   77 E   77 F   269·2 gm^{-2}h^{-1}

76 A   76 B   76 C   76 D   142·2 gm^{-2}h^{-1}

73 A   73 B   73 C   73 D   73 E   73 F   210·5 gm^{-2}h^{-1}

Cutaneous evaporative loss (Δ °C)

Time (hours)

0   1   2   3   4   5   6   7

INTRAFOLLICULAR REGION

Perifollicular zone

INTRADERMAL REGION

Duct body

Transition zone
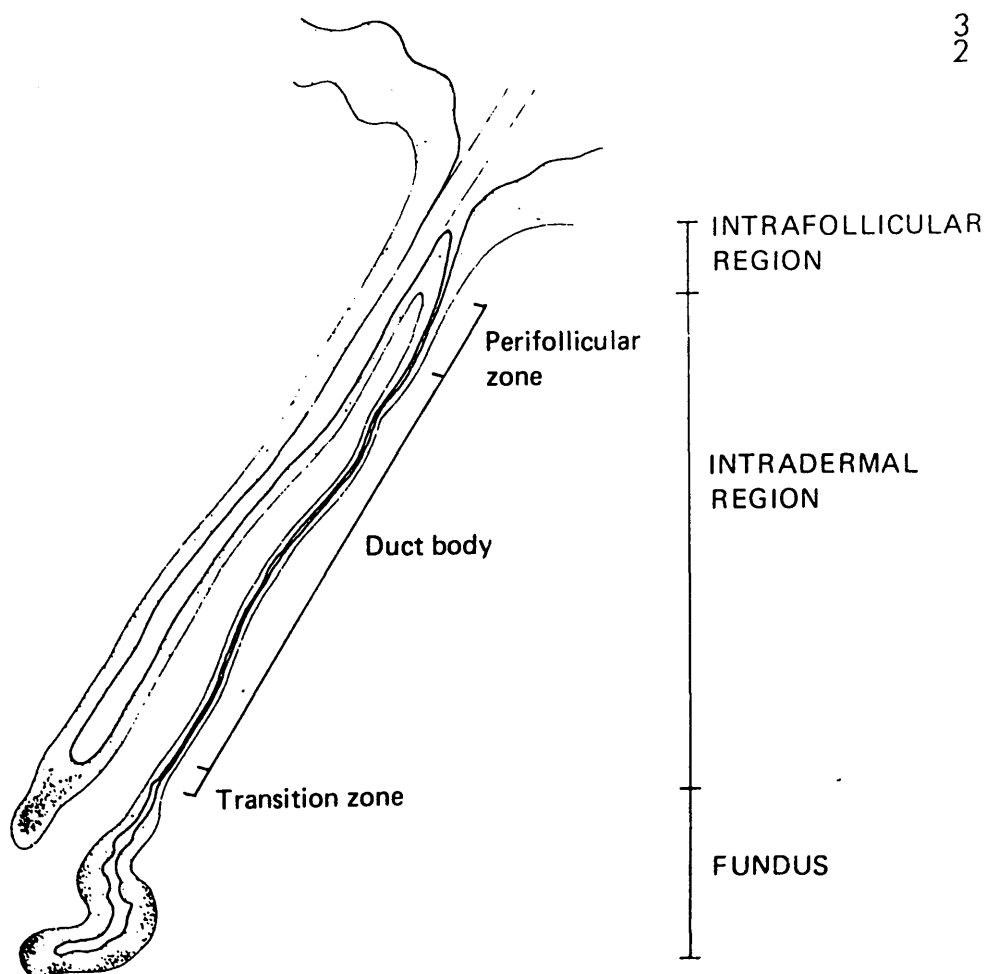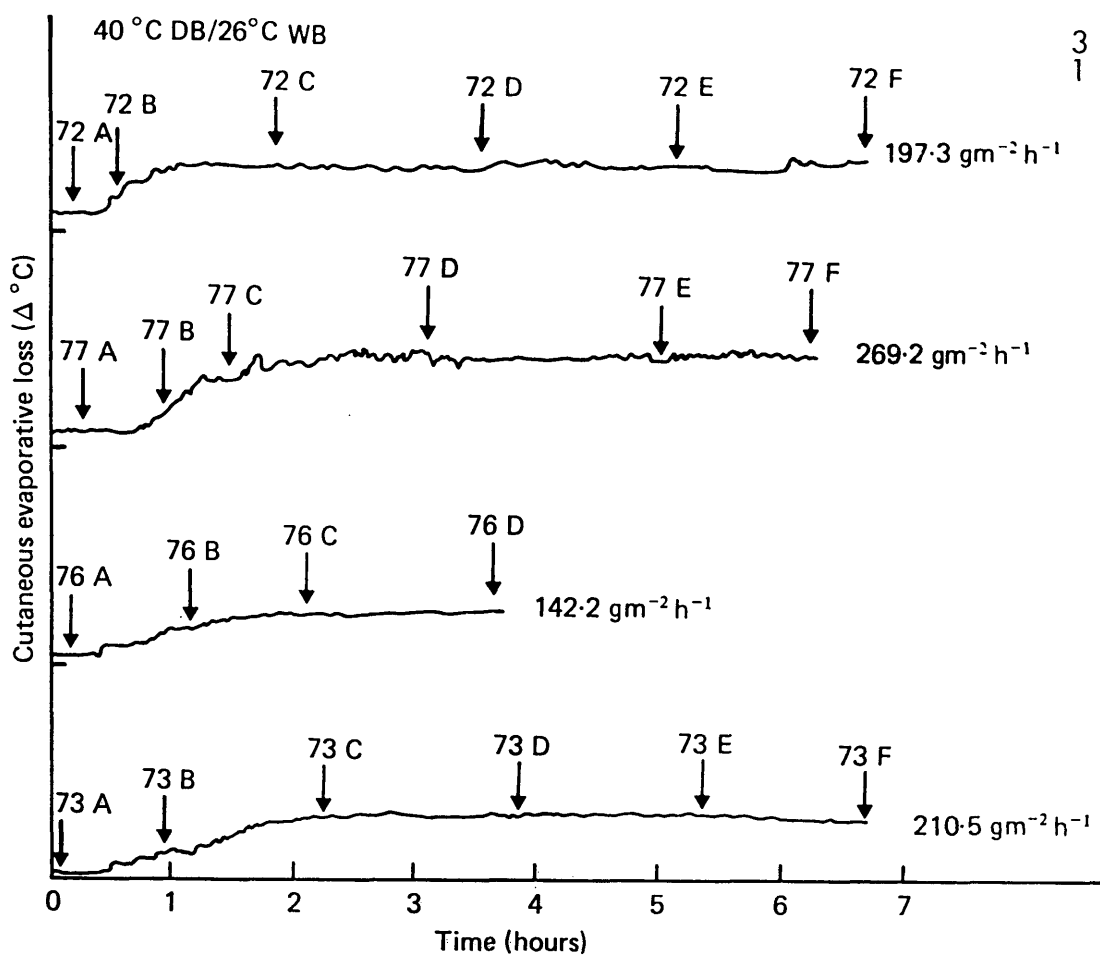
FUNDUS

2.) The intradermal region, which can be further divided into three zones.

A.) The perifollicular zone.

B.) The duct body, with a length of 380±58μm is the longest zone stretching from the hair follicle to a few layers from the fundus.

C.) The fundus/duct transition zone. This is a short portion of not more than six cells in length where the duct joins the fundus.

The unstimulated gland ultrastructure.

Fundus.

The ultrastructural appearance of the glandular fundus in samples taken before the onset of sweating at 40°C was indistinguishable from that in samples taken during exposure to the control temperature of 20°C. The fundus had a lumen containing a fibrous-like macromolecular colloid, a flattened secretory epithelium surrounded by a myoepithelium and a well developed basement membrane (Fig.3.3). The entire fundus was enveloped in a fenestrated sheath of fibrocytes. The secretory cells were each interlinked by an apical junctional complex complete with desmosome and zona occludens (Fig.3.4). The myoepithelial cells were

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Fig.3.3. Sweat gland fundus of an ox illustrating the secretory and myoepithelia and the surrounding fibrocyte sheath.

Fig.3.4. An electron micrograph demonstrating the connections between the epithelial cells.

attached to the secretory cells and to each other by desmosomes and to the basement membrane by hemidesmosomes (Fig.3.5).

The secretory cells had luminal microvilli, generally single, but sometimes branched (Fig.3.6) which appeared to be more numerous at the apical junctions (Fig.3.5). Additionally, at the bases of these cells, microvillous projections from the epithelium lay within narrow clefts between the secretory and myoepithelial layers (Fig.3.5). The dense granular appearance of the cytoplasm is probably due to polysomes as staining for glycogen proved negative. A small Golgi body was found situated lateral to the flat, oval, centrally placed nucleus (Fig.3.3). Coated vesicles were occasionally found in close association with the Golgi apparatus but more often proximal to membrane-bound vesicles with electron-dense contents. These electron-dense vesicles were most probably lysosomes, as indicated by autofluorescence and positive histochemical tests for acid phosphatase. Rough endoplasmic reticulum (RER), although not prominent, was generally present, especially in the perinuclear region. Microfilaments could be seen throughout the cell but were most pronounced in the apical region where they were orientated parallel to the luminal surface. Microtubules were found in a few secretory cells. The mitochondria were elongated, with a

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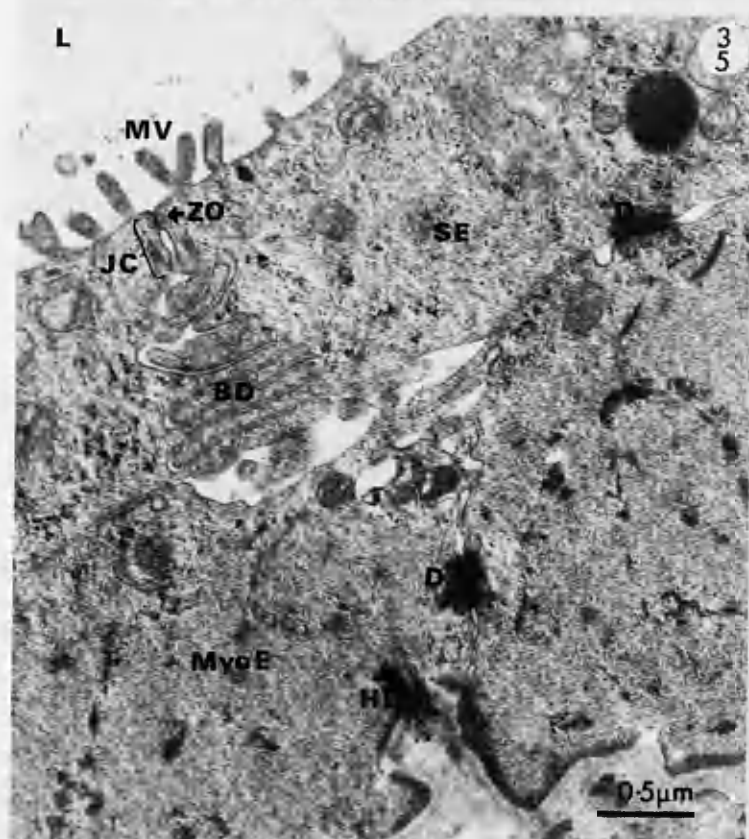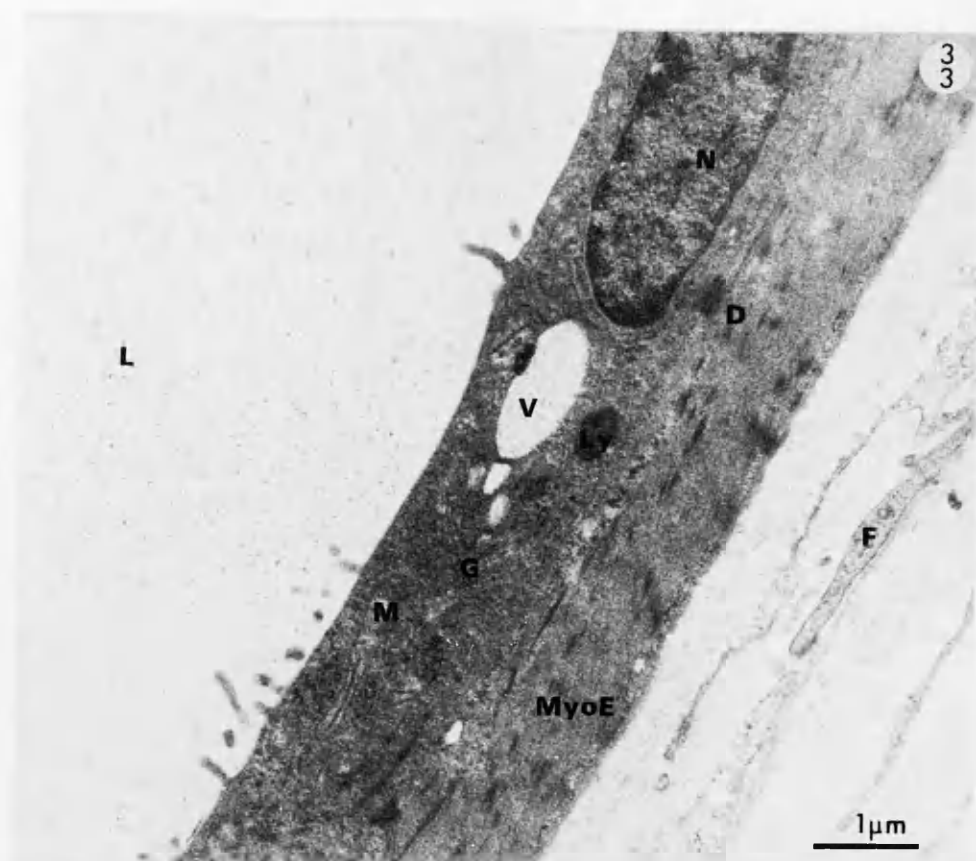Fig.3.5. High power view of the apical junctional complex.
Fig.3.6. Secretory cell illustrating vesicles and branched microvilli.

pale matrix, and plate-like cristae across their longitudinal axis. Prominent within the cells were membrane bound vesicles (Fig.3.6). These contained closely packed granular and filamentous material similar to the luminal contents. A number of these vesicles also contain electron-dense aggregates (Fig.3.7).

The myoepithelium was well-developed and equivalent in thickness to the secretory epithelium (Fig.3.3,6). It consisted of a single layer of spindle-shaped smooth muscle cells which overlapped at their tapered poles. The nucleus, also spindle-shaped, was central or eccentrally placed to a more luminal position in the cell. The apical cones at the nucleus contained RER and elongated mitochondria which had a matrix more electron-dense than that of the mitochondria in the secretory cells. Mitochondria were also found singly or in small groups at the periphery of the sarcoplasm. The apical cones also contained abundant electron-dense granules which resembled beta glycogen particles (Fig.3.7). Caveolae were found on the serosal membrane underlying the basement membrane and in the clefts between adjacent myoepithelial cells. Thick and thin filaments were found among the dense arrays of contractile proteins. Numerous dense bodies were seen in

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Fig.3.7. An electron micrograph of the Golgi apparatus and closely associated vesicles in a secretory cell. Some of the vesicles contain electron-dense aggregates, and others a filamentous material similar to that in the lumen.

3
3

N

D

L

V

Ly

F

G

M

MyoE

1μm

3
5

L

MV

+ZO

JC

SE

BD

D

MyoE

HD

0·5μm

3
4

L

ZO

D

0·1μm

3
6

L

MV

M

V

V

SE

MyoE

CT

F

1μm

3
7

SE

V

L

G

V

C

MyoE

V

Gly

N

M

1μm
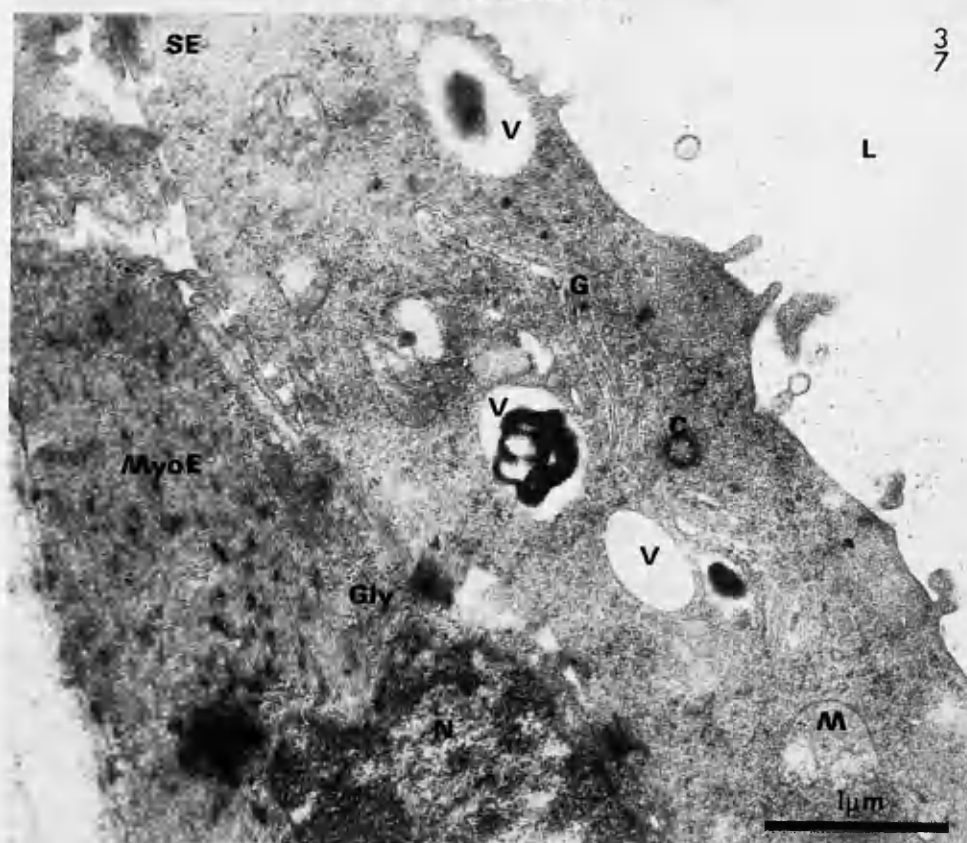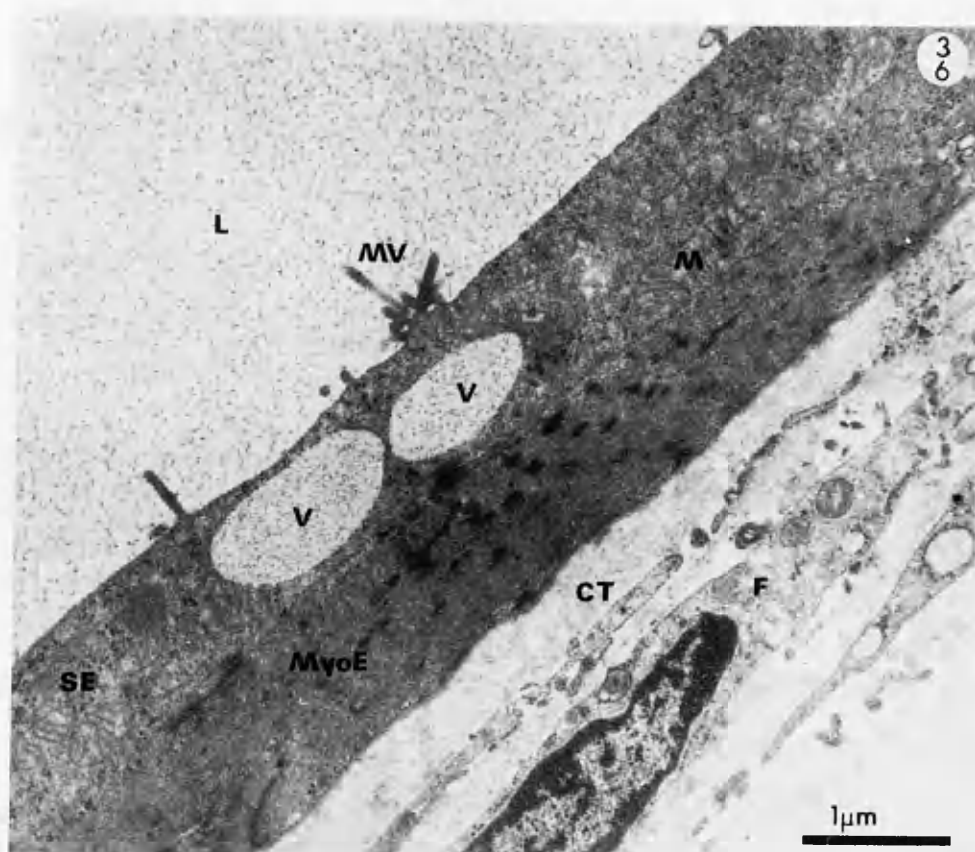
the sarcoplasm (Fig.3.3,5,6,7) and amongst the contractile filaments were elongated profiles of smooth membranous vesicles, probably sarcoplasmic reticulum.

Langerhans cells, identified by Birbeck granules, were also occasionally found between secretory and myoepithelial cells.

Duct.

The intrafollicular region.

Where the duct opened into the hair follicle orifice it was composed of three living cell layers with three to six cornified cells forming the lumen (Fig.3.8,9). At this point cornified cells of the duct were contiguous with the stratum corneum of the inner root sheath (Fig.3.8). The living cells were ultrastructurally indistinguishable from those of the stratum granulosum of the epidermis. The keratinized luminal surface deeper in this region was replaced by one lined with short densely-packed microvilli, usually from one cell which joined with itself

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Fig.3.8. The bovine duct at the point of entry into the hair follicle canal. The keratinized luminal surface is contiguous with the stratum corneum of the inner root sheath of the hair follicle. The granular cells which form the wall of the gland do not completely encircle the lumen.

Fig.3.9. In this electron micrograph from the intrafollicular region the duct still has a keratinized luminal surface but here no longer contiguous with the hair follicle.
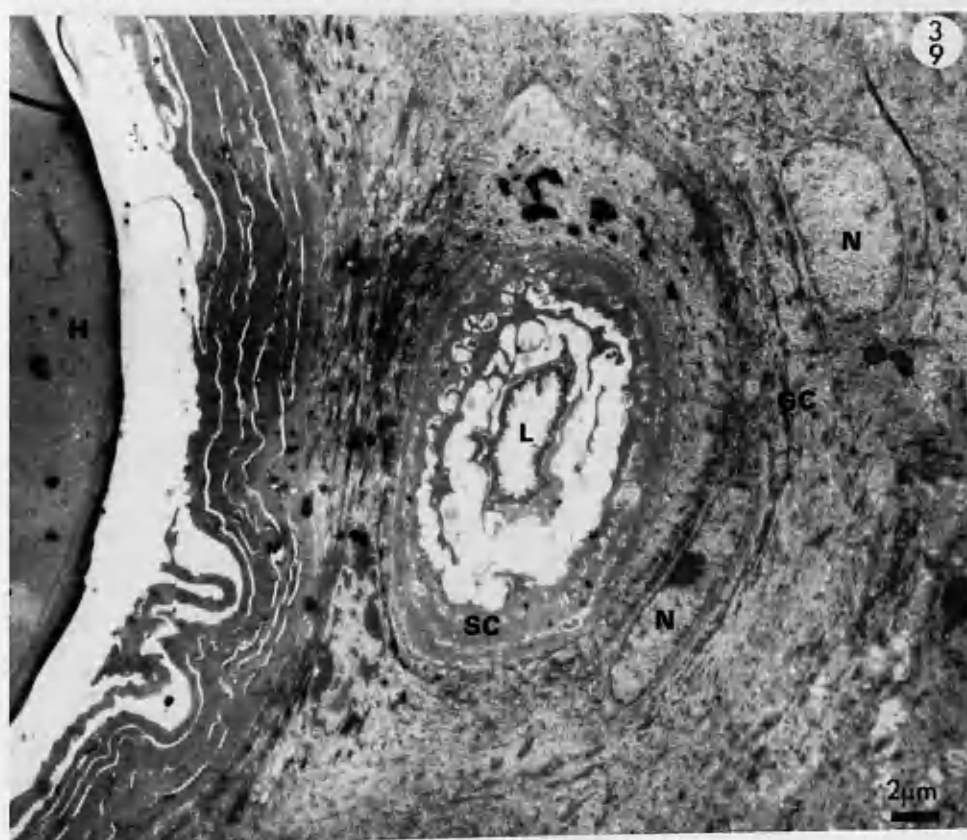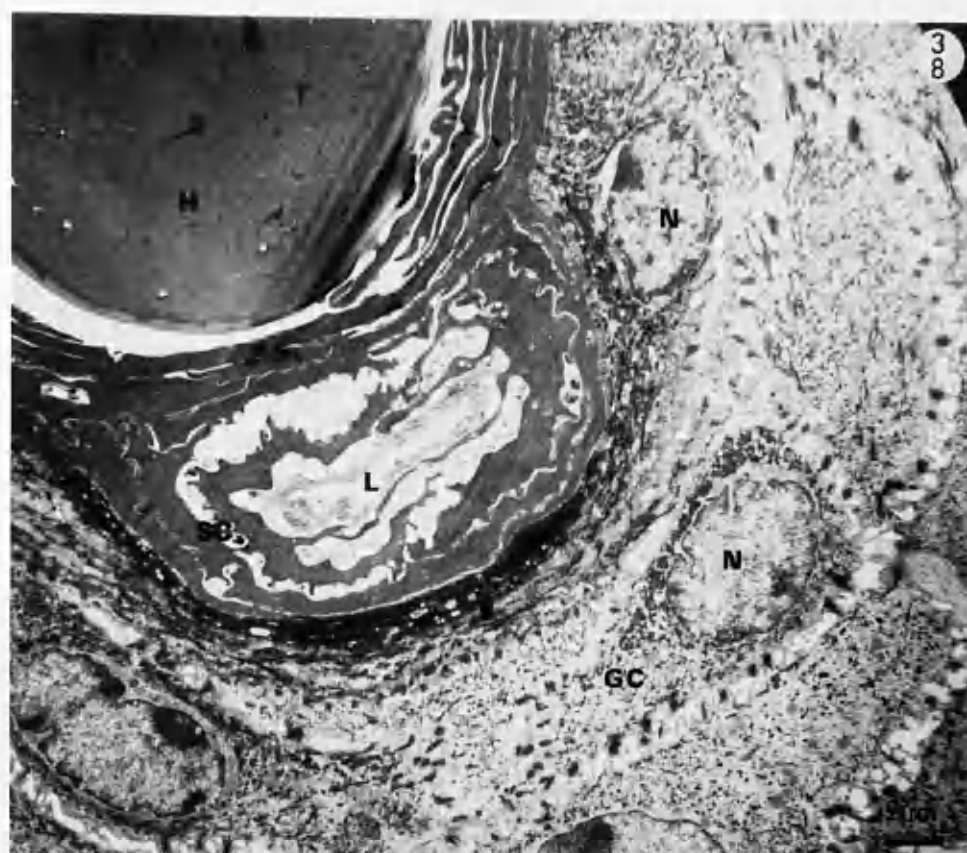
by means of a <u>zonula occludens</u>. At the apical junctional complex there was a prominent epithelial fold which extended into the lumen. This single luminal cell was surrounded by two more layers of granulosum cells interconnected by desmosomes. Towards the outer root sheath of the follicle the duct still had a single cell forming the lumen (Fig.3.10). This cell had a conspicuous epithelial fold at the luminal junctional complex and short microvill lining the entire lumen. Amongst these microvilli numerous small vesicles were observed (Fig.3.11 inset). Occasionally the lumen at this level was lined by two cells. The duct wall, including the luminal cell was generally found to consist of three to five cell layers. The outer cells still had an ultrastructure

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<u>Fig.3.10</u>. The bovine duct within the outer root sheath of the hair follicle is composed of three cell layers interconnected by desmosomes. The luminal surface is provided by the inner single cell which is connected to itself to form a ring. A prominent epithelial fold with a <u>zonula occludens</u> is present at the point of connection and the surface is lined by densely packed microvilli.

<u>Fig.3.11</u>. The bovine duct in the perifollicular region immediately outside the hair follicle. The appearance is similar to that found within the outer root sheath but the lumen is smaller and the entire duct is now surrounded by a fibrocyte sheath. Vesicles observed between the microvilli at the non-keratinized luminal surface throughout the intrafollicular zone were most numerous in this region (Inset).

Fig. 3/10

Fig. 3/11
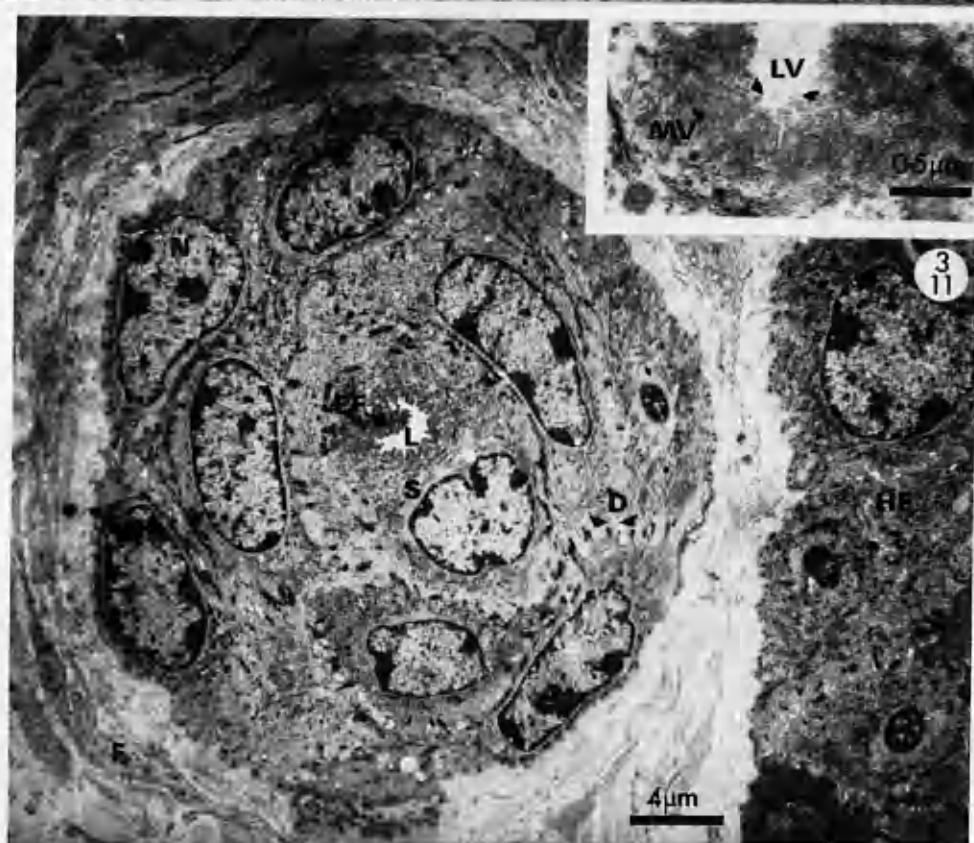
indistinguishable from the cells of the outer root sheath, although they tended to be flatter. The cells of the duct were connected by desmosomes to each other and to neighbouring cells of the outer root sheath.

The intradermal region.

The duct at the short perifollicular zone, where it separates from the hair follicle, had changed little in cellular ultrastructure (Fig.3.11). The cells of the three layers, although more flattened, were still similar to the granulosum cells of the outer root sheath. The inner cell layer surrounding the now small lumen was generally composed of two or three cells although ducts with a single cell could still be found (Fig.3.11). An epithelial fold was still prominent and the vesicles between the microvilli were now more numerous (Fig.3.11 inset). The duct was now entirely surrounded by a fenestrated sheath of fibrocytes.

The duct body, the longest portion, showed essentially the same structure along its entire length. The duct wall in this region consisted of two layers of epithelia (Fig.3.12), the luminal cells linked by apical junctional complexes with zonula occludentes (Fig.3.12


Fig.3.12. A section through the bovine duct in the body region. The cells of the inner of the two layers composing the duct wall are connected by zonulae occludentes (Inset) and have microvilli at their luminal surfaces. Tonofilament bundles are prominent around the margins of the basal cells.

inset). The epithelial folds were less pronounced and the
lumen now had contents similar to those in the fundus. The
luminal microvilli in this region were shorter and less
densely packed and there was no evidence of the vesicles
found in the intrafollicular duct. These luminal cells had
a spindle-shaped nucleus with a cytoplasm rich in
ribosomes and contained a few small round mitochondria.
The cells of the outer layer, connected by desmosomes,
became progressively flatter as the duct approached the
fundus. Gap junctions were observed between some but not
all of the basal cells in the lower portion of the duct
body (Fig.3.13, inset), i.e. in the region c.237µm from the
hair follicle to the transition zone. These basal cells
had an ovoid nucleus in a dense cytoplasm containing small
mitochondria. A prominent feature of the cells were
tonofilament bundles situated around the margins in a
direction parallel to the length of the duct (Fig.3.12).

Within the fundus/duct transition zone, which was two
to five cells in length (Fig.3.14), the luminal cells more
closely resembled the secretory cells of the fundus than
those of the duct body. Luminal cells in this zone had
present in their cytoplasm secretory vesicles and
degenerating mitochondria. Atretic luminal cells were also

Fig.3.13. Basal cells from the lower portion of the duct
body. Gap junctions occurred between the basal cells close
to the basement membrane. These basal cells also have a
prominent rough endoplasmic reticulum.
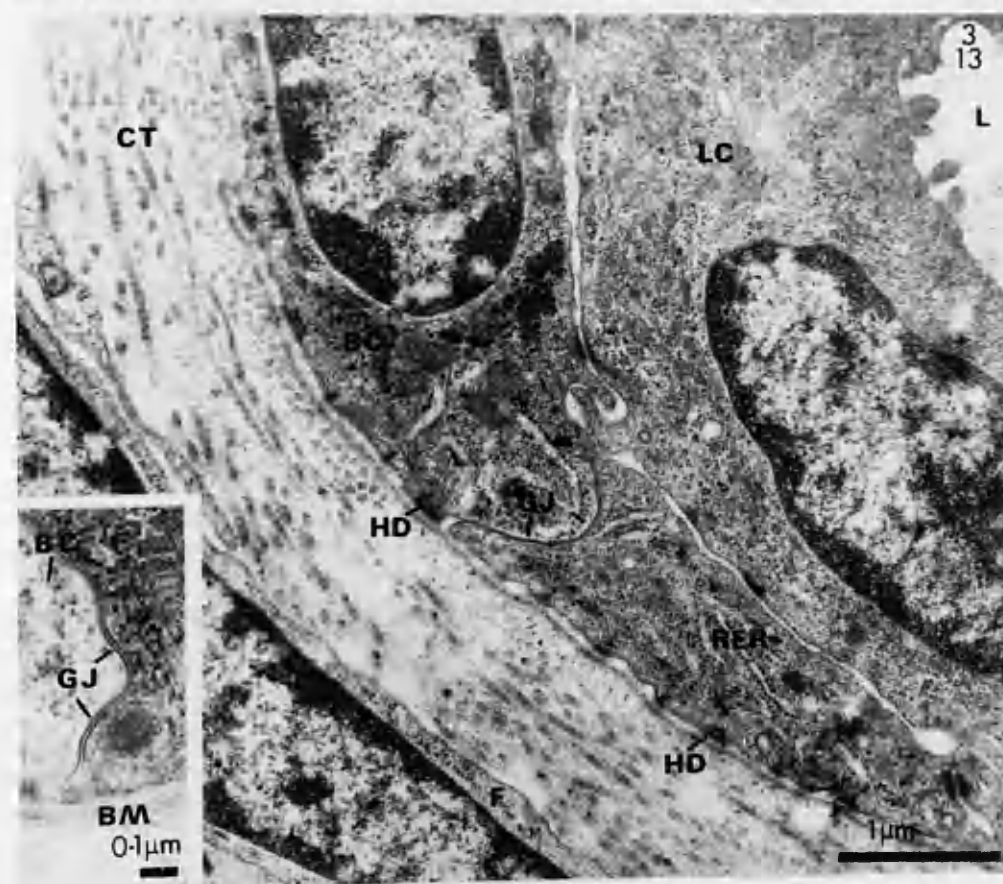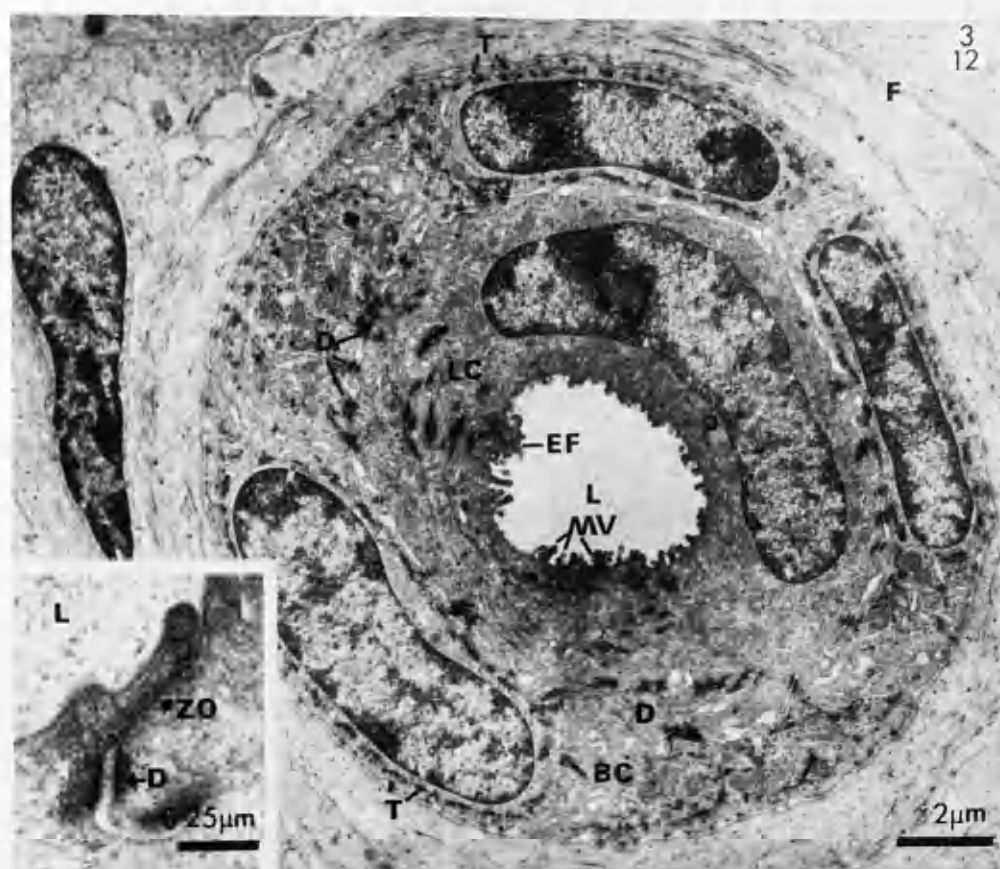
regularly seen in this zone (Fig.3.14,15). Occasionally
the serosal membrane of a luminal cell in this zone
reached the basal lamina (Fig.3.16). The most prominent
structural differences in this zone were found in the
elongated basal cells, which exhibited features
characteristic of myoblasts. Gap junctions were absent,
the basal cells were interconnected by desmosomes which at
points were differentiated into nexuses. Their cytoplasm
was rich in ribosomes, glycogen deposits and filament
bundles of varying size (Fig.3.17). These filament bundles

Fig.3.14. A longitudinal section of the fundus of the
bovine sweat gland with the duct leading from it. The
fibrocyte sheath and inner and outer cell layers can be
seen. The basal cells at this level are very flat with
ovoid nuclei. The duct luminal cells now have features in
common with the secretory cells such as secretory vesicles
and degenerating mitochondria. Atretic luminal cells were
regularly observed in this transitional region.

Fig.3.15. An atretic luminal cell from the fundus/duct
transition zone.

Fig.3.16. The fundus/duct transition zone. A luminal duct
cell with a portion of its serosal membrane on the
basement membrane between two basal duct cells.

Fig.3.17. A basal duct cell in the fundus/duct transition
zone, with a group of filaments of varying thickness,
similar to those of myoepithelial cells.

FUNDUS
L
LC

SE
A
DUCT
BC
F
5μm
3/14

3/15
LC
L
N
A
BC
F
1μm
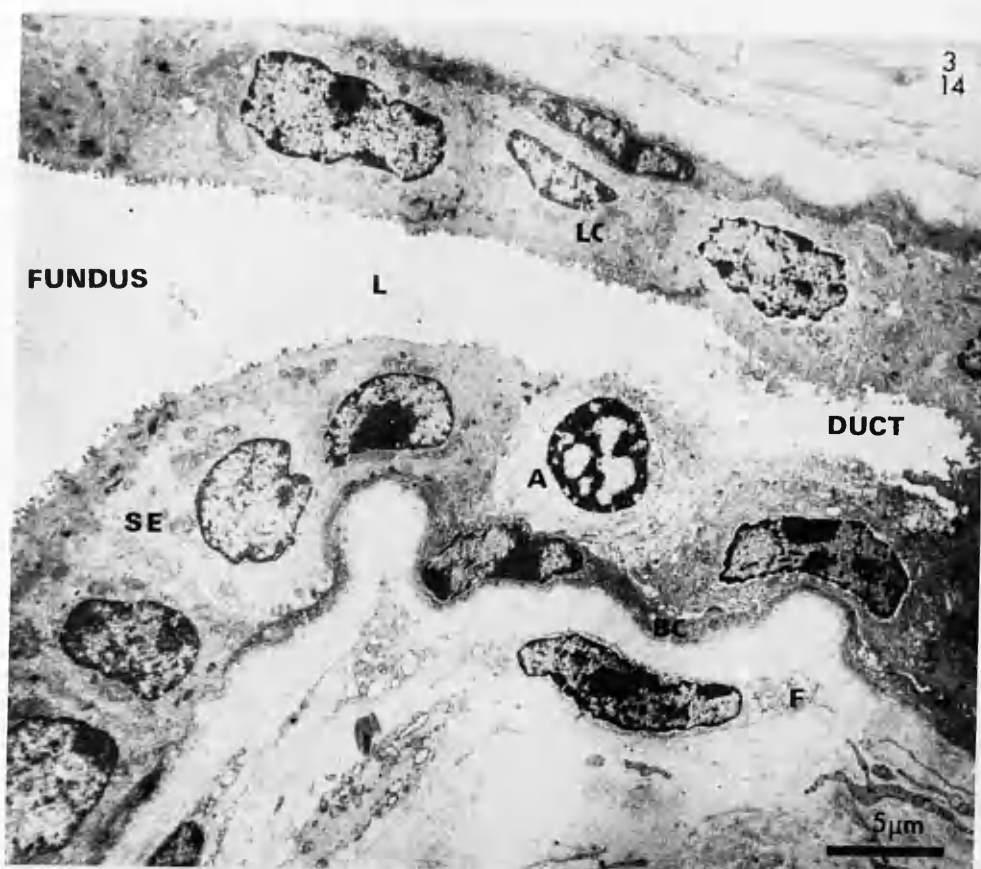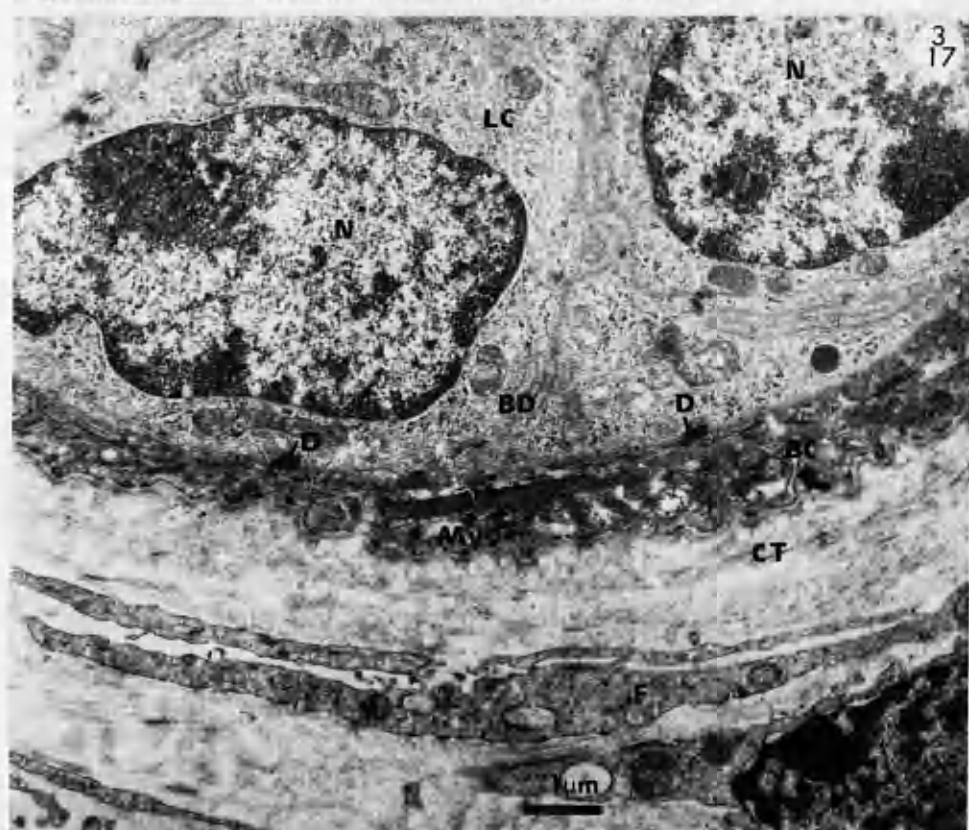
(Fig.3.18) were quite different from the tonofilaments of
the basal cells in the duct body and more closely
resembled those of the fundus myoepithelium. At the
junction between the duct and fundus there was no
appreciable morphological difference in the ultrastructure
of the luminal cells. The cells were connected by
junctional complexes with <u>zonulae occludentes</u> at the
luminal surface. The basal duct cells, although they
contained myofilament bundles, could still be
distinguished from the neighbouring myoepithelial cells
which at points overlapped (Fig.3.19).

Langerhans cells, identified by their Birbeck
granules, were located throughout the entire length of the
duct (Fig.3.20). Another structure found occasionally
along the length of the duct were the desmosome


<u>Fig.3.18</u>. A high power electron micrograph illustrating a
group of myofilaments. These are quite different from the
tonofilaments in the basal cells of the duct body.
<u>Fig.3.19</u>. The fundus/duct transition zone. The luminal
cell of the duct has a similar appearance to that of its
neighbouring fundus secretory cell. The fundus
myoepithelial cell with myofilaments, fusiform densities
and caveolae, overlaps with a basal duct cell rich in
ribosomes and containing glycogen deposits.
<u>Fig.3.20</u>. The body of a dendritic cell situated between a
luminal and basal cell in the duct wall. It is not
connected to the ductal cells and the presence in the
cytoplasm of the characteristic Birbeck granules identify
it as a Langerhans cell.

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connections between the fibrocytes of the sheath
surrounding the entire gland (Fig.3.21,22).

The active gland ultrastructure.

Fundus.

Upon activation of the glands few observable
differences were found in cellular appearance. An increase
in the diameter of the myoepithelial cells and crenation
of their nuclei were indicative of myoepithelial
contraction (Fig.3.23). The most conclusive evidence of
sweating activity was a widening of the intercellular
spaces between neighbouring cells of both epithelial
layers (Fig.3.24). In control and stimulated glands
membrane-bound vesicles containing filamentous material
and electron-dense inclusions were frequently seen

Fig.3.21. Fibrocyte sheath from the upper duct body.
Desmosomes connecting fibrocytes of the sheath.

Fig.3.22. Desmosome connecting fibrocytes from the sheath
of the lower duct body.

Fig.3.23. The active bovine sweat gland fundus. The
increase in diameter of the muscle cells is indicative of
myoepithelial contraction.

Fig.3.24. The active bovine sweat gland fundus. The most
significant sign of sweating activity was an increase in
the size of the spaces between the myo- and secretory
epithelia.

0·5μm

0·2μm

0·5μm

MyoE

SE

L

MyoE

N

N

2 μm

L

N

SE

SE

←D

M

MyoE

←D

1 μm
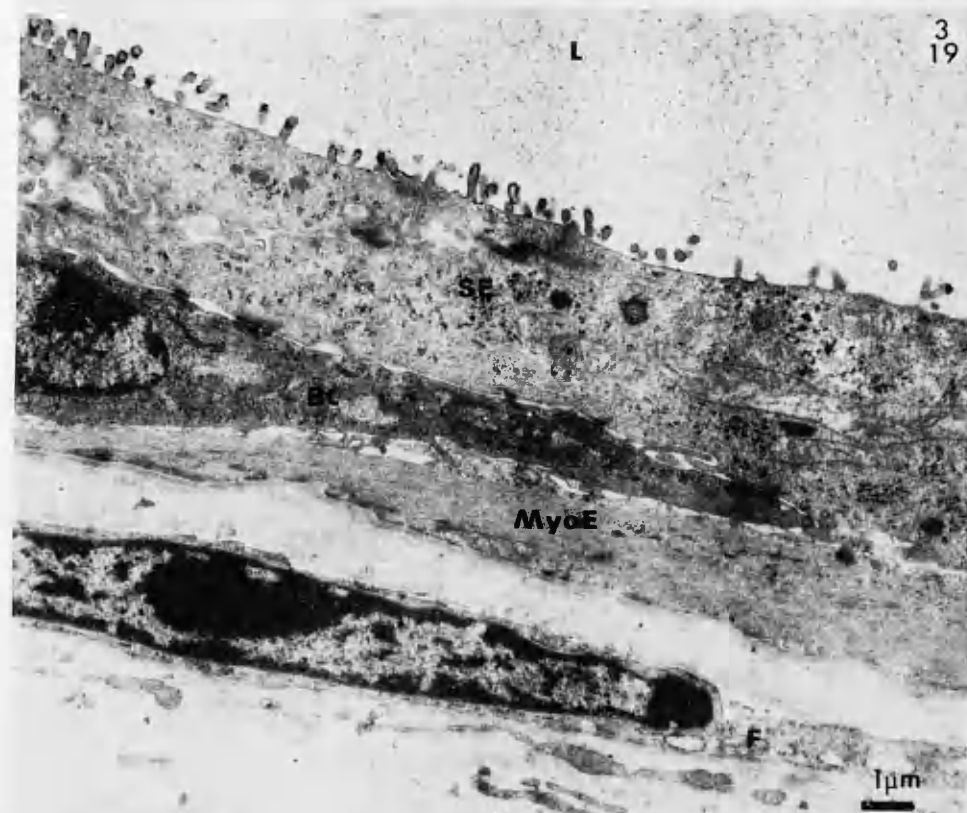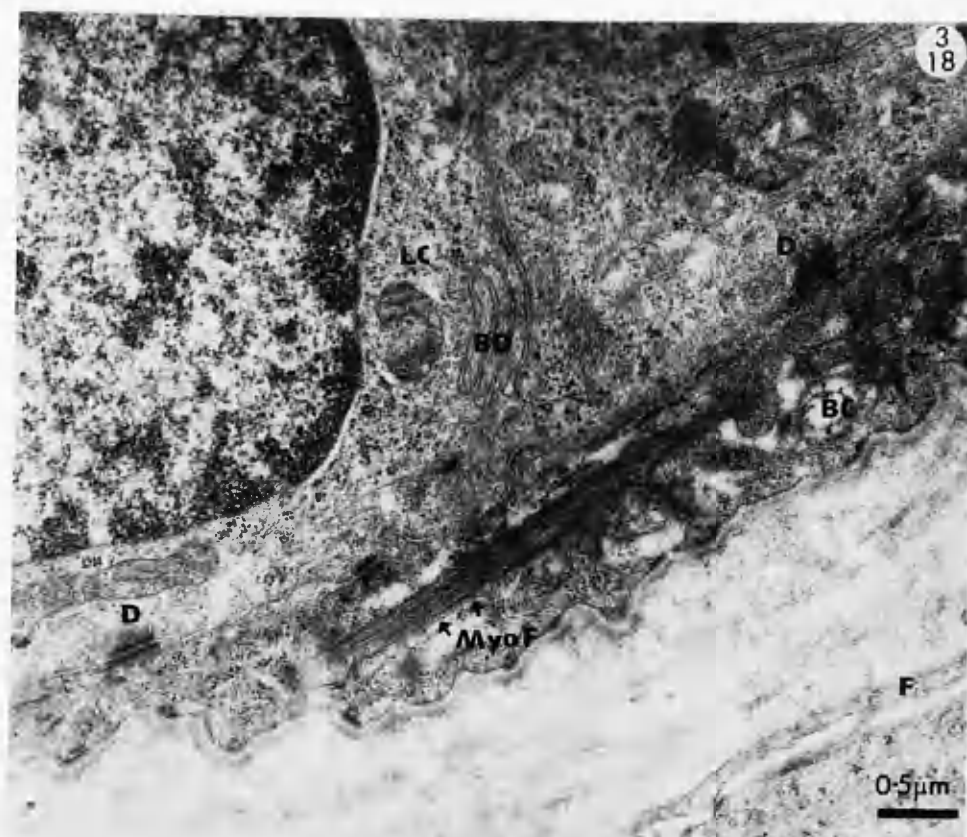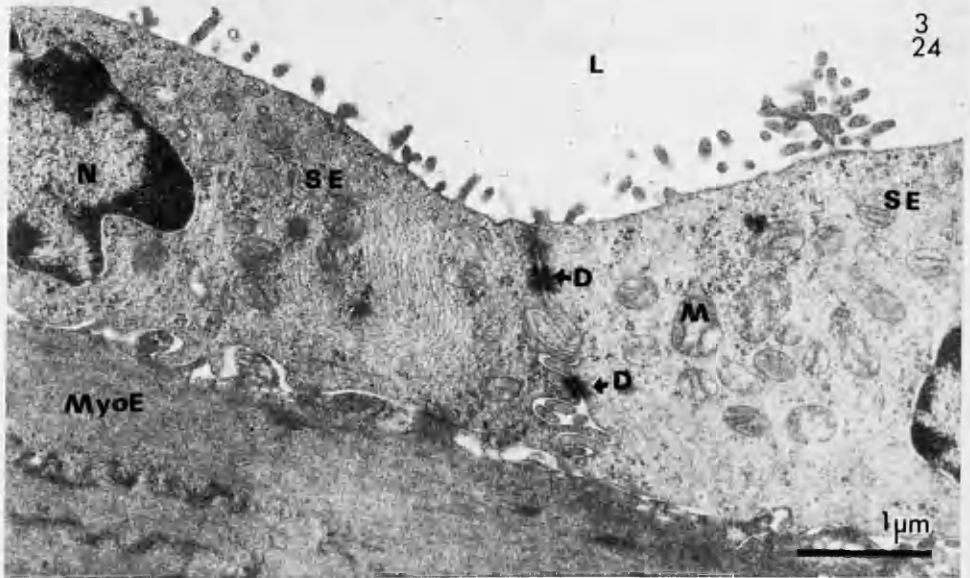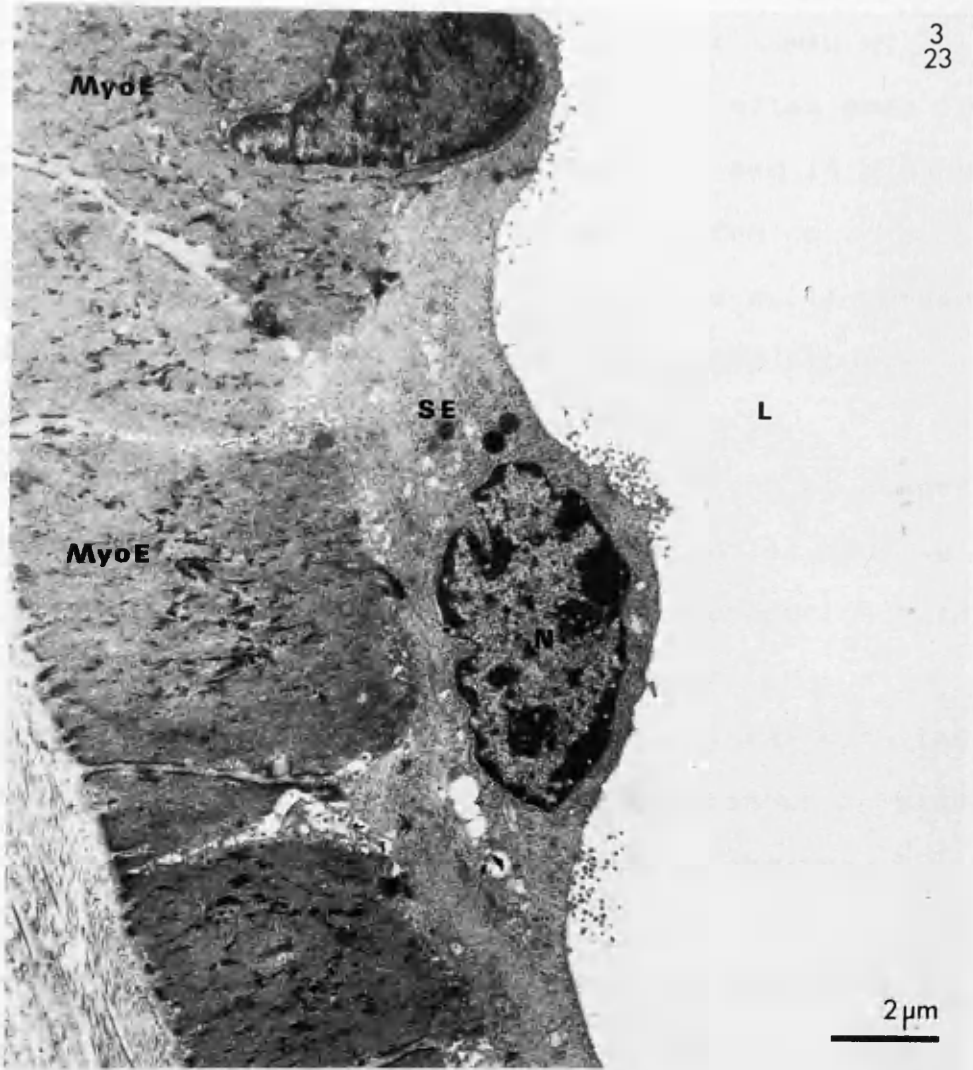
adjacent to the luminal membrane (Fig.3.25,26). They
appeared to release their contents into the lumen by
exocytosis (Fig.3.25). These vesicles were often seen in
close apposition to each other (Fig.3.27), and in a manner
which suggested the occurrence of vesicle fusion
(Fig.3.28). The source of these vesicles is still obscure.
Although vesicles of variable size were found closely
associated with the Golgi body and adjacent to
mitochondria (Fig.3.7), the glands at rest and at stages
of stimulation showed evidence of mitochondrial budding
and/or degeneration. Fig.3.29 shows a mitochondrion with
its 'bud' almost fully formed before separation.
Mitochondria exhibited changes in their cristae with the
appearance of microfilaments and long membranous cristae
(Fig.3.30,31). Some mitochondria were also observed to

Fig.3.25. An exocytotic vesicle at the luminal surface of
the secretory epithelium.

Fig.3.26. A vesicle containing an electron-dense aggregate
just below the luminal membrane of the secretory
epithelium.

Fig.3.27. Vesicles in a secretory cell apparently about to
fuse.

Fig.3.28. Fusing vesicles within a secretory cell.

Fig.3.29. An example of mitochondrial budding.

Fig.3.30. A large mitochondrion from a secretory cell
illustrating cristal changes.

Fig.3.31. A large dense inclusion and filaments in a
mitochondrion.

contain small electron-dense bodies (Fig.3.32).
Mitochondria with large dense aggregates and very few,
apparently degenerating, cristae were also found
(Fig.3.33). These mitochondria were similar in appearance
to the vesicles containing the electron-dense aggregates.

Secretory cell death was occasionally observed in the
active gland (Fig.3.34,35). The dead cells were more
frequently found close to the fundus/duct junction.

Duct.

Heat-induced sweating had little visible effect on the
ultrastructure of the glandular duct in cattle. The lumen,
particularly within the intrafollicular region, was
noticeably wider in the samples taken after stimulation.
The numerous small luminal vesicles observed amongst the
microvilli of the intrafollicular region were still
present. The luminal contents of the duct body which were
similar to those of the fundus were still seen. The
intercellular spaces in some ducts appeared to be wider,
but this was not established as a general feature during
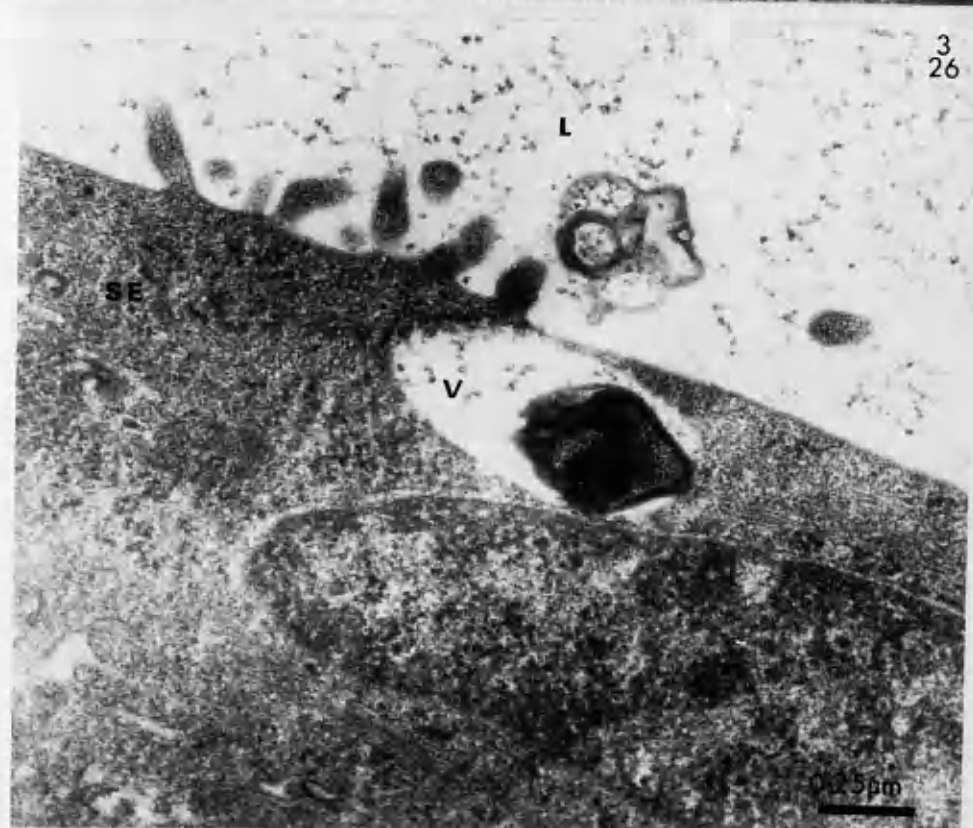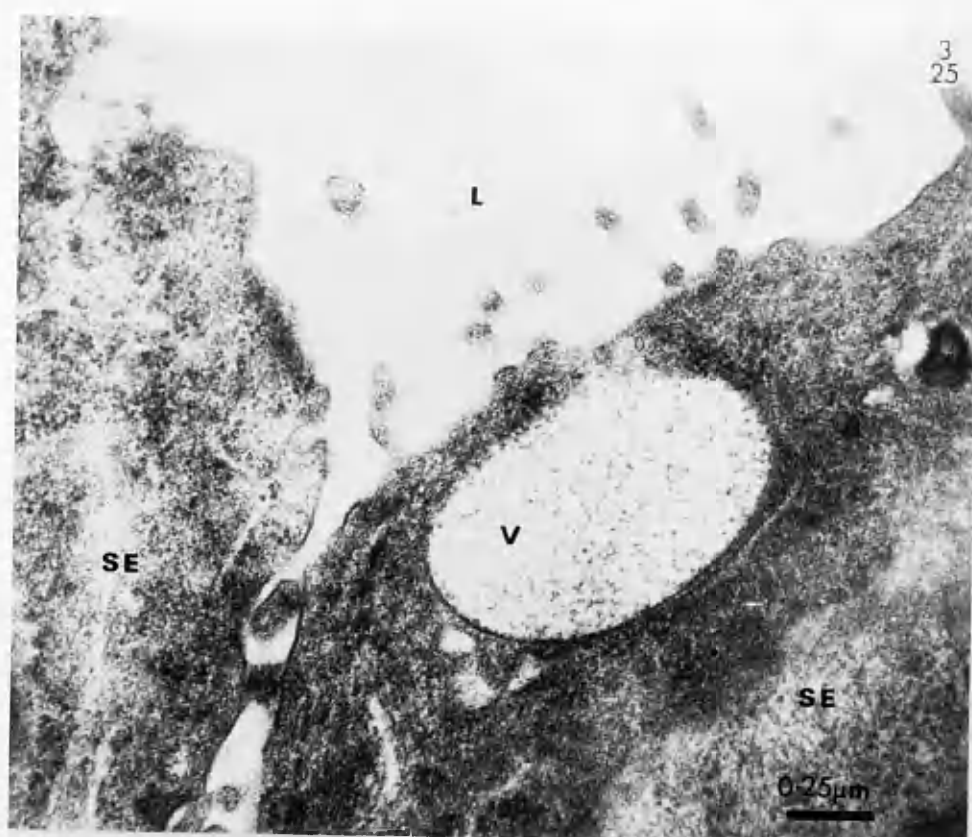sweating.

Fig.3.32. Mitochondria containing small electron-dense
inclusions.

Fig.3.33. A mitochondrion with a large inclusion and few
recognisable cristae.

Fig.3.34. A low power view of a disrupted secretory cell
from the active fundus.

Fig.3.35. Dead secretory cell from the active fundus. The
luminal membrane is disrupted.

3
29

N L M SE M M 0·5μm

3
30

N L RER MyoE RER 0·5μm
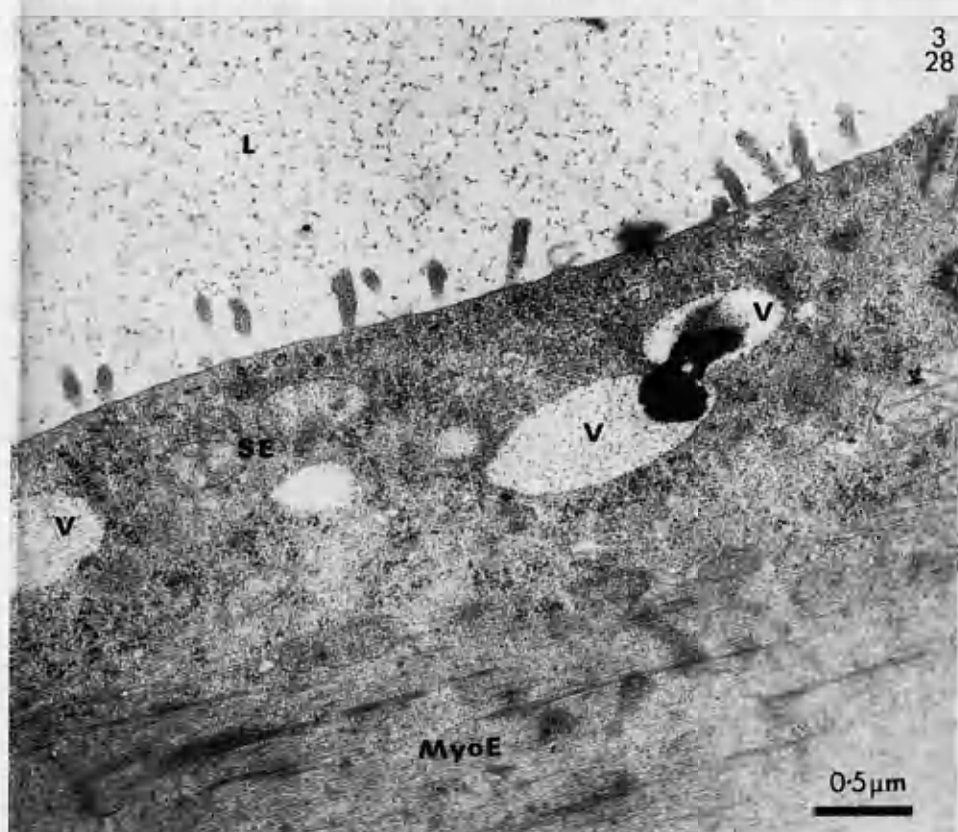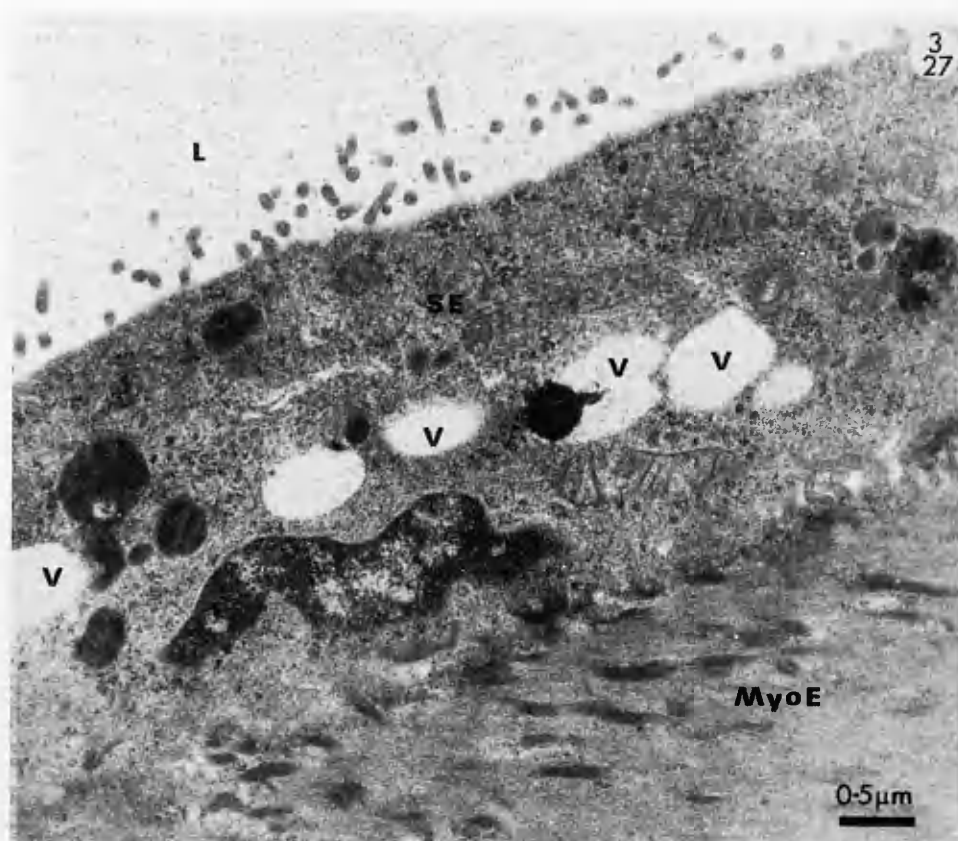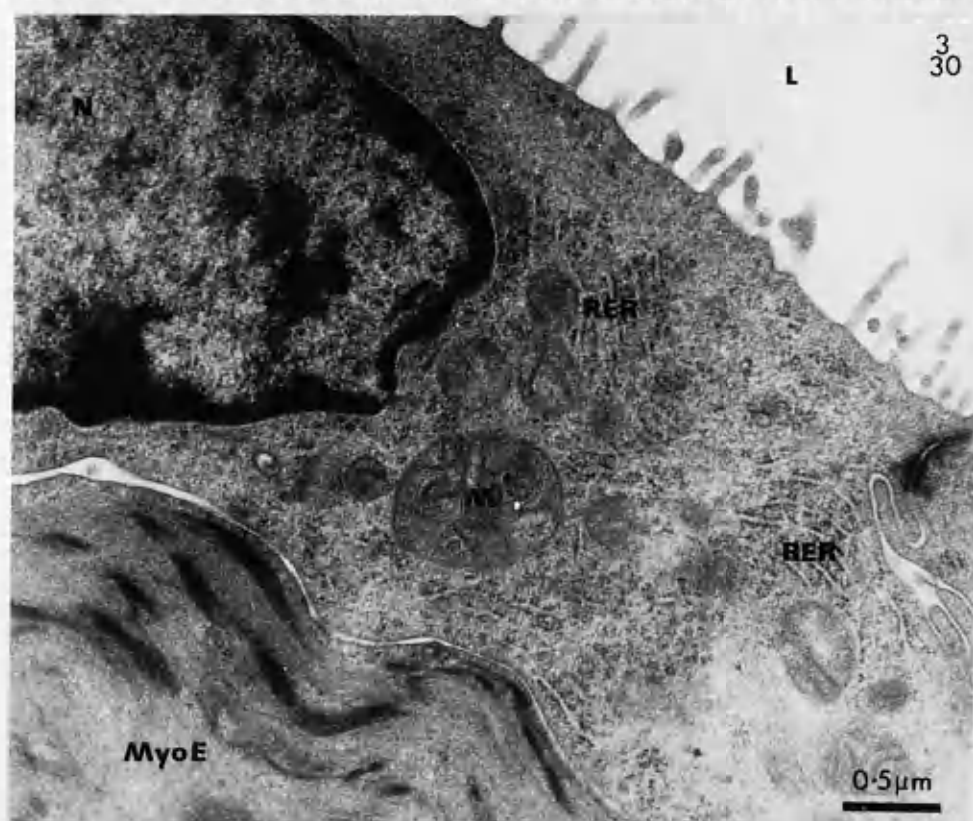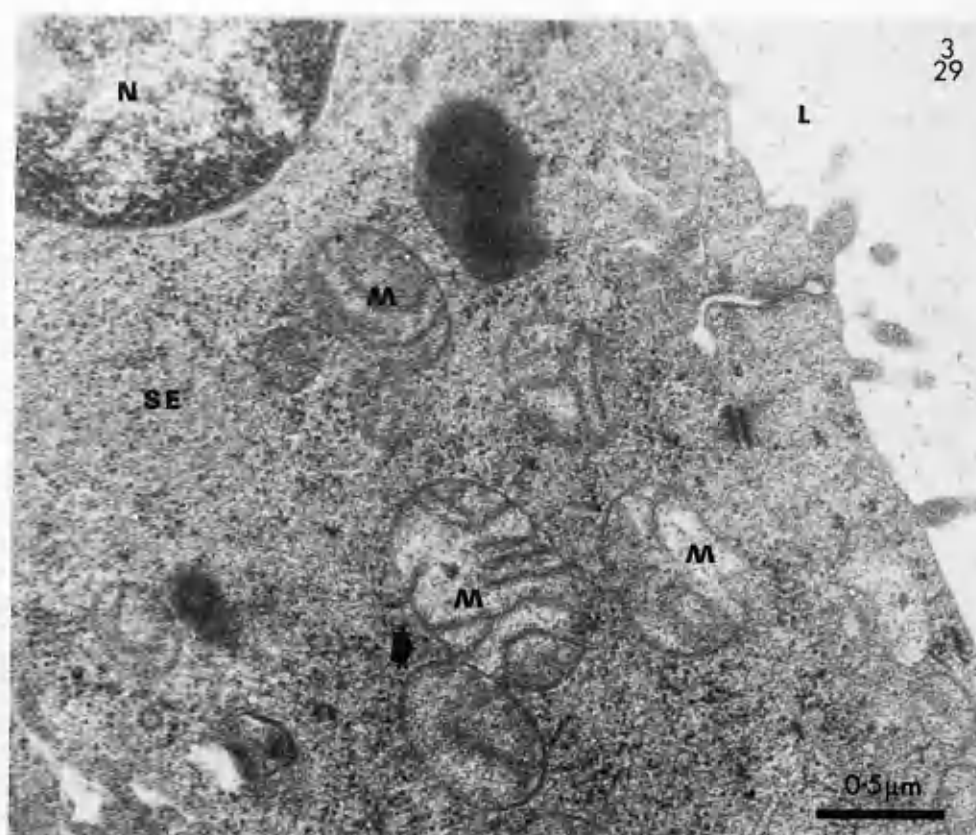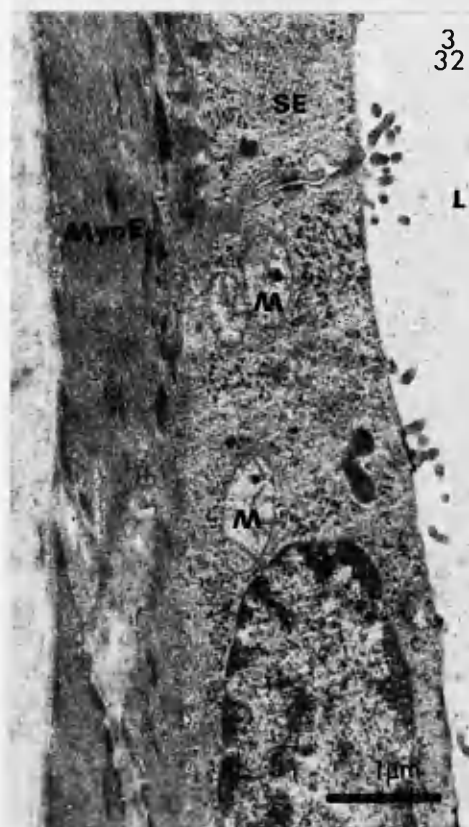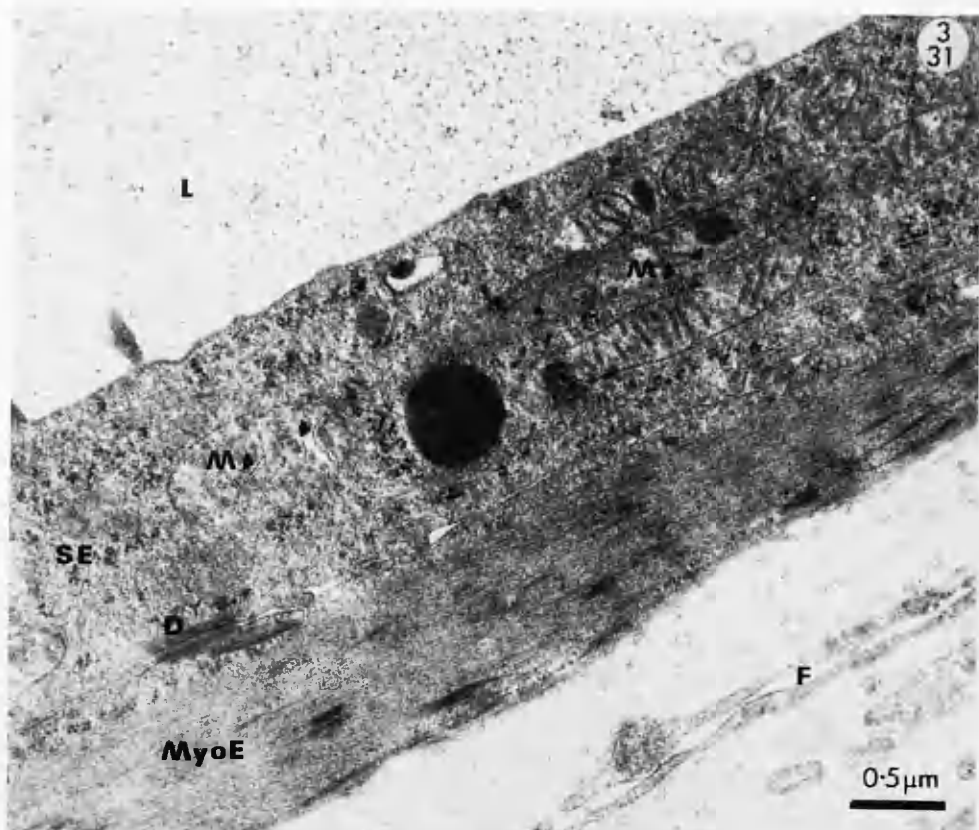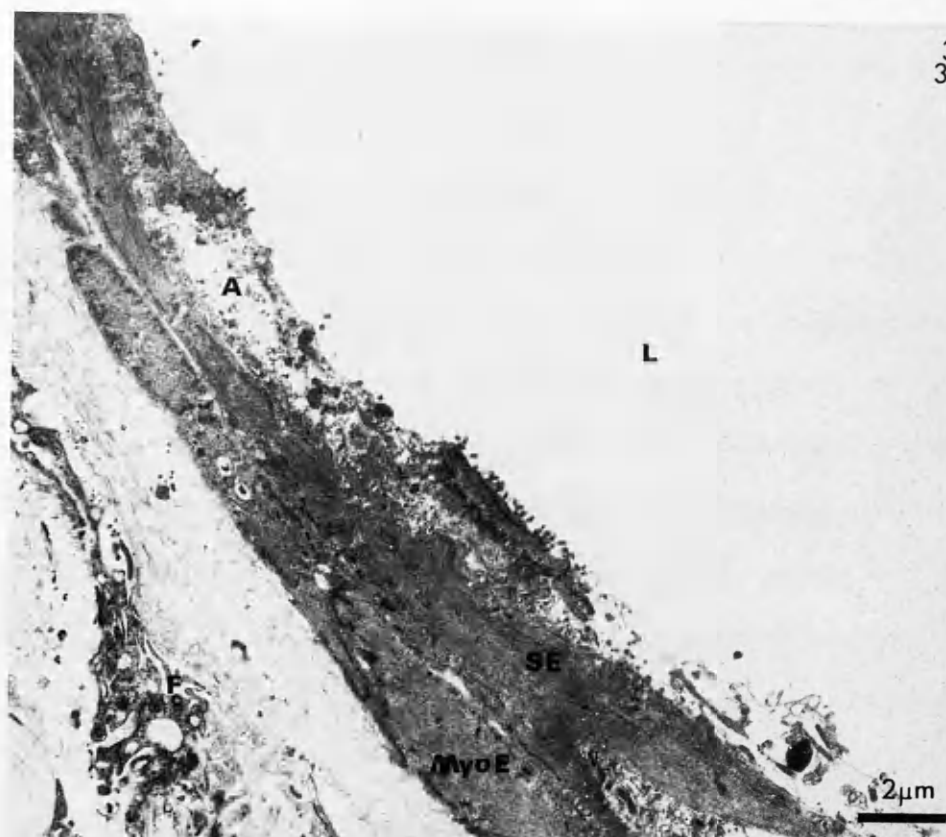
DISCUSSION.

The cytological appearance of the unstimulated cattle sweat gland fundus was similar to that found by Prasad (1973). There were few detectable cytological changes associated with sweating. The nuclear and cytological changes observed in the myoepithelium during sweating support the earlier conclusion, based on evidence of a reduction in glandular volume (Findlay & Jenkinson, 1964), that the myoepithelium seemed contracted throughout activity. However myoepithelial contraction could not be directly correlated with any specific stage of secretion. Columnar secretory cells were never seen, and the absence of apical necrobiosis confirms the view of Findlay & Jenkinson (1964) that the sweat glands of cattle are not apocrine glands as defined by Schiefferdecker (1917). The colloidal luminal contents, which are similar to those illustrated by Prasad (1973), appear to be derived from secretory vesicles, the probable source of the protein found in cattle sweat (Jenkinson, Mabon & Manson, 1974a). Their contents appear to be released by exocytosis in a manner similar to that described for the sweat glands of the primate axilla and cat (Biempica & Montes, 1965; Munger, 1965a). There was no evidence from this morphological study to show whether or not this process, or that of vesicle formation is accelerated during sweating. Indeed the mode of vesicle formation, and the source of the vesicles is still unknown. Vesicles, smaller than those at the luminal surface, were frequently seen close to the Golgi body. Thus they may be derived, as suggested for the sweat glands of other species (Charles,

26

1959; Biempica & Montes, 1965; Munger, 1965a,b), from the Golgi apparatus. However, secretory vesicles were also seen near to mitochondria and could be a product of mitochondrial transformation. These configurations were seen within mitochondria more frequently than could be explained by an ageing process. In the apocrine sweat gland of the human axilla mitochondria containing osmiophilic inclusions have been observed (Biempica & Montes, 1965), and associated with secretory granule formation (Charles, 1959; Biempica & Montes, 1965; Munger, 1965b; Hashimoto, Gross & Lever, 1966b). Although secretory granules were not present in cattle sweat glands, it is conceivable that the secretory vesicles with dense contents is the bovine equivalent. The close apposition of these vesicles with and without dense contents strongly suggested the occurrence of vesicle fusion, and so a third possibility is that the larger vesicles at the luminal surface were produced by fusion of either or of both of the preceding types, perhaps in association with lysosomal action.

It is difficult to see how any of these observations can account for the remarkable sustained production of sweat of which the cow is capable. The most noticeable feature of the gland is the increase in the size of the intercellular spaces between the myo- and secretory epithelia. This increase in the intercellular spaces, which also occurs in man (Sato, 1974), appears to be a characteristic of the active sweat gland. Therefore, the conclusion can be made that secretion involves fluid

transport and exocytosis but that the products of cell death also contribute to sweat composition.

CHAPTER 4.

THE OVINE AND CAPRINE GLAND.

INTRODUCTION.

It seems reasonable to suppose that sheep and goats with a different sweating pattern to that of cattle may have a different mode of secretion. As described in Chapter 1 sheep and goats show discrete intermittent discharges of sweat which gradually decline in size to what has been called 'fatigue' (Bligh, 1967; Robertshaw, 1968). The 'fatigue' of the glands is apparently due to the rate of expulsion exceeding that of sweat production (Jenkinson & Robertshaw, 1971; Johnson, 1973). In the goat, as in cattle, light microscopy shows a decrease in glandular volume during sweating supporting the view of sweat expulsion by myoepithelial action. The ultrastructure of the gland in sheep and goat, even at rest, has not been described and the mechanisms of sweat production and expulsion are still incompletely understood.

Therefore by studying the ultrastructural appearance of the sweat glands, before, and at intervals during sweating in a controlled warm environment, information on the modes of secretion and sweat expulsion can be obtained and compared with the mechanisms found in cattle.

PROCEDURE.

Five adult castrated male Dorset Horn X Finnish Landrace sheep and two adult castrated British Saanen goats were subjected to 15°C Dry Bulb (DB)/10°C Wet Bulb (WB) for 3 hours and cutaneous evaporative loss was

monitored continuously. Skin samples were taken
immediately upon entry and before removal of the animals
from the chamber. The following day the animals were
induced to sweat by placing them in an environment of
40°C DB/26°C WB. Cutaneous evaporative loss was again
measured continuously and samples were taken from the
contralateral side at the times shown in Fig.4.1.

Three additional female British Saanen goats were
subjected to 40°C DB/26°C WB and sampled before and at the
end of the exposure after sweat gland 'fatigue'. These,
with one of the other goats and three of the sheep, were
sampled to provide a range of samples from 'fatigue'
during the recovery period at the times shown in Fig.4.2.
These samples were taken in a cool environment and the
intervals were measured from the moment the animals left
the hot environment. All samples were fixed and processed
for transmission electron microscopy as described in
appendix 1.

RESULTS.

Patterns of evaporative loss.

The patterns of cutaneous evaporative loss from the
five sheep and two goats in an environment of 40°C DB/26°C

Fig.4.1. The patterns of cutaneous evaporative loss from 5
sheep and 2 goats in an environment of 40°C DB/26°C WB
Skin samples were taken from the contralateral side at the
times shown (numbered arrows).

Fig.4.2. Skin sampling times during the sweat gland
recovery period, measured from the time of leaving the hot
environment.
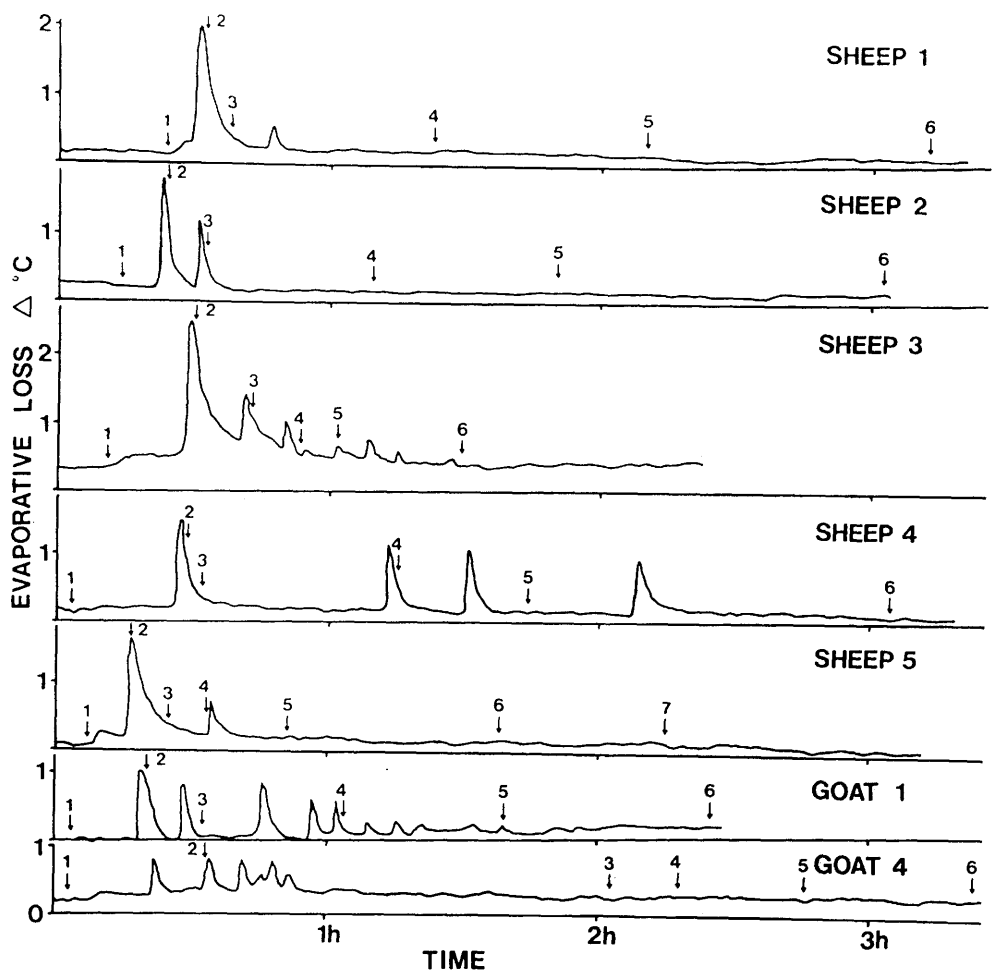
Animal	Environmental temperature max/min (°C)	Sampling times (h-min)						
		1.	2	3	4	5	6	7
Sheep 3	12 (controlled)	0–15	0–45	1–30	3–0	9–0	24–0	
Sheep 4	12 (controlled)	0–10	0–40	1–0	1–45	2–30	7–0	22–0
Sheep 5	11/9	0–25	3–0					
Goat 1	21/19	1–0	3–0	6–0	10–0	24–0		
Goat 2	13/9	1–0	2–0	3–0	5–0	10–0	24–0	
Goat 3	13/9	0–15	0–30	1–0	4–0	5–0	10–0	24–0
Goat 5	12/10	0–10	0–40	1–45	2–30	4–0	9–0	24–0

WB are shown in Fig.4.1. Although there was substantial inter-animal variation the decremental pattern of discrete synchronous sweat discharges to 'fatigue' was found in all animals. Sweating was not observed during the control experiment at 15°C using the same animals.

Sweat gland structure.

The ovine and caprine sweat gland consists of a coiled fundus with a duct which passes through the dermis alongside the hair follicle between lobes of the sebaceous gland. The duct penetrates the cell layers of the hair follicle close to the surface and opens into the follicular orifice. The structure of the duct in sheep and goat was essentially similar to that found in the cattle. In both species the intrafollicular/perifollicular region was 75±41μm in length. The duct body in sheep was 555±46μm long and 450±41μm long in the goat.

The unstimulated gland ultrastructure.

Fundus.

Sheep and goats had an analagous ultrastructure in the specimens taken before sweating at 40°C, and during and after exposure to 15°C. The fundus of both species consisted of a cuboidal secretory epithelium surrounded by a myoepithelium and basement membrane (Fig.4.3,4). The whole gland was enveloped by a fenestrated fibrocyte

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Fig.4.3. Electron micrograph of a section through the sweat gland fundus of the sheep showing the low cuboidal secretory epithelium characteristic of the unstressed gland, the surrounding myoepithelium and fibrocyte sheath.

sheath (Fig.4.3). The fundus lumen in sheep and goat did not always contain colloidal filamentous material but when present it was similar to, but less densely packed than, that in cattle. The junctional complexes between epithelial cells were comparable to those found previously in cattle. Myoepithelial cells had hemidesmosomes on the basement membrane and desmosomes between neighbouring muscle cells and the secretory epithelium (Fig.4.5). Secretory cells were linked by apical junctional complexes, with desmosomes and zonulae occludentes (Fig.4.6). Basal dovetailing was present, although it was much less pronounced than in cattle. The microvilli of the secretory cells were more numerous, shorter, and more frequently branched than those of cattle (Fig.4.7). Microvilli were also present in the clefts between the secretory and myoepithelial cells.

Fig.4.4. This electron micrograph from the unstressed sweat gland from the goat illustrates the species similarities. The fundus has a low cuboidal secretory epithelium and surrounding myoepithelium.

Fig.4.5. Electron micrograph illustrating the cellular connections between myoepithelium and secretory epithelium in the goat.

Fig.4.6. High power view of an apical junctional complex between secretory cells of a goat.

Fig.4.7. High power view of luminal microvilli from a secretory cell of the goat.

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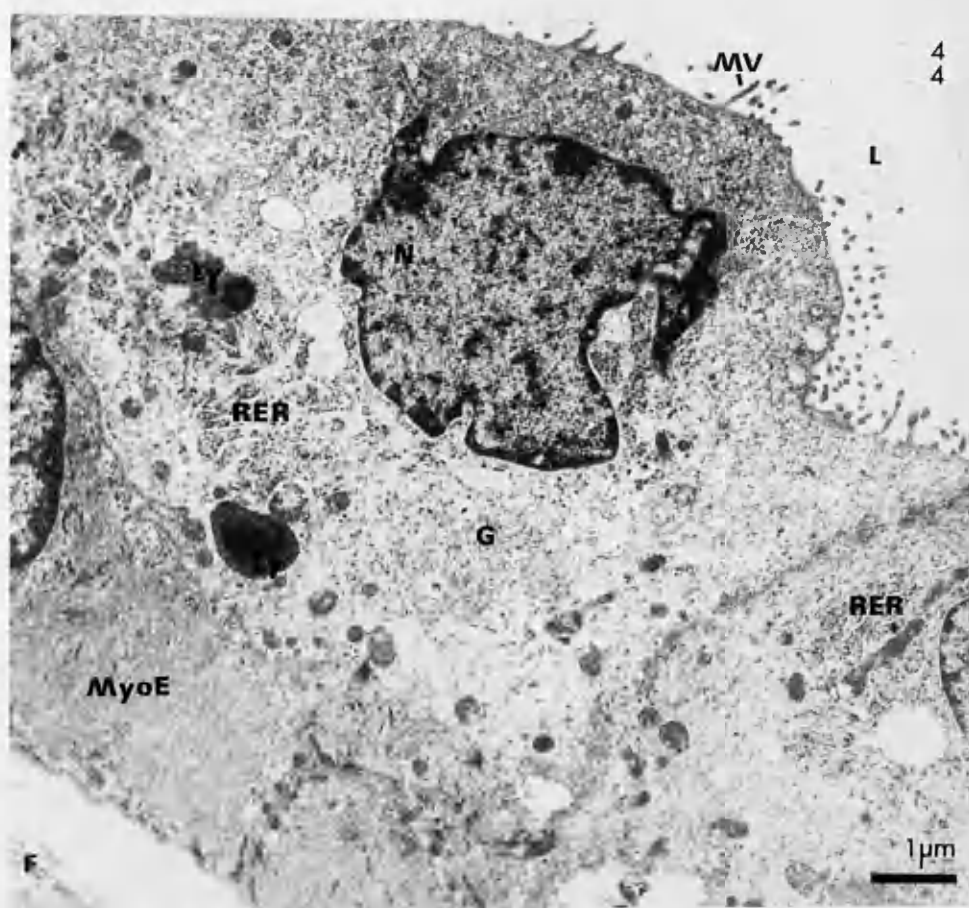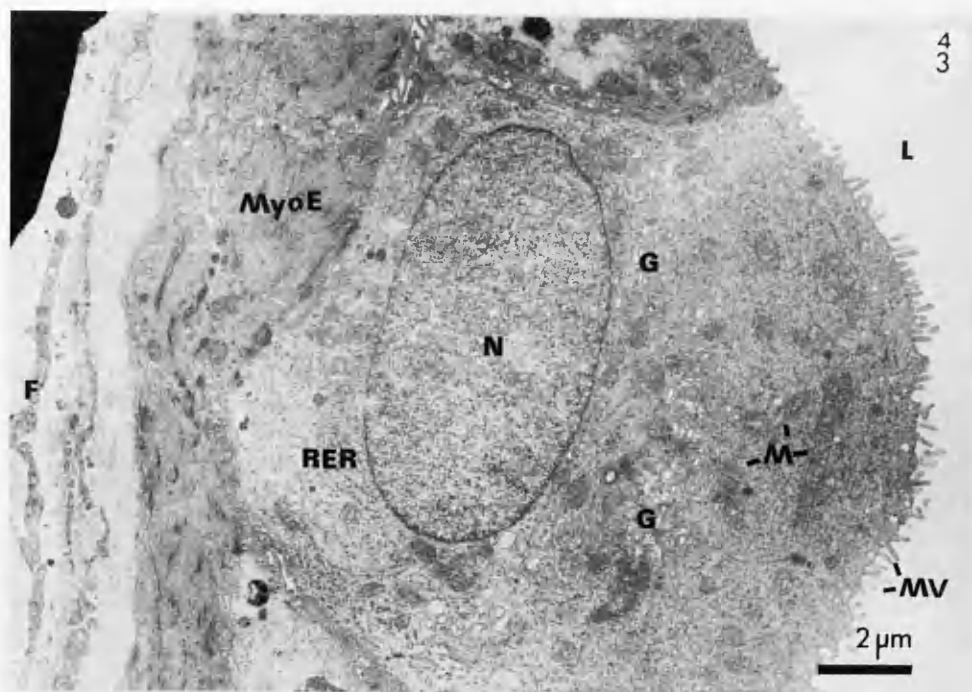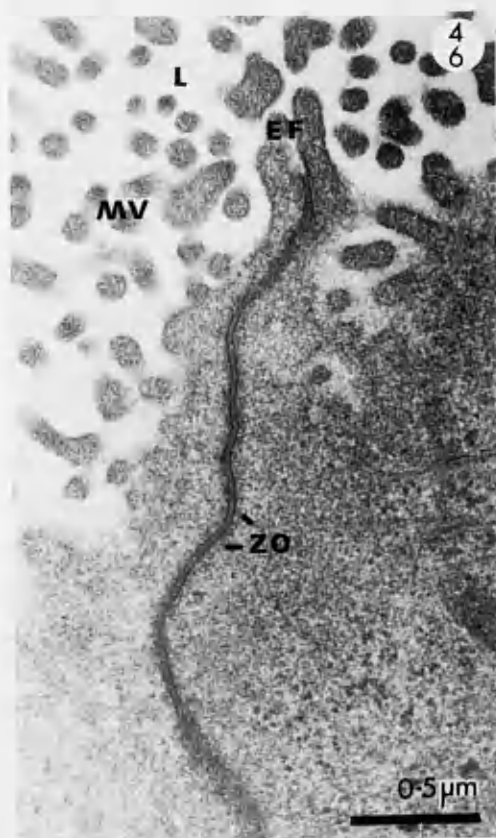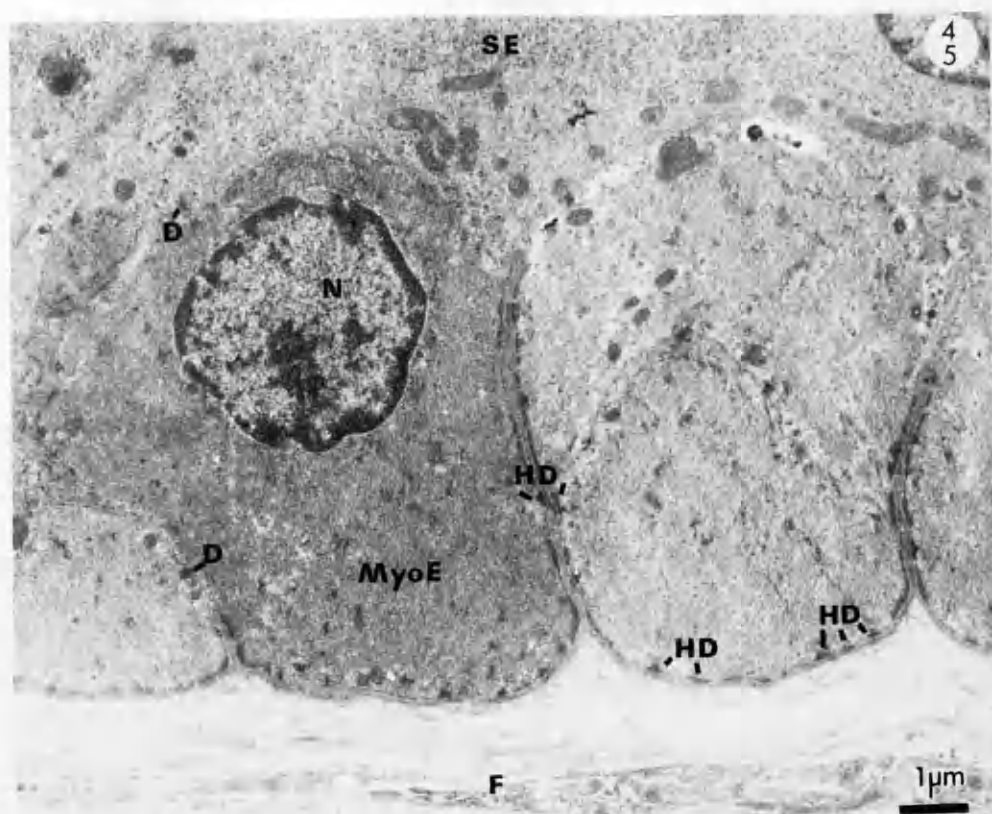
The cytoplasm of the secretory cells had a fine granular texture due to polysomes. The ovoid nucleus contained mainly euchromatin with a little heterochromatin (Fig.4,3,4). In some cells a prominent nucleolus was present. The Golgi apparatus was a prominent organelle, generally lateral to the nucleus, although sometimes in a more luminal position (Fig.4.8). A more extensive RER was found located towards the base of the cell and in a perinuclear position (Fig.4.9). The small mitochondria were ovoid and had an electron dense matrix. The cristae, lying in a transverse position, were of the flat lamellar type. The lysosomes present were small and inconspicuous. Coated vesicles were occasionally seen closely associated with them. Microfilaments were a prominent feature of the secretory cells and were more noticeable in the basal regions and the apex where they lay parallel to the luminal membrane. Closely associated with the Golgi apparatus were membrane-bound vesicles (Fig.4.8), often found immediately below the luminal membrane and appearing to be undergoing exocytosis (Fig.4.10).

The myoepithelium consisted of a single layer of well-developed smooth muscle cells, which were thinner than the overlying cuboidal secretory cells and more

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Fig.4.8. Electron micrograph of the Golgi and its associated vesicles from a secretory cell of the sheep.

Fig.4.9. Rough endoplasmic reticulum from a secretory cell of the goat.

Fig.4.10. Membrane-bound exocytotic vesicles at the luminal surface of the secretory epithelium of a goat.

L V G V G G N 4 8 1μm

N RER M M 4 9 μm

L V V V 4 10 0·5μm
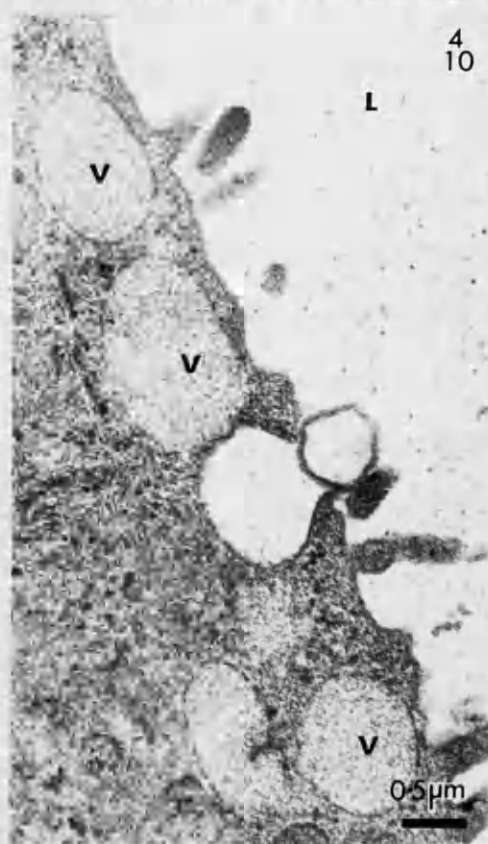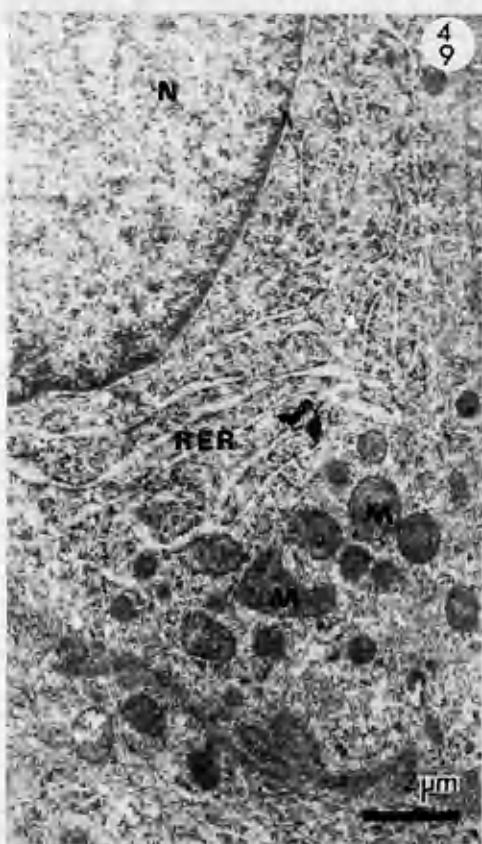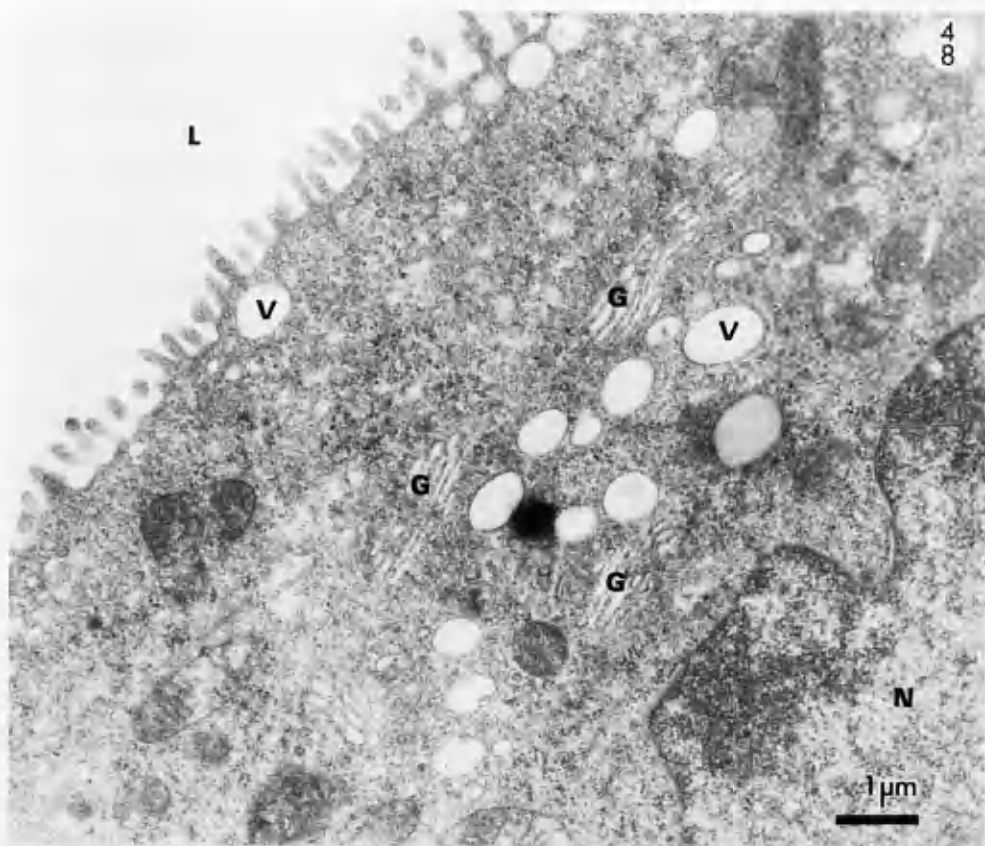
angular than those of cattle. They also exhibit a greater overlapping at their poles than cattle. The cells had thick and thin filaments with what appeared to be sarcoplasmic reticulum between them. The apical cones at the nuclei contained RER and mitochondria which were also found randomly in the peripheral sarcoplasm. Caveolae were also present on the basal surface and granules possibly of beta-glycogen were found throughout the cytoplasm.

Langerhans cells were seen occasionally between the secretory epithelium and myoepithelium.

Duct.

The intrafollicular duct had a keratinized lumen with some six cornified layers surrounded by four layers of granular cells (Fig.4.11,inset). In the goat the keratinized lumen usually extended through the intrafollicular region to the perifollicular zone (Fig.4.12). The single living cell composing the luminal wall of the duct in the perifollicular zone was replaced by two or three luminal cells (Fig.4.13). The duct lumen of the perifollicular zone in the sheep was generally lined by three cells with a three cell layer duct wall (Fig.4.14). Amongst the luminal microvilli of both species in this zone numerous small vesicles were observed (Fig.4.13 inset,14 inset).

Fig.4.11,inset. Keratinized lumen of the intrafollicular duct of the goat.

Fig.4.12. Keratinized lumen of the perifollicular duct in the goat.
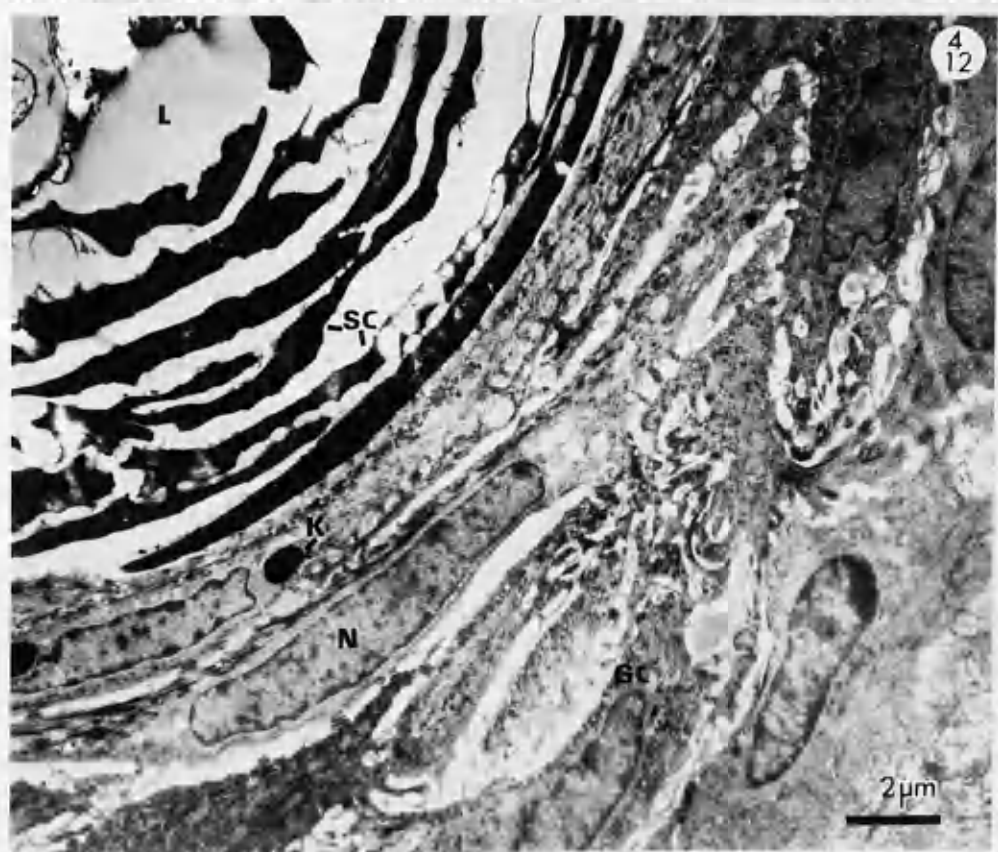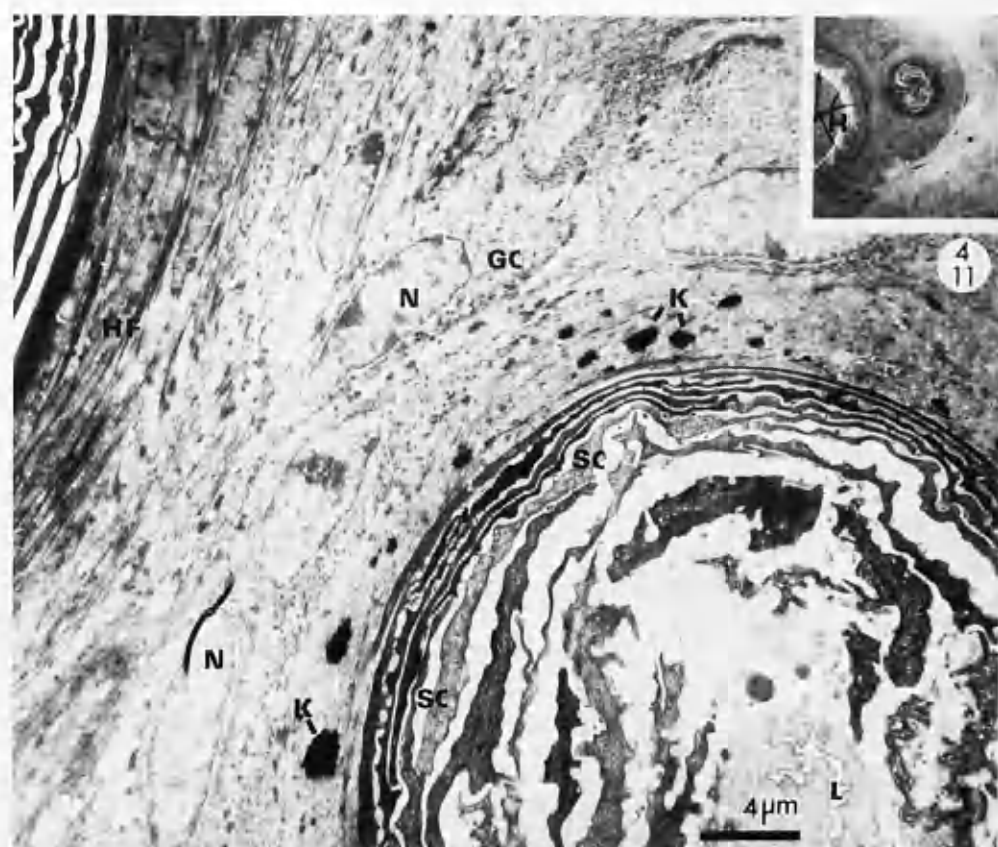
The ultrastructure of the duct body in both the sheep and goat was similar to that of the cow, although the tonofilament bundles of the basal cells were less conspicuous and appeared to be less well developed (Fig.4.15). Dovetailing of lateral microvilli, particularly towards the base of the luminal cells, not a noticeable feature in the cow, was present in the goat, and was even more prominent in the sheep (Fig.4.16). In the lower duct body, desmosomes and gap junctions were present between basal cells (Fig.4.17,inset).

Fig.4.13,inset. Perifollicular zone of the duct from a goat. The inner two cells form a ring lined with microvilli. The outer layers of granular cells are connected by desmosomes. Vesicles were observed between the microvilli at the luminal surface throughout this zone.

Fig.4.14,inset. The perifollicular zone of the sheep. The lumen is lined by three cells with a three cell layer duct wall. Langerhans cells and their processes can be seen between cell layers. Vesicles were observed between the microvilli at the luminal surface throughout this zone.

Fig.4.15. The duct region in a goat illustrating the two epithelial layers and surrounding fibrocyte sheath. A Langerhans cell process can be seen between the inner and outer cell layers.

Fig.4.16. The duct body region in a sheep illustrating the dovetailing of lateral microvilli towards the base of luminal cells.
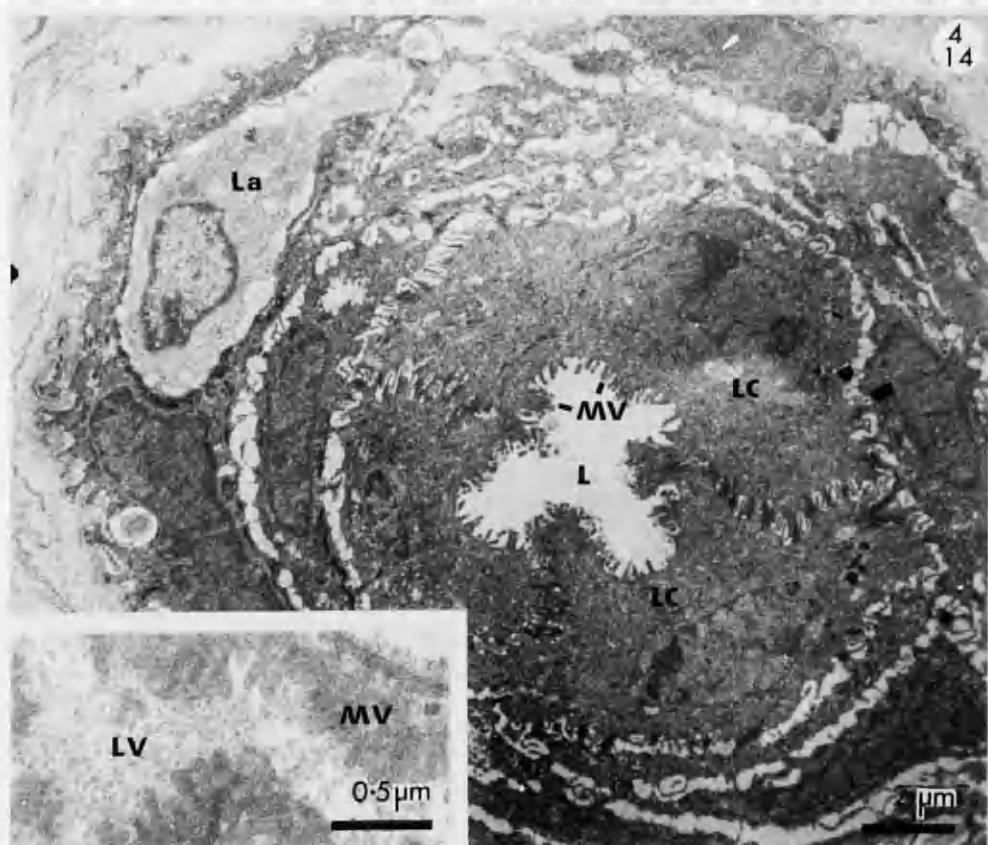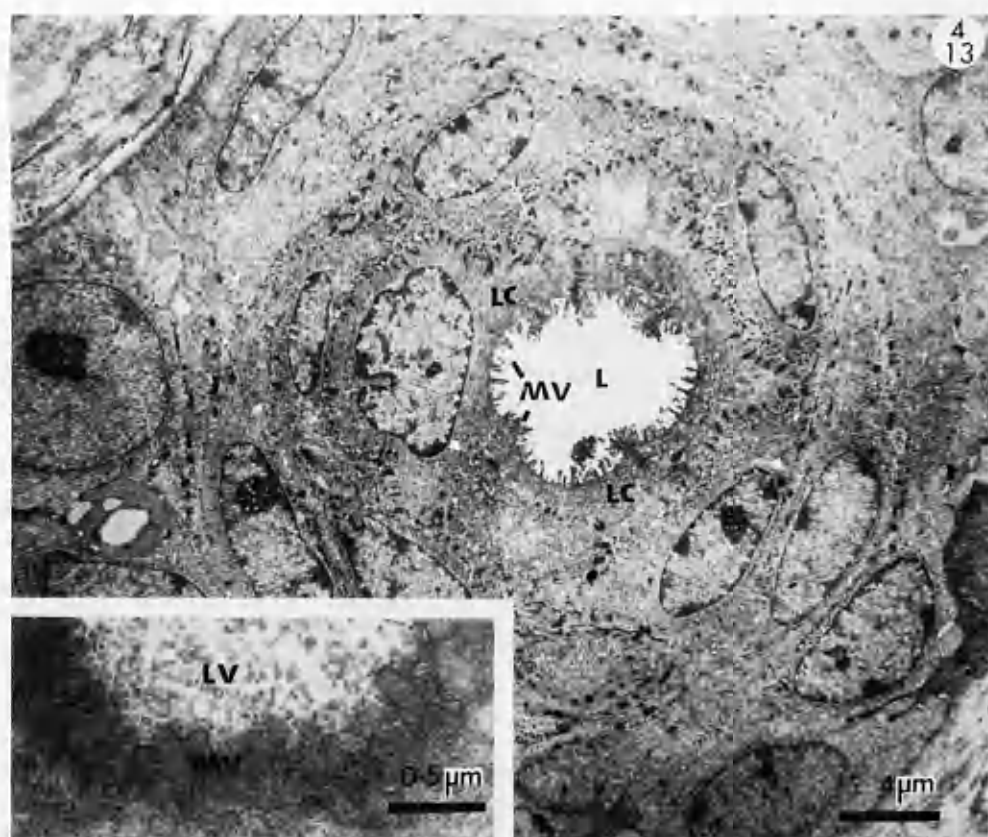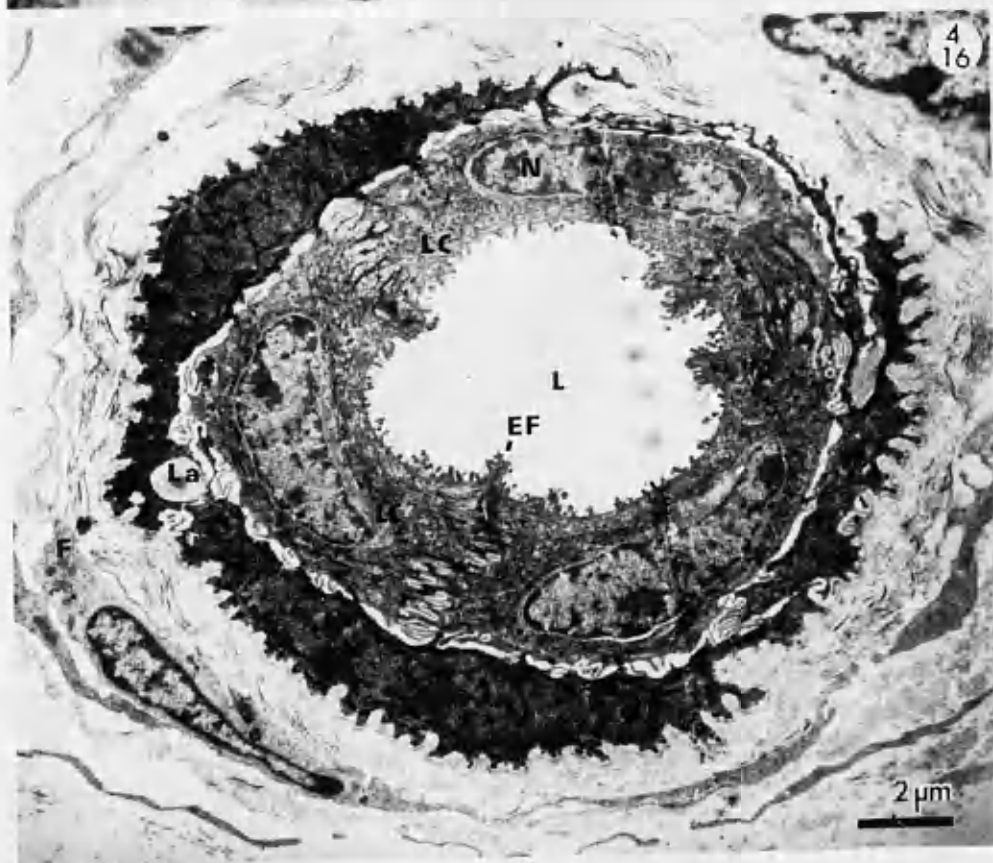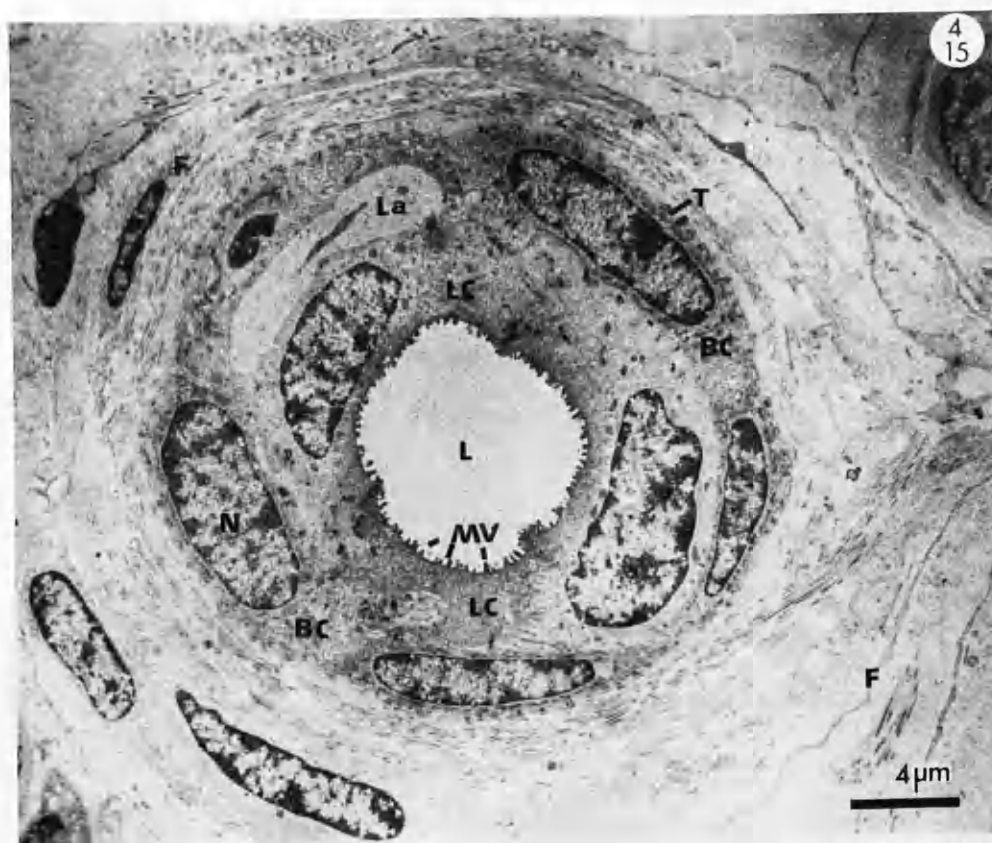
At the transition zone the luminal cells increasingly became more cuboidal and reminiscent of secretory cells as they neared the fundus. Basal cells like those of the cow contained myofilament bundles and exhibited features characteristic of myoblast cells (Fig.4.18). Langerhans cells were also found between duct wall cells throughout the duct. In the sheep they were more prominent and exhibited more dendritic processes.

The active gland ultrastructure.

Fundus.

The glands of the sheep and goat exhibited qualitatively similar changes on stimulation. These were, however, much more pronounced in the goat. During the first sweat discharge (Fig.4.1) there was little change in the appearance of the secretory cells in either species (Fig.4.19). The Golgi apparatus was large with numerous secretory vesicles. Some of the cells, especially those in samples taken during the downward slope of the peak, had a convex luminal membrane with misshapen apical microvilli.

Fig.4.17. In this electron micrograph from the lower duct body of the goat a gap junction and desmosome is present between basal duct cells.

Fig.4.18. Fundus/duct transition zone from the goat. Luminal cells are reminiscent of fundus secretory cells while basal cells exhibit features characteristic of myoblast cells.

Fig.4.19. The fundus of the goat after the first sweating peak showing little change in the appearance of the secretory cells.

By the beginning of, and during, the second sweat
discharge, the myoepithelium appeared contracted and the
secretory cells were now columnar (Fig.4.20). Microvilli
on the lateral membranes of the columnar secretory cell
were still numerous. Those at the apex were sparse,
sometimes distorted, and in some instances absent
(Fig.4.21). The columnar secretory cells had a very
prominent Golgi apparatus and numerous secretory vesicles.
Glands were also observed during the second and third
discharge where the secretory cells remained cuboidal.
These cuboidal cells were more frequently found in the
sheep (Fig.4.22) than in the goat, although, cells were
found with a misshapen luminal membrane (Fig.4.23). In

Fig.4.20. The fundus of the sheep during the upward slope
of the second sweating peak (sheep 4, sample 4). The
secretory cells have become columnar and the myoepithelium
appears to be contracted.

Fig.4.21. Apical portion from secretory cells of the sheep
at the second sweating peak. Microvilli on the lateral
surfaces are numerous while those at the apex are sparse
or absent.

Fig.4.22. In this electron micrograph of the goat fundus
taken after the second sweating peak the lumen is packed
with secretory product making it difficult to determine
the exact shape of the secreory cell.

Fig.4.23. This micrograph of the fundus from a sheep taken
at the second sweating peak has an active secretory cell
with a misshapen luminal membrane.

some glands the amount of secretory product and cellular

debris in the lumen made it difficult to easily determine

the exact shape of the secretory cell (Fig.4.24). During

and from the third sweat discharge (the second in goat 1)

until 'fatigue' secretory cells varied markedly in

appearance, even within a particular gland. They could be

classed into three types, all of which were columnar.

Cells of the first type were similar to those found at the

second sweating peak except that the secretory vesicles in

their apical cytoplasm were more numerous. The

intercellular spaces between the secretory and

myoepithelial cells exhibited a marked dilatation

suggesting the accumulation of intercellular fluid. In the

second type (Fig.4.25) the Golgi apparatus was very large

and disorganised. The RER was strikingly distended and

appeared to be vesiculating in the apical region of the

cell. Microvilli on the lateral membrane were normal

whereas those at the apical membrane were uneven, angular

and often missing. These cells also had a marked

dilatation of the perinuclear cisternae. The third cell

Fig.4.24. Electron micrograph of an active secretory cell

showing stress of the apical membrane from a sheep at the

second sweating peak.

Fig.4.25. Electron micrograph of the fundus of the sweat

gland of a goat taken on the downward slope of the fifth

sweating peak (goat 1, sample 4). In the much contracted

gland, columnar cells show marked Golgi and RER dilatation

and activity. Note the nucleus in the lumen.

4
17

0·25 μm

0·1 μm

4
18

0·5 μm

L

MV

G

SE

N

G

N

MyoE

F

2 µm

SE

N

L

N

MV

SE

G

MyoE

N

F

1 µm

4
23

L

SE

N

>MV

G

MyoE

1 µm

4
24

MV

L

SE

SE

SE

N

0·5 µm
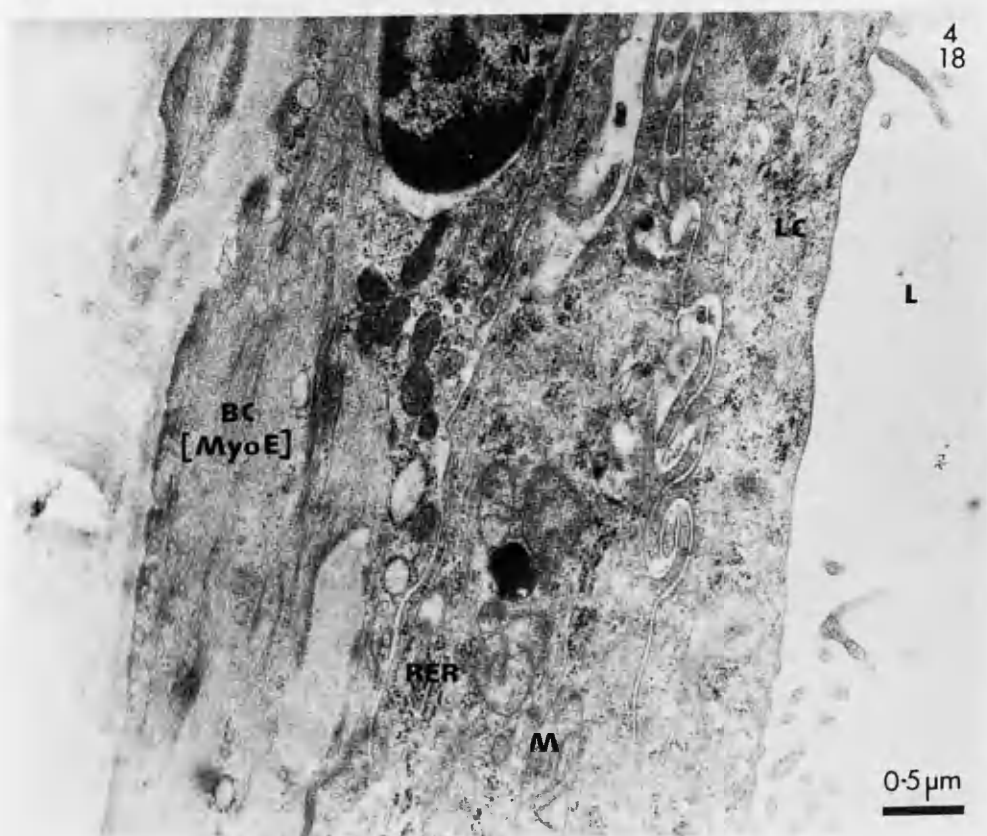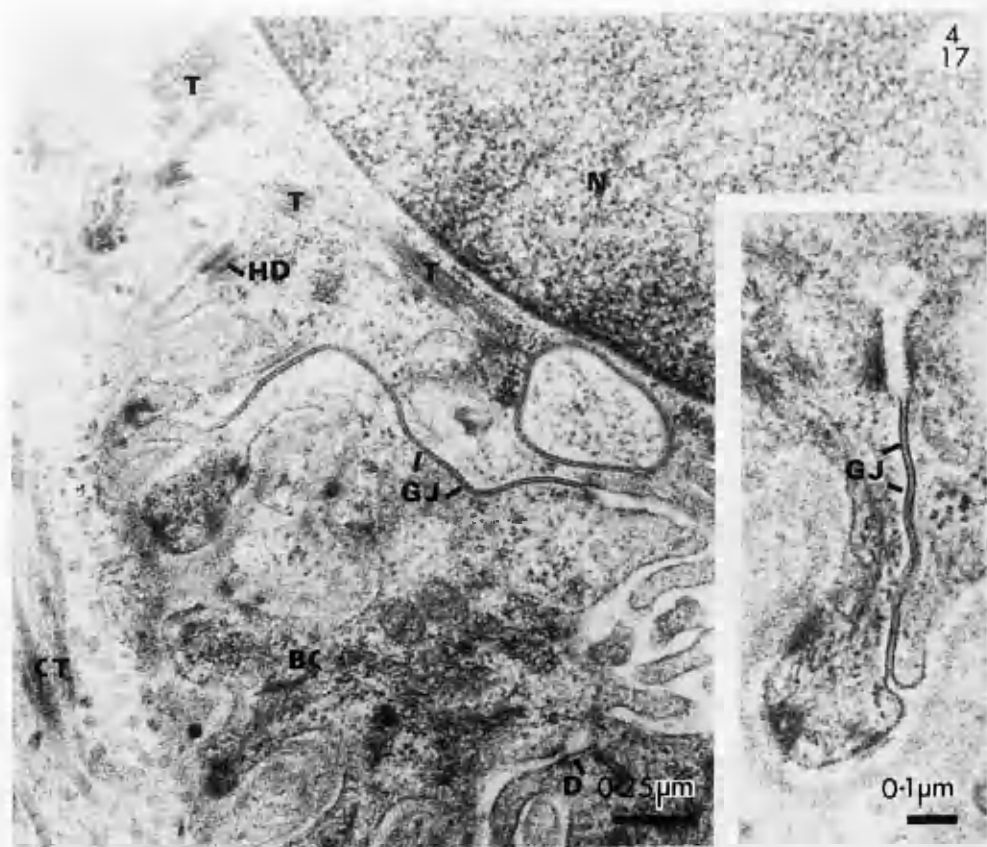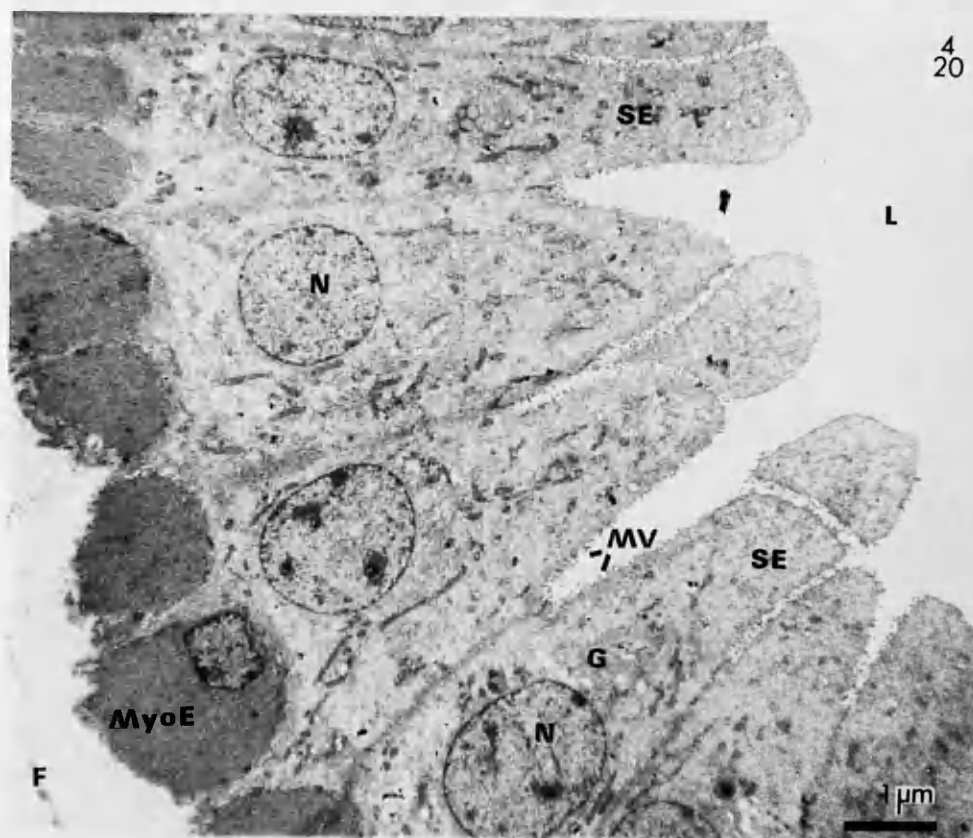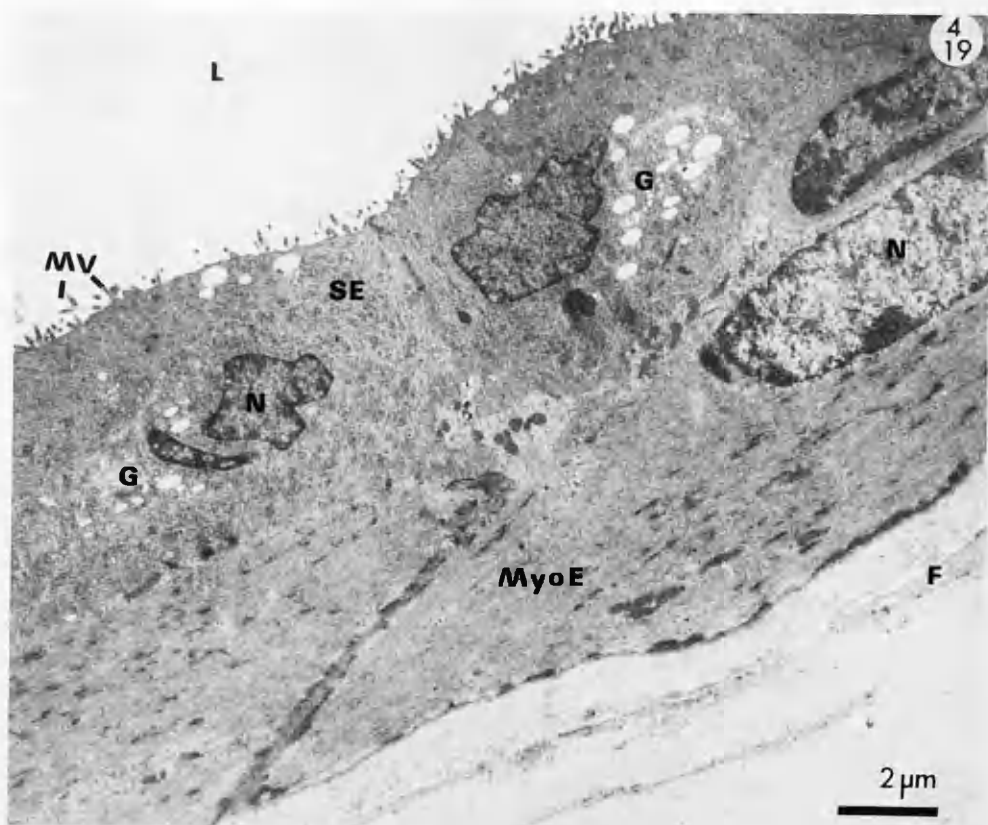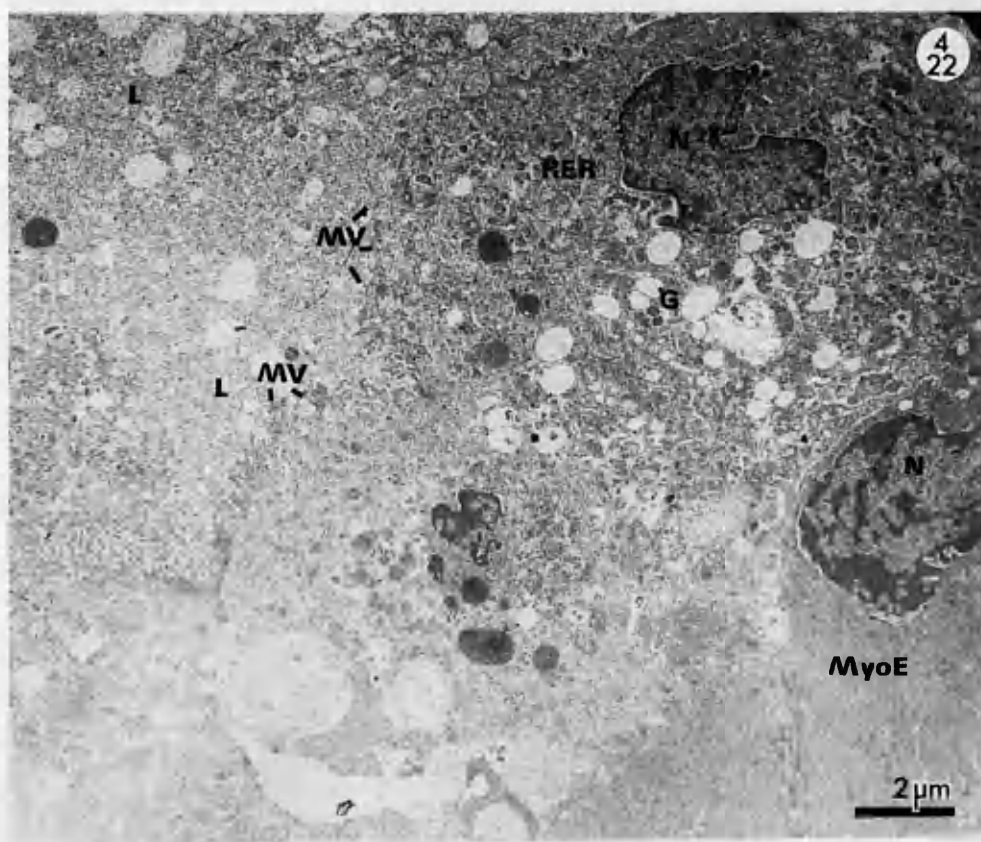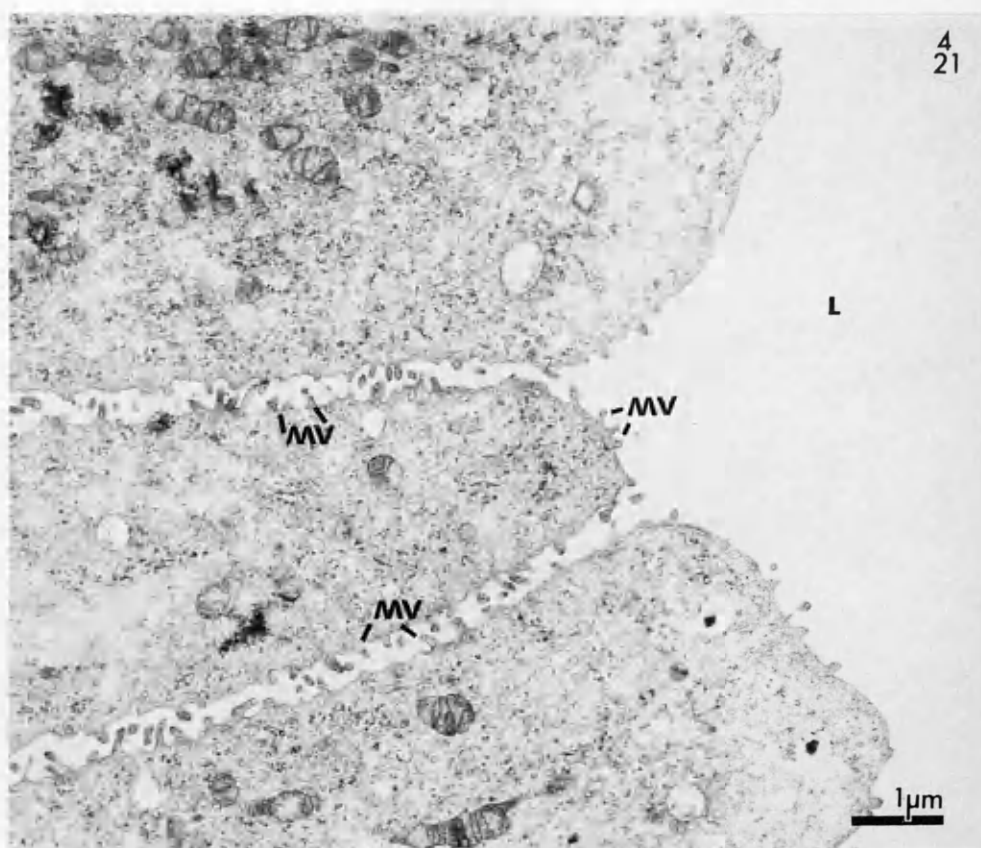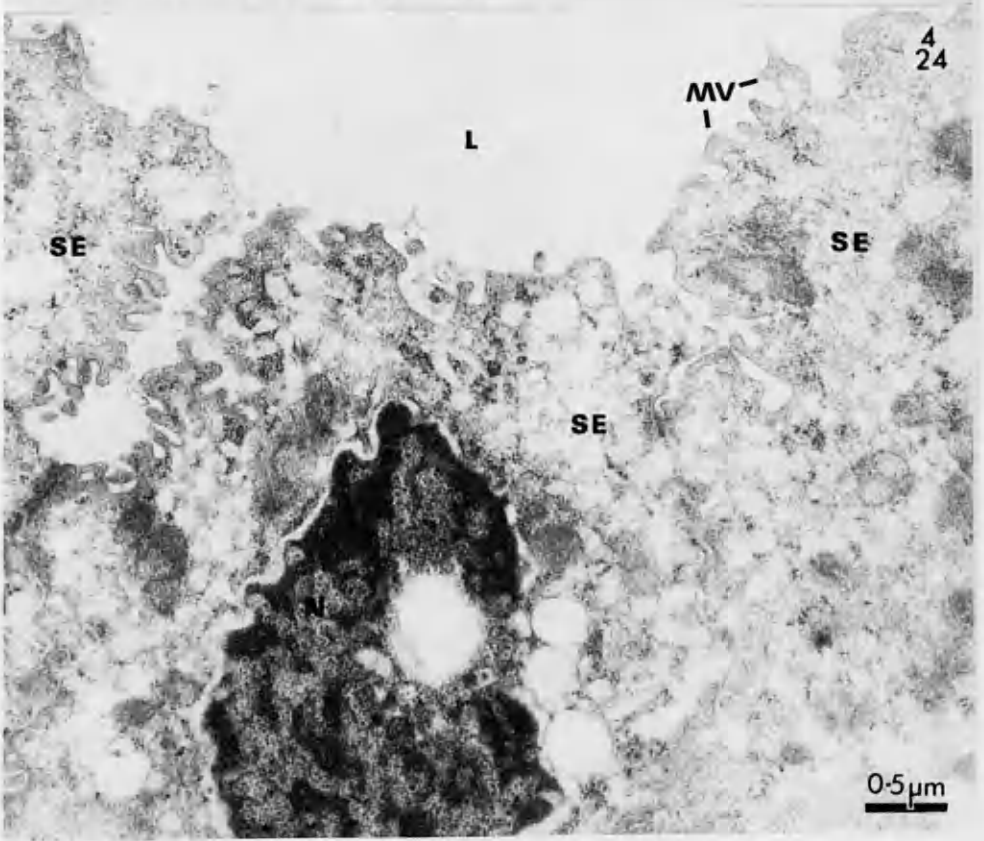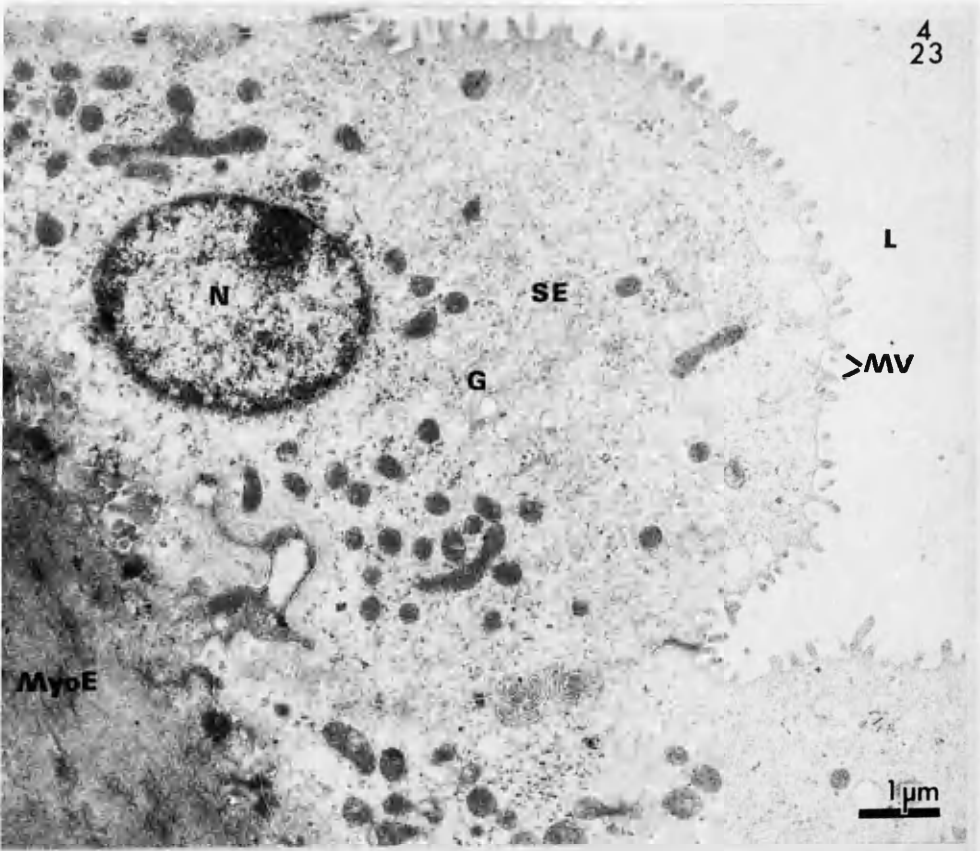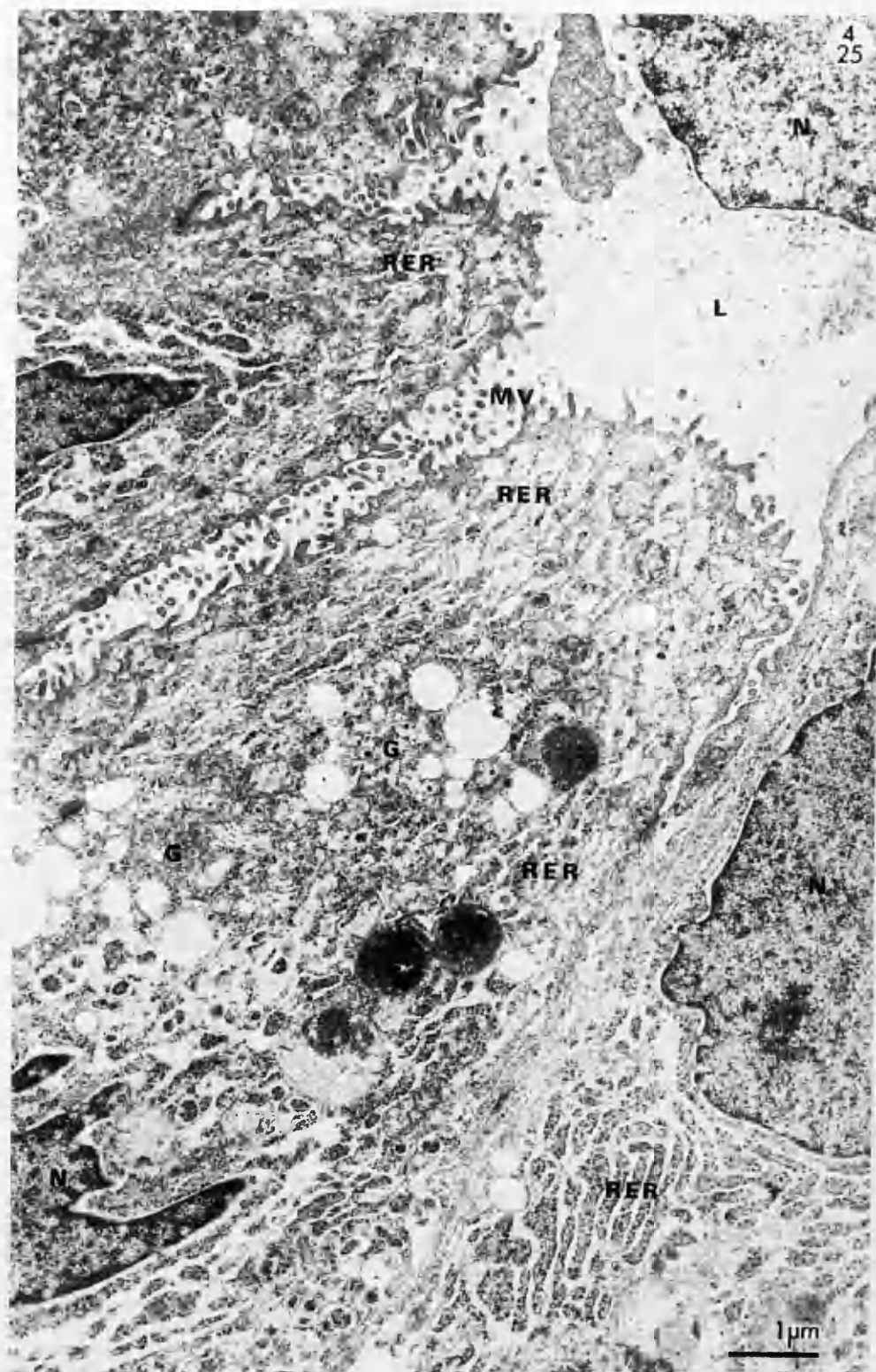
type, more commonly found in the goat, appeared to have taken this seemingly high level of activity to a more extreme stage where cellular disruption occurred (Fig.4.26). During this period, and at glandular 'fatigue', the lumen contained cellular debris, including nuclei, which, particularly in the goat often filled the lumen (Fig.4.27). The myoepithelium was contracted with some cells with markedly crenated nuclei. The intercellular spaces between neighbouring cells of both layers were greatly dilated.

At 'fatigue' there was still dilatation of the intercellular spaces and cellular debris in the lumen (Fig.4.28). The smooth muscle cells appeared to be contracted. Some of the secretory cells still had a distinctly vesicular appearance, especially in the apical

---

Fig.4.26. Electron micrograph of the gland in a sample taken from a goat at a small peak close to 'glandular fatigue' (goat 1, sample 5), showing a partly disrupted secretory cell being shed into the lumen.

Fig.4.27. Electron micrograph of the gland at 'fatigue' (goat 1, sample 6). Amongst the luminal debris, nuclei and organelle remnants can be seen.

Fig.4.28. Low power electron micrograph of a sweat gland from goat 2 at 'fatigue' showing debris in the lumen, numerous secretory vesicles in the apical cytoplasm, and marked dilatation of the spaces between the secretory and myoepithelia.

region, and were apparently active (Fig.4.29). In many of these cells the vesicular membranes were incomplete and it was not obvious whether this vesiculation was caused by Golgi activity or associated with the dilation of the RER. Other cells, particularly in the sheep, were similar in appearance to those in the unstimulated gland (Fig.4.30) except that in some there were still conspicuous gaps between them and the myoepithelium. Some of the gaps were filled by large membrane-bound bodies containing cytoplasmic debris. There were also, most noticeably in the goat, areas of the secretory epithelium that showed considerable damage with an almost squamous epithelium overlying large accumulations of membrane-bound bodies (Fig.4.31).

Duct.

The duct in the sheep and goat showed little ultrastructural difference on heat-induced sweating. The only noticeable changes was a wider lumen with the intercellular spaces in some ducts appearing wider.

Fig.4.29. Sheep sweat gland taken at 'fatigue'. The secretory cells are still active with a distinctly vesicular appearance.

Fig.4.30. Electron micrograph of the sheep fundus at 'fatigue'. This secretory cell is similar to those of the unstimulated gland.

Fig.4.31. Electron micrograph of the goat fundus at 'fatigue'. The almost squamous epithelium overlies large accumulations of membrane-bound bodies.

μm

μm
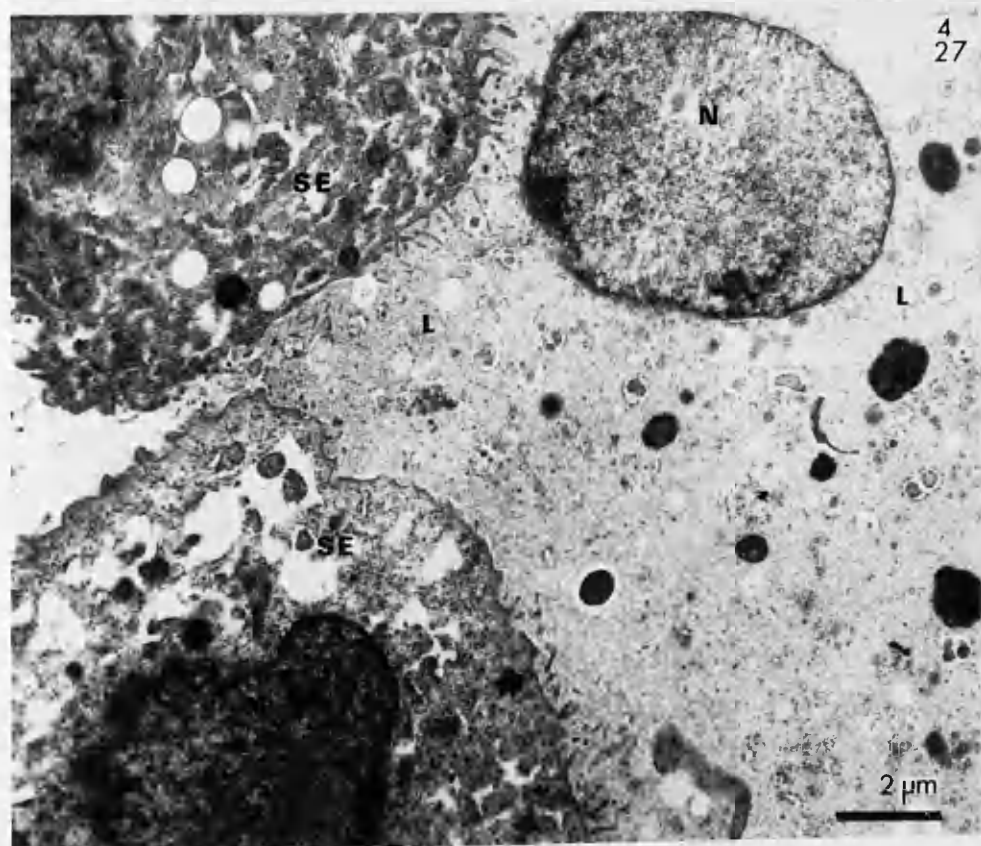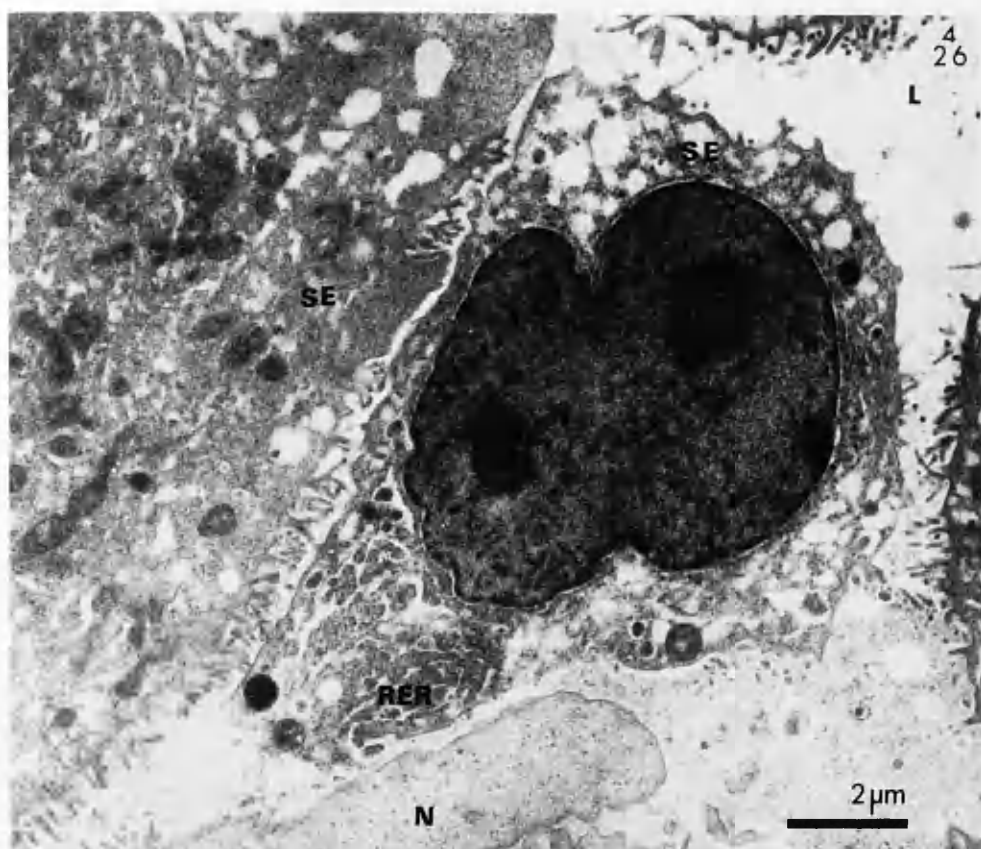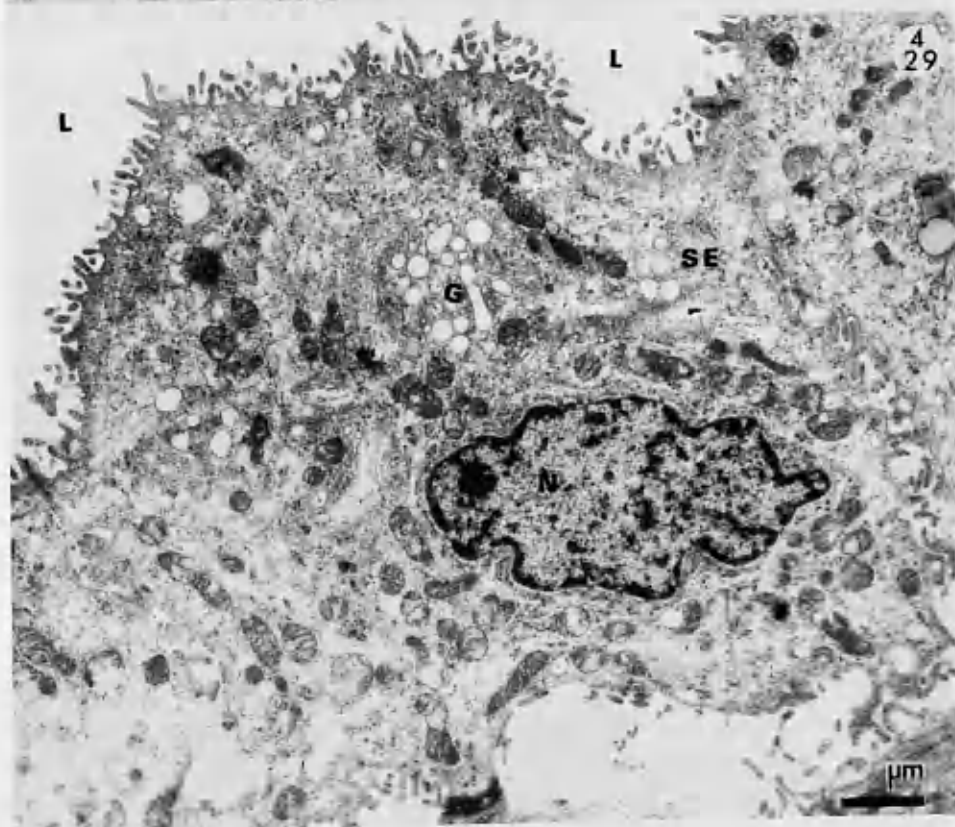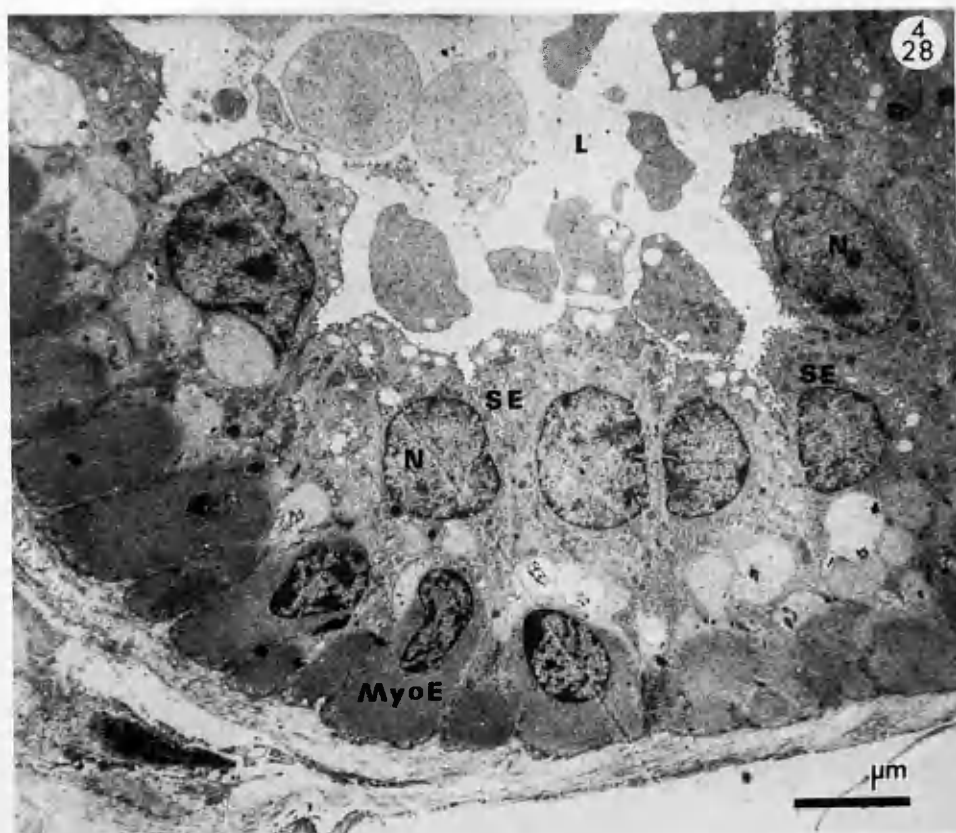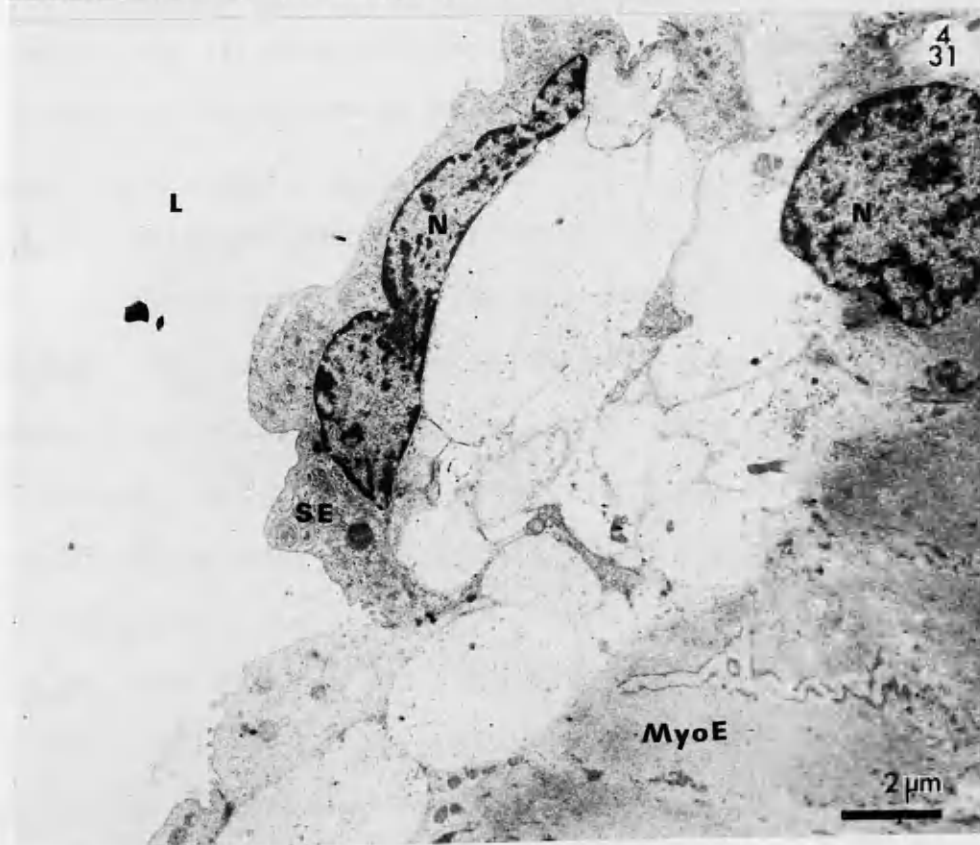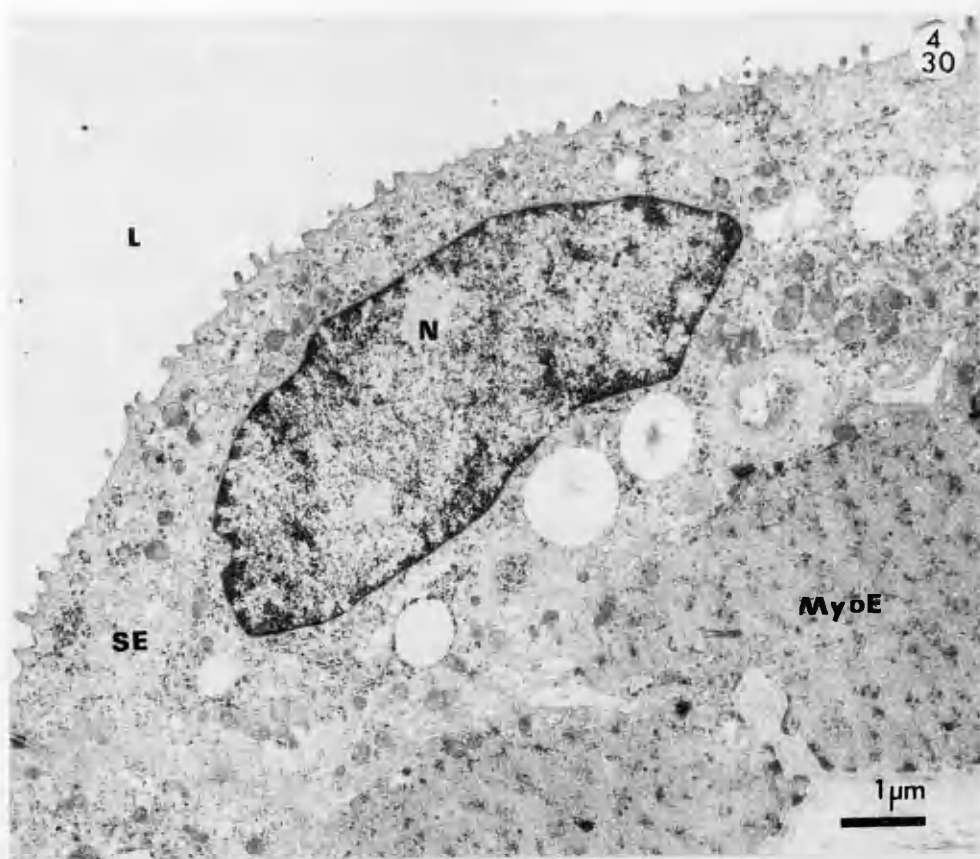
<u>Recovery</u>.

During the early part of glandular recovery in a cool environment the ultrastructure was similar to that at 'fatigue', except that there was usually little or no debris in the lumen. There was still considerable intercellular vacuolisation and evidence of membrane-bound bodies in the gaps. In samples taken within the first hour of recovery the secretory cells were largely cuboidal rather than columnar, although some were still vesiculated. Degenerating or atretic cells were also found in both species between the vesiculated secretory cells (Fig.4.32,33). Myoepithelial cells appeared to be more relaxed with a less crenated nuclei. One hour after removal of the animals from the heat the secretory cells still varied in shape from flat attenuated cells (Fig.4.34,35) to those which were almost 'normal' cuboidal while showing evidence of exocytosis and apical

---

<u>Fig.4.32</u>. Electron micrograph of the gland in a sample from a sheep taken during recovery, 45 minutes after 'fatigue'. There are extensive dilatations between the secretory and myoepithelial cells. An atretic cell can be seen between two vesiculated secretory cells.

<u>Fig.4.33</u>. An atretic cell from a sheep gland 45 minutes after 'fatigue'.

<u>Fig.4.34</u>. The sweat gland in a sample from goat taken 1 hour after 'fatigue'. The cells of the now relaxed gland show little evidence of secretory activity, and are attenuated.

vesiculation (Fig.4.36). Two hours after the start of recovery the now relaxed gland still had attenuated secretory cells and cells which were in a reasonably 'normal' state (Fig.4.37). There were areas where cells showing no secretory activity had dilatations between cell layers. Vacuoles and membrane-bound bodies containing cytoplasmic debris were prominent in the secretory epithelium (Fig.4.38). The myoepithelium at this time was indistinguishable from that of the unstimulated gland. After 3 hours, cuboidal secretory cells with vesiculated cytoplasm could still be observed with large membrane-bound vesicles closely resembling those seen earlier. The majority of the cells by this time, especially in the sheep, appeared similar to those of the unstimulated gland although at this stage of recovery the Golgi apparatus was not prominent and there was little

---

Fig.4.35. The sweat gland in a sample from goat taken 1 hour after 'fatigue'. The secretory cells in this electron micrograph are squamous.

Fig.4.36. The sweat gland in a sample from goat taken 1 hour after 'fatigue'. In this eletron micrograph the secretory cells almost have the 'normal' cuboidal shape.

Fig.4.37. The sweat gland in a sample from sheep 2 hours after 'fatigue' with a reasonably 'normal' secretory epithelium.

Fig.4.38. The sweat gland in a sample from sheep 2 hours after 'fatigue'. In this electron micrograph the secretory cell has present in the basal cytoplasm vesicles and membrane-bound bodies containing debris.
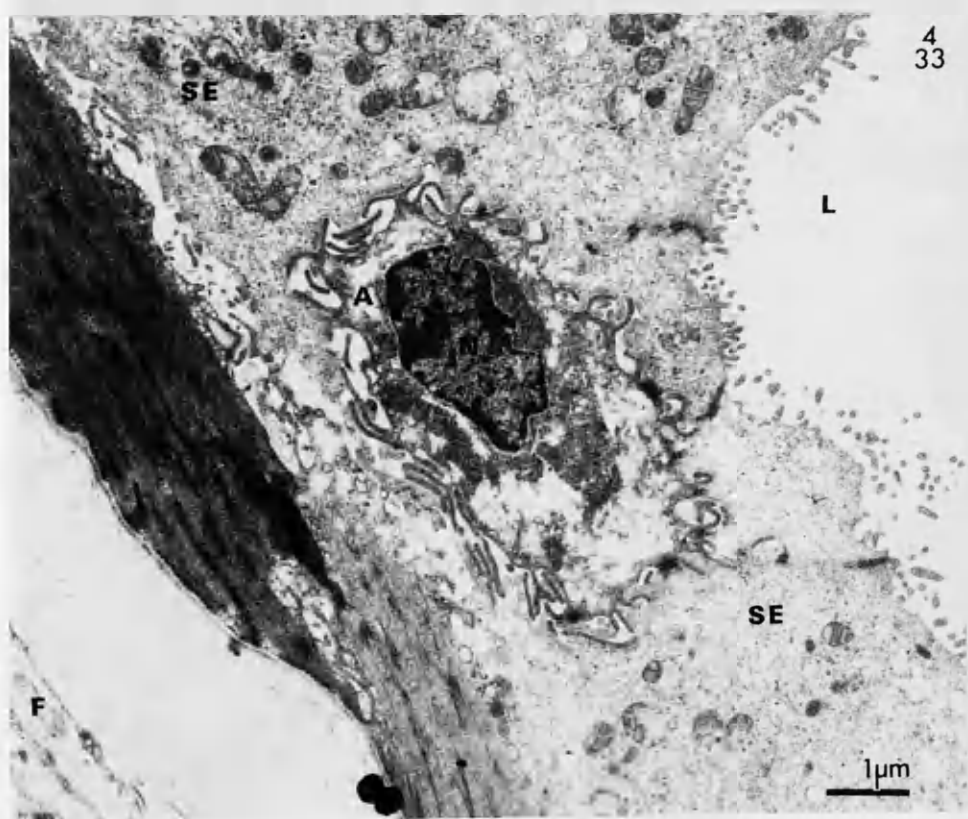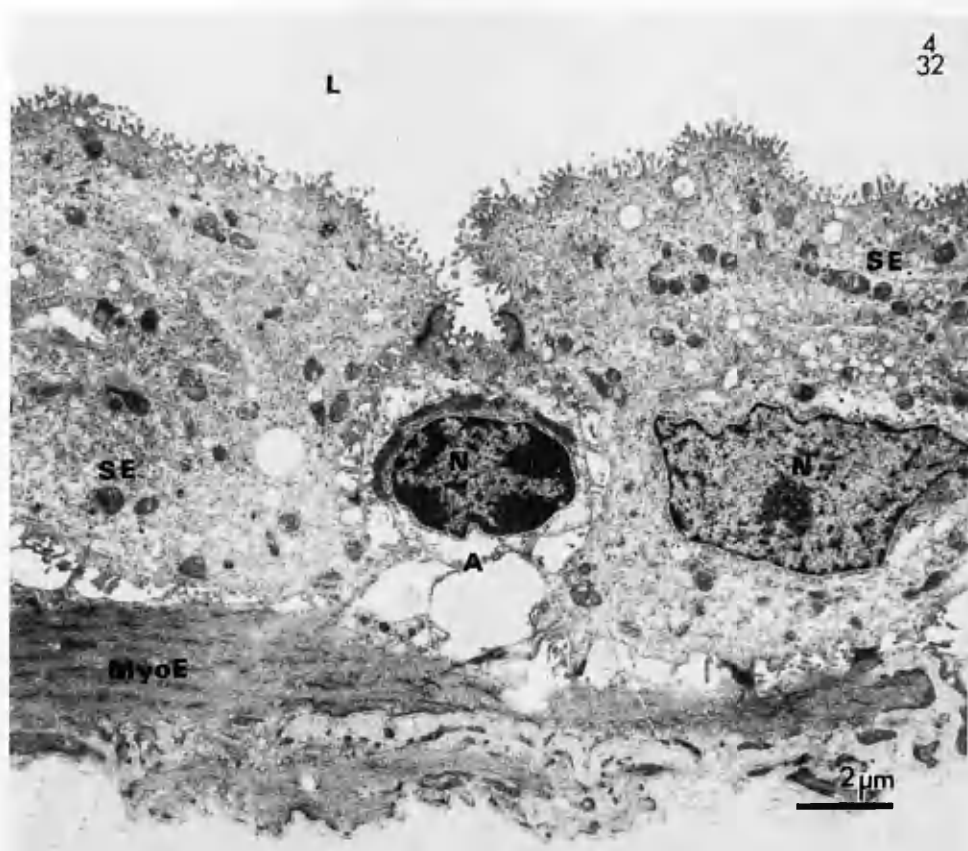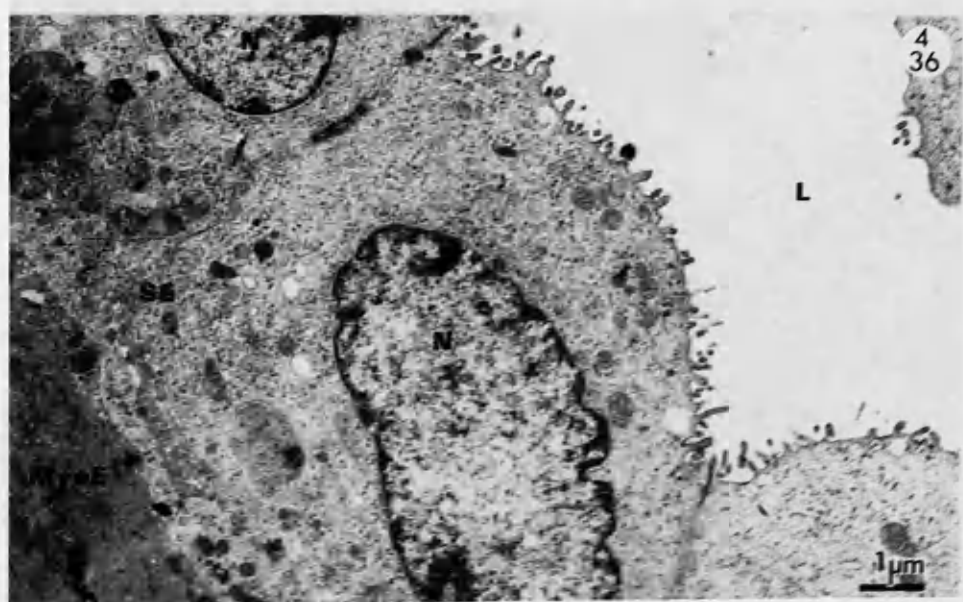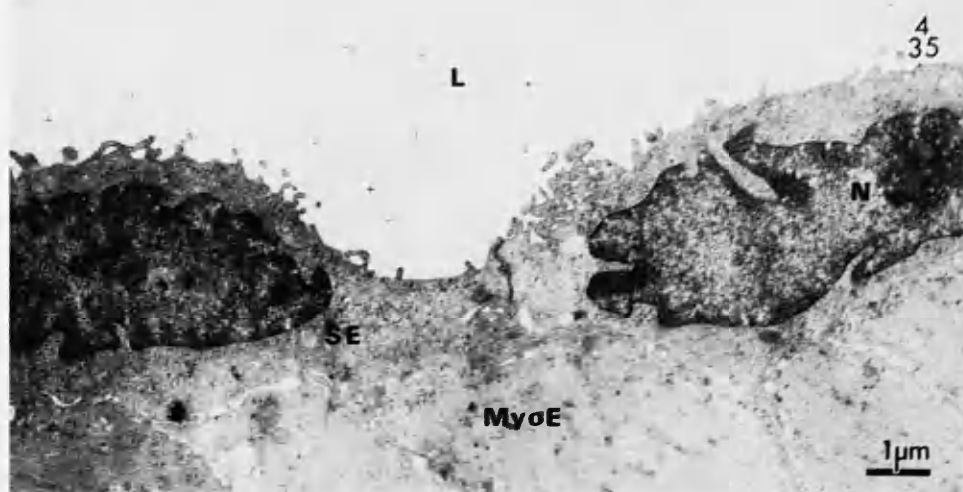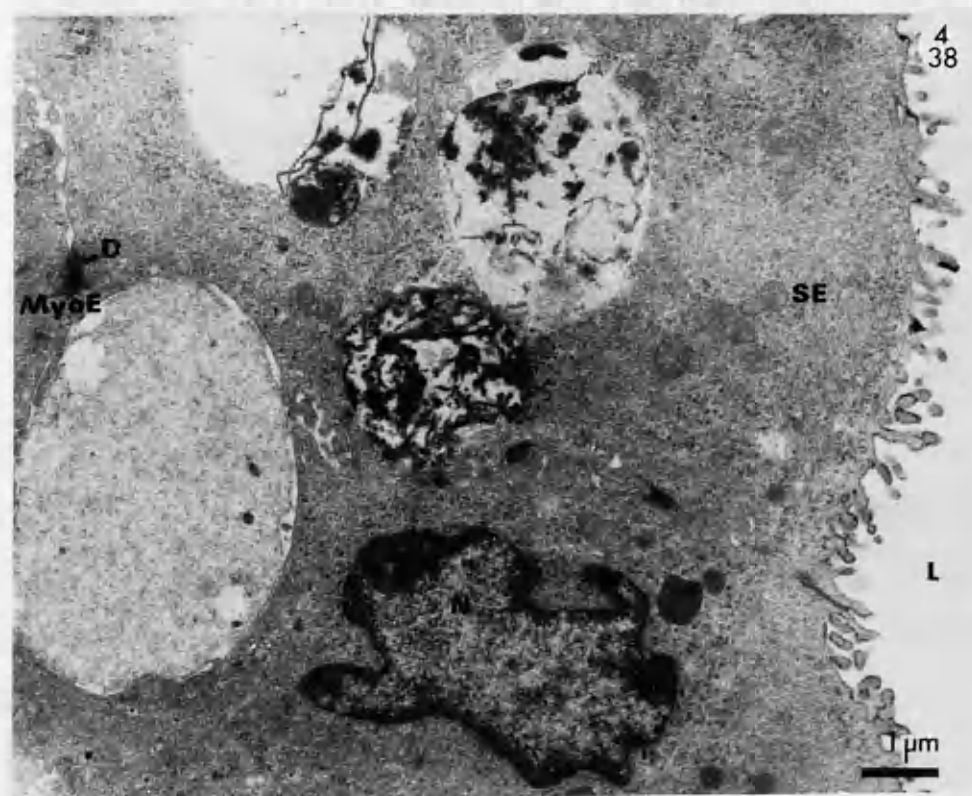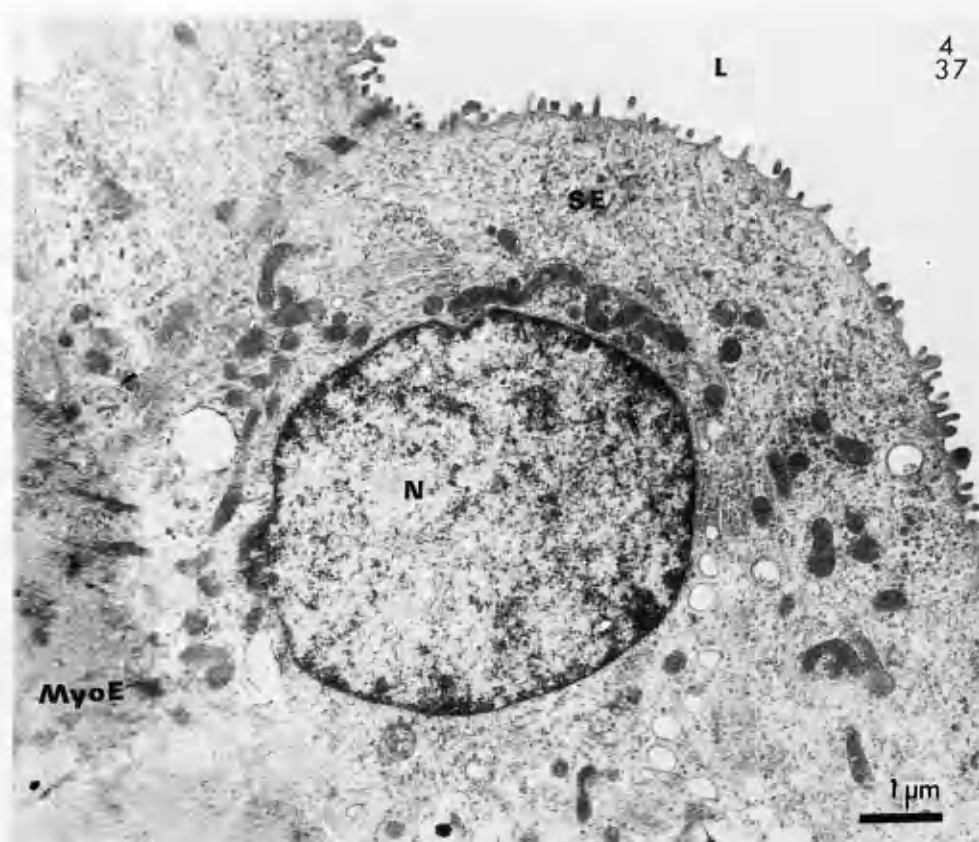
evidence of vesicle production. Intercellular spaces were still wider than in the unstimulated gland but considerably less pronounced than at 'fatigue'.

8-10 hours after the animals were removed from the heat the majority of the secretory cells, particularly in the sheep, had returned to 'normal'. The number of cells containing membrane-bound bodies had decreased with the gaps between cell layers becoming less pronounced. Flattened secretory cells could be found (Fig.4.39), although these had a fairly 'normal' appearance. The cuboidal secretory cells had lysosomes, now smaller, and a reorganising Golgi with its associated vesicles (Fig.4.40) more prominent in a higher proportion of the cells. By 18-24 hours of recovery the glands of both sheep and goat had become indistinguishable from those of the unstimulated animals.

Fig.4.39. The sweat gland in a sample from sheep 9 hours after 'fatigue'. In this electron micrograph the myoepithelium appears relaxed and the secretory epithelium although flattened had a fairly 'normal' appearance.
Fig.4.40. The sweat gland in a sample from sheep 9 hours after 'fatigue'. The secretory cell in this electron micrograph has returned to 'normal'. The now cuboidal secretory cell has a reorganised Golgi with associated vesicles.

L

N

G

MyoE

N

F

SE

2 µm

L

SE

G

G

M

N

1 µm
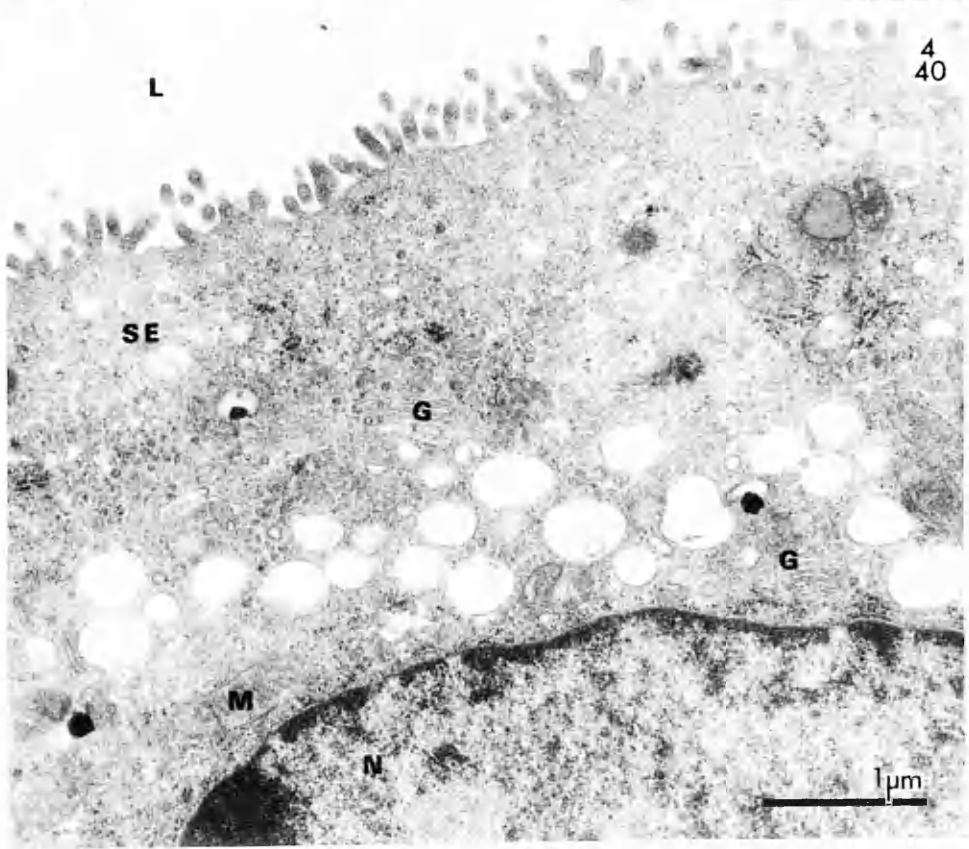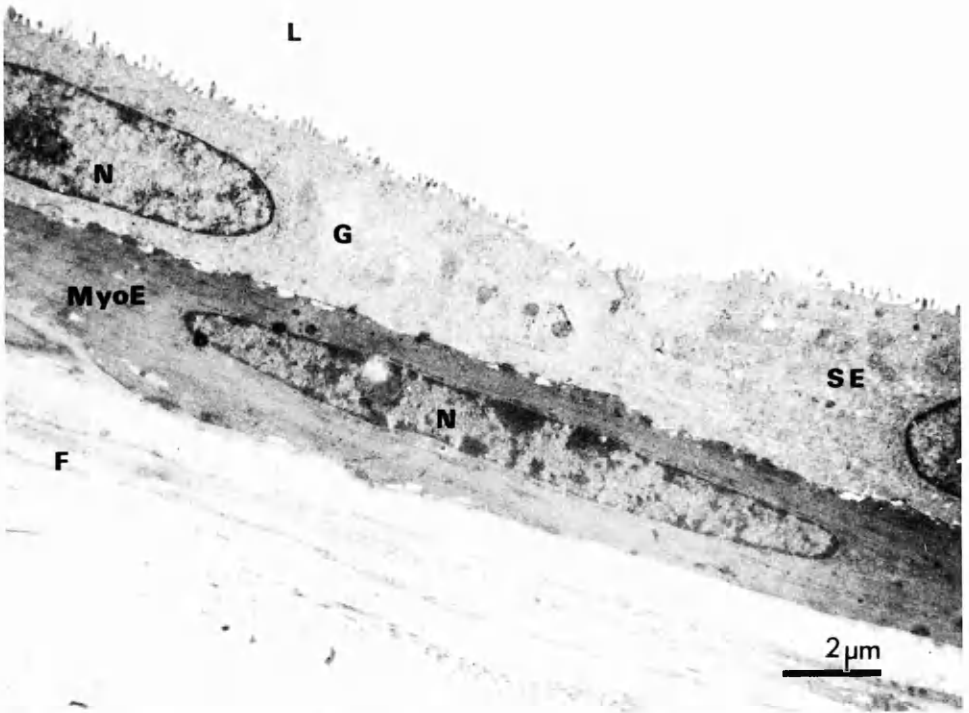
<u>DISCUSSION</u>.

The secretory mechanism in the sheep and goat also seems to be one of fluid transport and exocytosis of secretory vesicles. These vesicles were apparently produced by the well-developed Golgi body and possibly directly from the RER. The mitochondrial transformation found in cattle was not present in sheep and goats. The changes which occurred in the secretory cells during the early part of the decremental sweating response were strongly indicative of increased vesicle production and enhanced secretory activity. There was no correlation between the obvious cytological changes and the peaks and troughs of the sweating pattern. The myoepithelium generally appeared to be contracted from the first sweating peak to 'fatigue'. The pale apical zones, such as those described by Bell (1974) in the myoepithelium of the human axillary apocrine gland after stimulation by adrenaline were never seen. The changes in the luminal membrane of the secretory cells, and the marked distension of the RER seen at and between the smaller sweating peaks, seem to indicate the beginnings of secretory failure. Some, but not all, cells, particularly in the goat, appear to fail to the point of destruction, extruding their contents, including nuclei, into the lumen.

It is still not clear if or how an intact epithelium is maintained during the later stages of decremental sweating while disrupted cells are being extruded into the lumen. The evidence from 'fatigued' glands and during the subsequent recovery period suggests that neighbouring cells spread, make contact, and encase the remnants of

atretic cells between them and the underlying myoepithelium. The debris now located in the intercellular spaces then appears to be gradually phagocytosed by the secretory cell as the epithelium reconstitutes itself when the animals are again in a cool environment. Whether neighbouring cells can come into contact rapidly enough to maintain tight junctions during the later phases of sweating is still debatable. However, the absence of breaks in the secretory epithelium would seem to indicate that rapid reconstitution occurs. Jenkinson, Nimmo, Jackson, McQeen, Elder, Mackay and Montgomery (1983) using lanthanum injected intradermally, concluded that the luminal intercellular connections between epithelial cells are tight and remain so during sweating despite the cell death.

The occurrence of cell death and loss during activity suggests the existence of a mechanism of cell replacement. Although centrioles were found in the secretory cells, mitotic configurations were never seen. Montagna and Parakkal (1974) indicate that mitotic figures were rarely seen in the human apocrine sweat gland, in spite of clear evidence of high cytoplasmic activity and cellular sloughing. Both of these components may well be present in all mammals. Evidence for the existence of cell loss from the secretory epithelium has been published for the human apocrine gland (Schaumburg-Lever & Lever, 1975), and for cattle in Chapter 3.

Recovery of at least some of these cells appears to be rapid, since secretory activity returns within 15 minutes of the goats returning to a cool environment

(Jenkinson & Robertshaw, 1971). It is evident from these results that the full sweating response obtained 5 hours after 'fatigue' in the goat (Jenkinson & Robertshaw, 1971) occurred in glands which had not returned fully to the pre-stressed state.

The second component, secretion, appears to be brought about by two mechanisms, namely vesicle exocytosis and fluid transport. The particulate matter could be protein, which is known to be present in the sweat of a number of species (Jenkinson, Mabon & Manson, 1974b), and has been demonstrated in the fundus of cattle and sheep (Lloyd, Mabon & Jenkinson, 1977). The site of fluid transport still remains to be determined, but active pumping may occur in the glandular fundus, as in the rat plantar gland (Sato & Sato, 1978).

The results from the sheep and goat, while agreeing with the view of Jenkinson and Robertshaw (1971) that secretion occurs during sweating, also illustrate the importance of pre-formed sweat to subsequent activity in the heat, as suggested by Allen and Bligh (1969) and Johnson (1973). They also demonstrate that the 'fatigue' is not as postulated by Johnson (1973), simply an imbalance between the secretory and discharge mechanisms but due to secretory cell exhaustion and failure. The evidence of myoepithelial contraction throughout sweating is not consistent with Bligh's thesis of myoepithelial action in expulsion of sweat.

Therefore, the mode of secretion in the sheep and goat as in the cow has three components, fluid transport,

exocytosis and cell death despite the different patterns

of evaporative loss.

CHAPTER 5.

THE EQUINE GLAND.

INTRODUCTION.

Equidae on exposure to heat display a gradual
increase in cutaneous water vapour loss over a period of
about 1 hour. This rises to a maximum which can be
sustained for several hours (Allen & Bligh, 1969;
Robertshaw & Taylor, 1969; Kerr et al., 1980). The
myoepithelium of the glandular fundus is believed to play
little part in the discharge of horse sweat, the output of
which seems to be largely dependent on secretory cell
activity (Takagi & Tagawa, 1959; Allen & Bligh, 1969).
Information on the nature of the secretory process in this
species is conflicting and there is apparently none on
ductal activity, although the composition of the
hypertonic sweat (Kerr et al., 1980) is probably modified
by cellular activity in the duct, as is the hypotonic
sweat of the primate (Sato, 1977; Morimoto, 1978).

The physiological experiments of Allen and Bligh
(1969) support the view of Takagi and Tagawa (1959), based
on light microscopic studies of the equine sweat gland
that the mode of secretion is not apocrine. Alternatively,
evidence of cytoplasmic protrusions on the secretory
cells, obtained from light (Talukdar, Calhoun & Stinson,
1970) and electron micrographs of unstimulated horse sweat
glands (Kurosumi, Matsuzawa & Saito, 1963; Sorensen &
Prasad, 1973), indicate an apocrine process. However
Sorensen and Prasad (1973) failed to detect 'pinched-off'
cytoplasm in the lumen and noted that apical swelling of

the secretory cells can be induced by fixation. The
presence of low, more vacuolated, sometimes completely
ruptured, secretory cells with fragmented nuclei, in the
glands of skin from regions rendered anhidrotic by
repeated intradermal injections of adrenaline (Lovatt
Evans et al., 1957), is indicative of cell death during
activity. Thus, although it is possible that this
appearance results from excessive stimulation since the
doses of adrenaline used were not given, the evidence
suggests that sweat formation in the horse may also have
two components, namely secretion (exocytosis and fluid
transport) and cell death, as postulated for the cow,
sheep and goat (Chapters 3,4).

The ultrastructure of the horse sweat gland was
therefore studied before and during sweating to test this
hypothesis and provide anatomical evidence on the nature
of the changes in both the duct and fundus for further
studies of ionic transport during secretion.

PROCEDURE.

One thoroughbred gelding aged 11 years, two Shetland
mares and a Welsh cross gelding all aged 4 years had skin
samples taken at the beginning and after six hours
exposure to 18°C Dry Bulb (DB)/11°C Wet Bulb (WB) Two days
later the horses were exposed to 40°C DB/23°C WB in the
climatic chamber for 6 hours. Cutaneous moisture loss was
again monitored continuously and samples were taken from
contralateral areas of the body surface at the times shown
in Fig.5.1. All samples were fixed and processed for
transmission electron microscopy as described in Appendix
1.

RESULTS.

Patterns of evaporative loss.

The patterns of cutaneous loss, each to its own base
line, found in the four horses when exposed to an
environment of 40°C DB/23°C WB are illustrated in Fig.5.1.
The sweat patterns of the four animals were essentially
similar, although there were variations in the magnitude
of the response and in two of the animals there was a fall
in output towards the end of the experimental period. In
the cool environment sweating did not occur in any of the
four animals.

Sweat gland structure.

The equine sweat gland (Fig.5.2), which was always
found in close association with the hair follicle,
traversed from the tightly coiled fundus, through the
dermis, penetrated the hair follicle and finally opened
into the follicular canal close to the surface of the
skin. The duct entered the hair follicle in a direction
almost parallel to it (Fig.5.2) giving a short

Fig.5.1. The patterns of cutaneous evaporative loss from
four Equidae in an environment of 40°C DB/23°C WB Skin
samples were taken from the contralateral side at the
times shown (numbered arrows).

Fig.5.2. A diagram illustrating the fundus and different
zones of the duct from the equine sweat gland and their
situations with respect to the hair follicle.
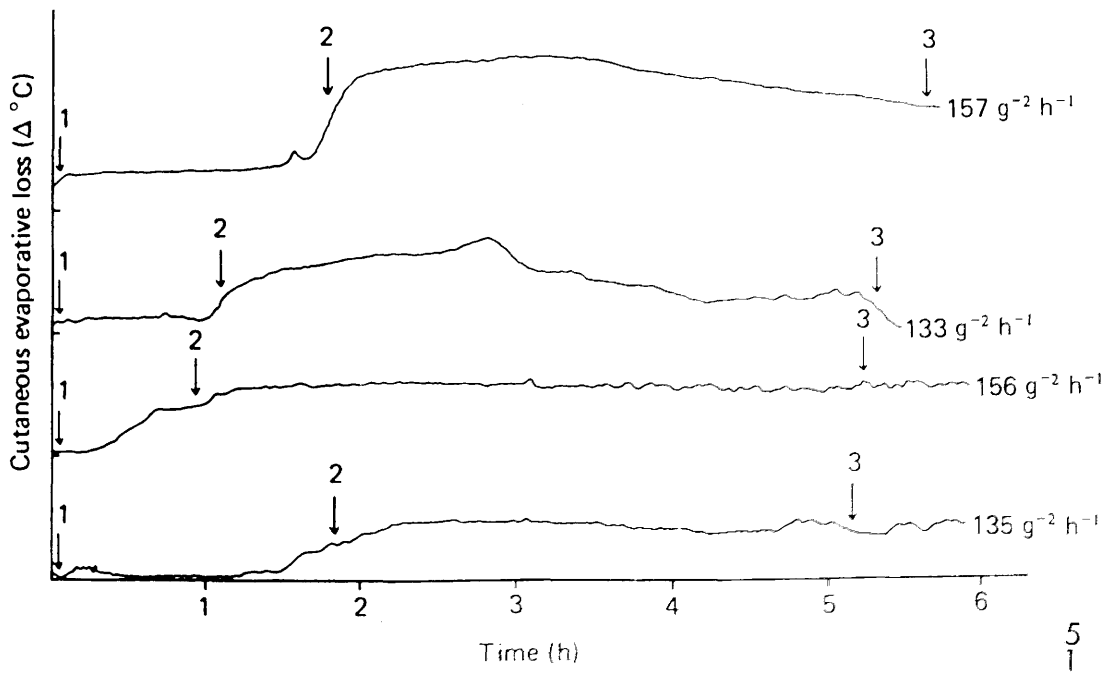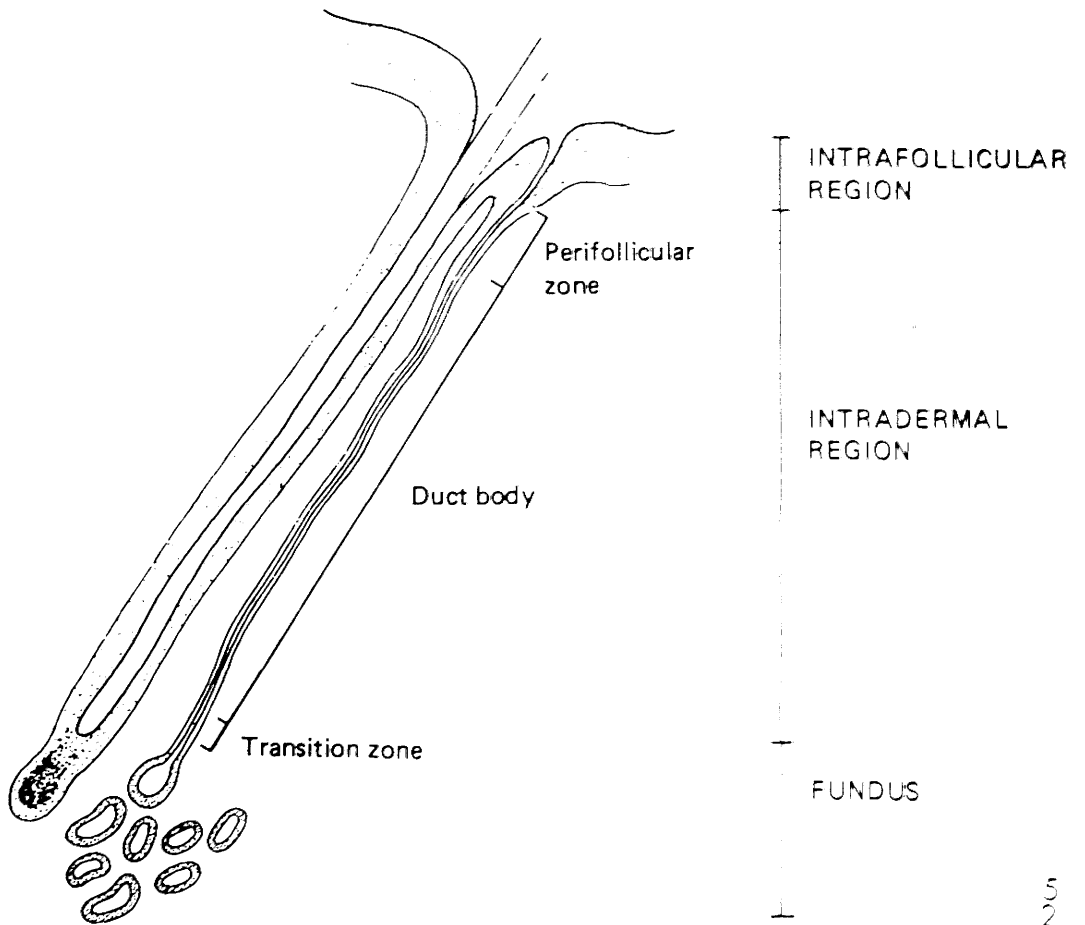
intrafollicular region of 89±35μm. The intradermal duct
can again be sub-divided into three morphological discrete
zones, a perifollicular zone, duct body and duct/fundus
transition zone (Fig.5.2).

Sweat gland ultrastructure.

Fundus.

The sweat gland fundus of the horse had the same
structure as that of the cow, sheep and goat. The
secretory epithelium was surrounded by a myoepithelium
(Fig.5.3) and enveloped in a fenestrated sheath of
fibrocytes. Unmyelinated nerve fibres associated with
capillaries were present immediately outside and
occasionally in the connective tissue between the sheath
and myoepithelium. The cuboidal secretory cells had a
cytoplasm densely packed with secretory vesicles
(Fig.5.3,4) and their nuclei were located in the basal
portion of the cell. The electron dense or opaque vesicles
seem to represent differences in the degree of storage
concentration rather than a range of vesicle type.

Fig.5.3. A section through the fundus of the resting
gland. The cuboidal secretory cells are densely packed
with vesicles and have their nuclei situated towards the
base of the cell.

Fig.5.4. Section of a secretory cell from a resting gland.
Densely packed secreory vesicles interspersed with small
mitochondria can be seen. The nucleus has a 'dented'
appearance due to pressure from secretory vesicles. The
cell also has long densely packed micovilli.

Figure 5 3

MV L 5/3

SE

SE

SE

N

V

BMV +LMV

MyoE

CT 2μm

Figure 5 4

MV G 5/4

N

V

M

RER

V M

BMV

1μm
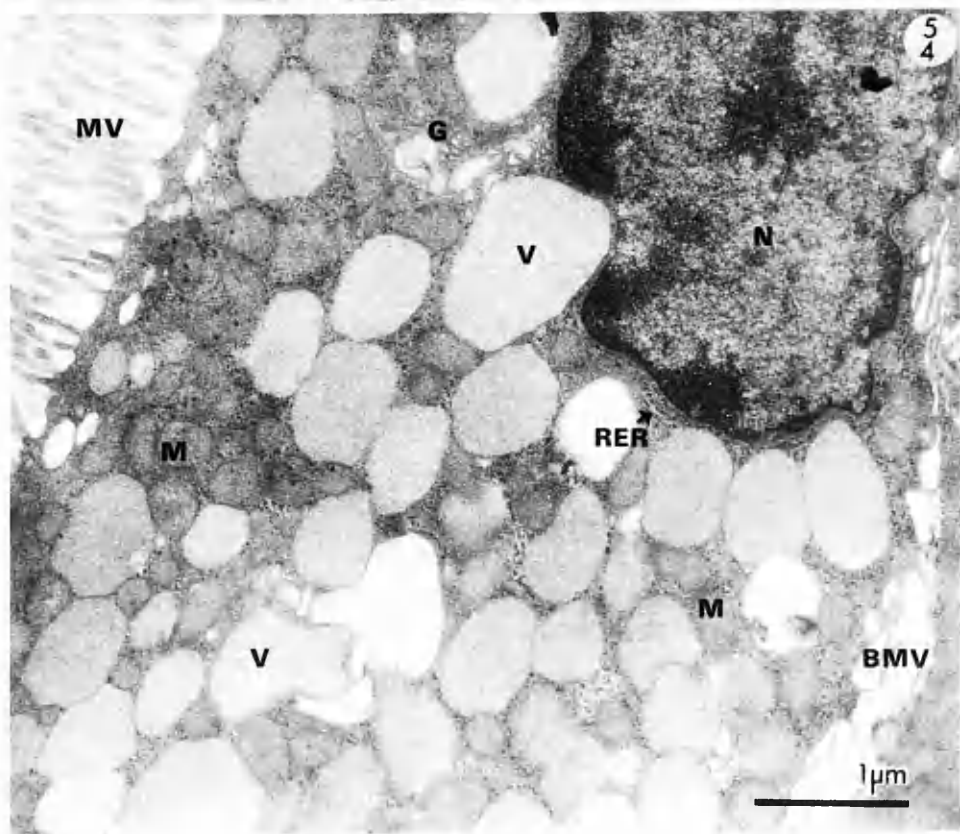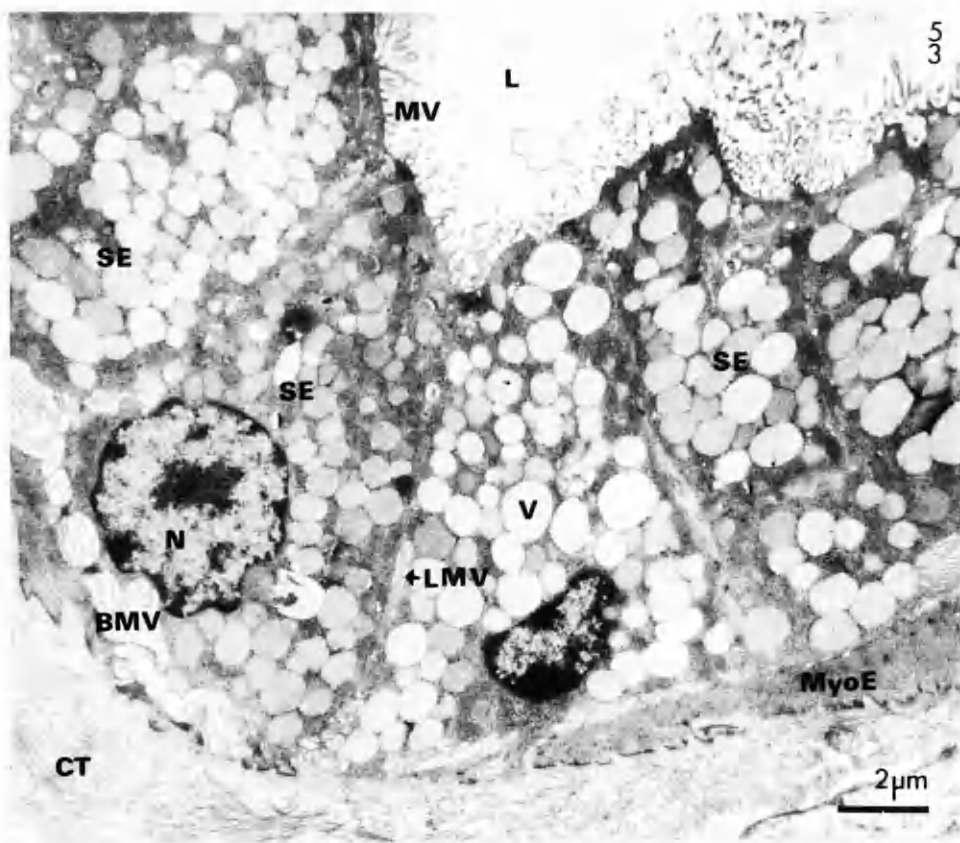
The secretory cells exhibited an extensive and well developed Golgi apparatus with associated vesicles of varying size. The mitochondria were small and round or ovoid with flat lamellar cristae and an electron dense matrix. Between the tightly packed secretory vesicles, RER, ribosomes and glycogen particles were identified (Fig.5.4,5,6). The microvilli, both single and branched, on the luminal membrane of the secretory cells were more numerous and considerably longer than those found in cattle, sheep and goats. A secretory vesicle was occasionally seen protruding from the membrane at the luminal surface (Fig.5.5). However, this feature, reminiscent of micro-apocrine secretion, was not seen being 'pinched off' and no other form of cytoplasmic protrusion was ever observed at the luminal surface.

Secretory cells were connected at the lumen by apical junctional complexes each with a _zonula occludens_ and desmosome. The lateral surfaces had well developed

---

Fig.5.5. Section of a secretory cell from a resting gland with a protruding secretory vesicle at the luminal surface. This appearance, only occasionally seen, is reminiscent of micro-apocrine secretion. None of these vesicles was actually seen being 'pinched off' and no other form of cytoplasmic protrusion was observed at the luminal surface.

Fig.5.6. Secretory cell from the basal portion of a resting gland illustrating basal microvill which at points reach the basement membrane between myoepithelial cells.

5
5

L

MV

G   V

MAS

V

0·5μm

5
6

N

V

Gly

V

MyoE

Gly

CT

F

BMV

1μm
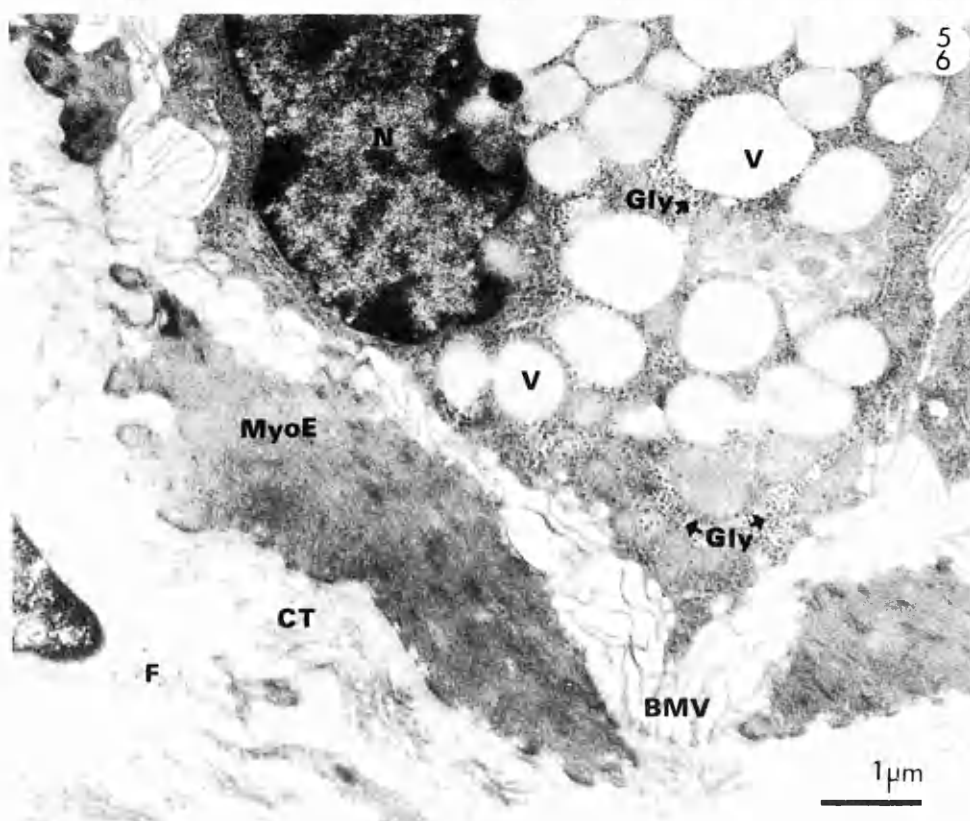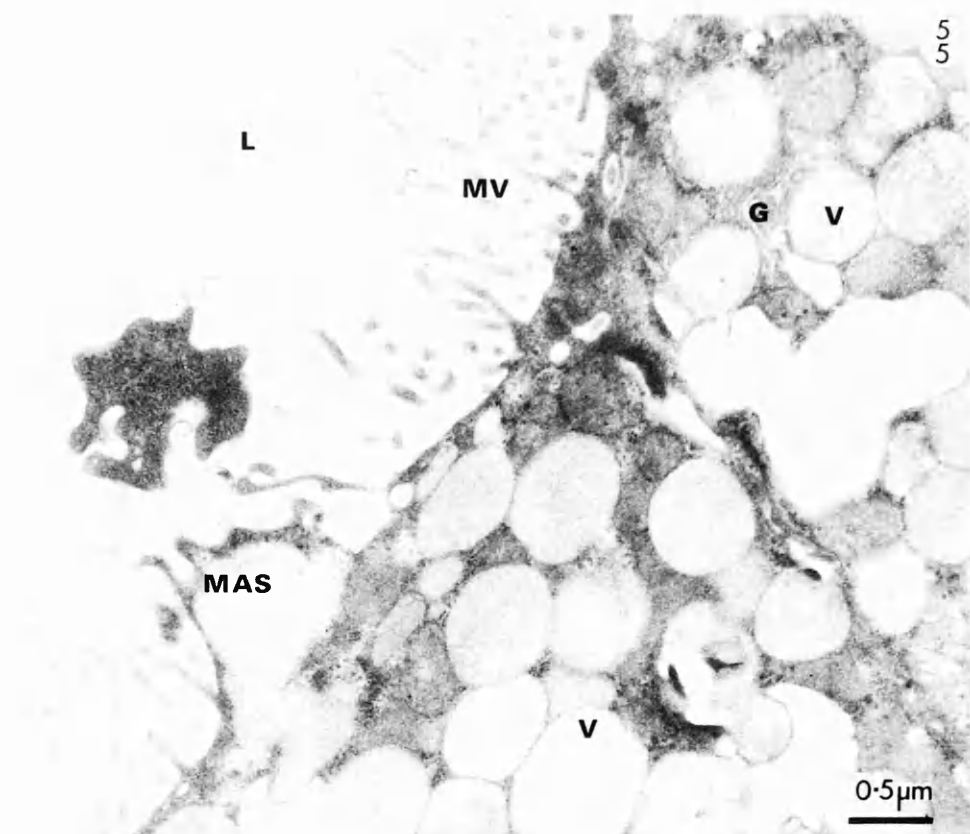
interdigitations which at the basal margin traversed
between neighbouring myoepithelial cells to the basement
membrane (Fig.5.6). Cellular projections were also found
along the serosal membrane adjacent to the myoepithelium.

The myoepithelium was composed of small
well-developed smooth muscle cells. Although separated by
the basal infoldings of the secretory cells they did
occasionally touch and link with desmosomes and formed a
wide-meshed network. The muscle cells were also connected
to neighbouring secretory cells by desmosomes.

Duct.

Intrafollicular region.

At the opening into the hair follicle the lumen was
lined with 8-10 layers of cornified cells, surrounded by
4-6 layers of living cells (Fig.5.7). The latter which
closely resemble the granulosum cells of the follicle were
rich in keratohyalin and pigment granules. The duct midway
through the follicle was still 4-6 cells thick and had a
lumen surrounded by living cells (Fig.5.8). These cells,
usually two, had on their luminal surface, microvilli,
which were associated with small vesicles at their bases
(Fig.5.8 inset). They also had prominent epithelial folds
(Fig.5.8) with apical junctional complexes. Langerhans
cells and their processes were commonly found between the

Fig.5.7. The keratinized luminal surface of the duct in
the upper part of the hair follicle. The cornified
squamous cells are surrounded by cells indistinguishable
from the granulosum cells of the hair follicle.

cells in this region (Fig.5.8).

Intradermal region.

The duct in the perifollicular zone which was
approximately 30µm long, acquires a fibrocyte sheath upon
leaving the hair follicle and narrows due to a gradual
reduction in the size of the lumen and the number of cell
layers composing its wall. The appearance of the duct in
this upper portion (Fig.5.9) was similar to that observed
within the lower follicle, except, that it was now
surrounded by a fibrocyte sheath and the small vesicles
observed at the base of the microvilli were generally more
numerous. The connective tissue layer outside the basement
membrane was not prominent and large gaps were present in
the fibrocyte sheath. A little deeper, the lumen was still
approximately the same size with the inner ring of 2-3
living cells surrounded by a further 2-4 layers of
granulosum-type cells. The lining of the lumen was similar
to that in the lower follicular zone. In the lowest
portion of the perifollicular zone, the number of cell
layers in the wall fell from 5 to 3 and the lumen became
narrower. There were now, generally, three cuboidal cells

Fig.5.8,inset. The duct mid-way across the width of the
hair follicle. The lumen, formed by two cells with
prominent epithelial folds, has a lining of densely packed
microvilli. Langerhans cells and their processes are
present between the cell layers of the duct wall. Vesicles
were observed between the microvilli at the luminal
surface throughout this zone.
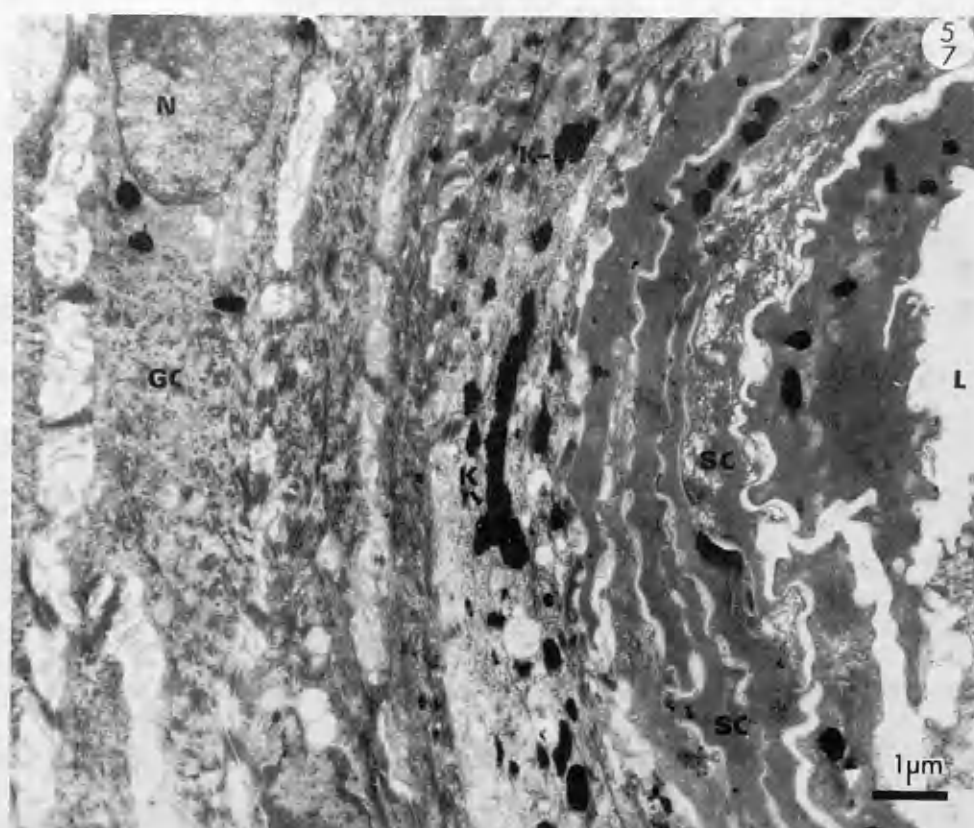
with short densely packed microvilli at their luminal surface forming the inner ring. These were interconnected at the luminal surface by junctional complexes each with a zonula occludens. There were small but definite infoldings into the lumen and lateral dovetailing of intercellular fingers occurred particularly near the base of the cells. The underlying cells, now more flattened in appearance, were connected to the luminal cells and those of the lower layers by desmosomes. The fibrocyte sheath was still loose and apparently not well developed. Occasionally secretory vesicles from the fundus secretory cells were observed in the lumen of the resting gland (Fig.5.9). Langerhans cells and their dendritic processes were still found between the duct cells at this level (Fig.5.9). These cells could be recognised by their characteristic Birbeck granules and the absence of junctions with neighbouring cells (Fig.5.10).

The duct body throughout its entire length of 474± 67µm was composed of two cell layers surrounded by a dense connective tissue layer and fenestrated fibrocyte sheath.

Fig.5.9. The perifollicular zone. The duct has now separated from the hair follicle. The lumen is still composed of two cells but the duct wall now has six cell layers.

Fig.5.10. A Langerhans cell in the perifollicular zone of the duct. The cell can be recognised by the absence of cell junctions and the presence of Birbeck granules in the cytoplasm.

L

MV

LC

La

HF

2μm

BC

LaG

LaG

La

0·5μm
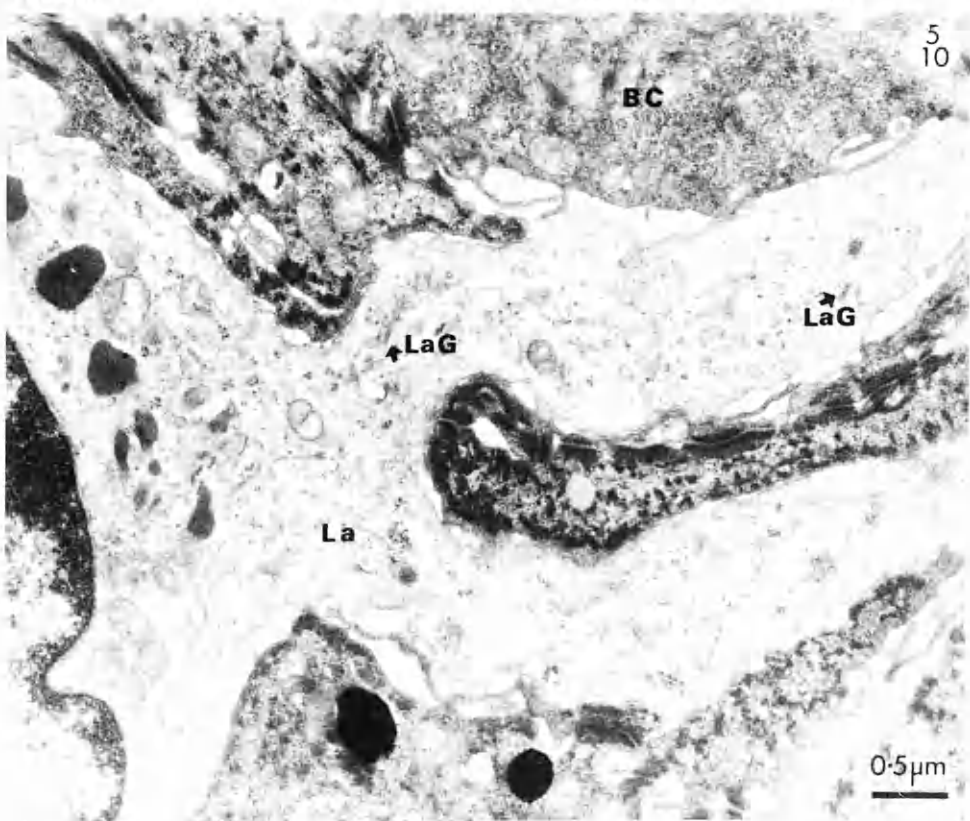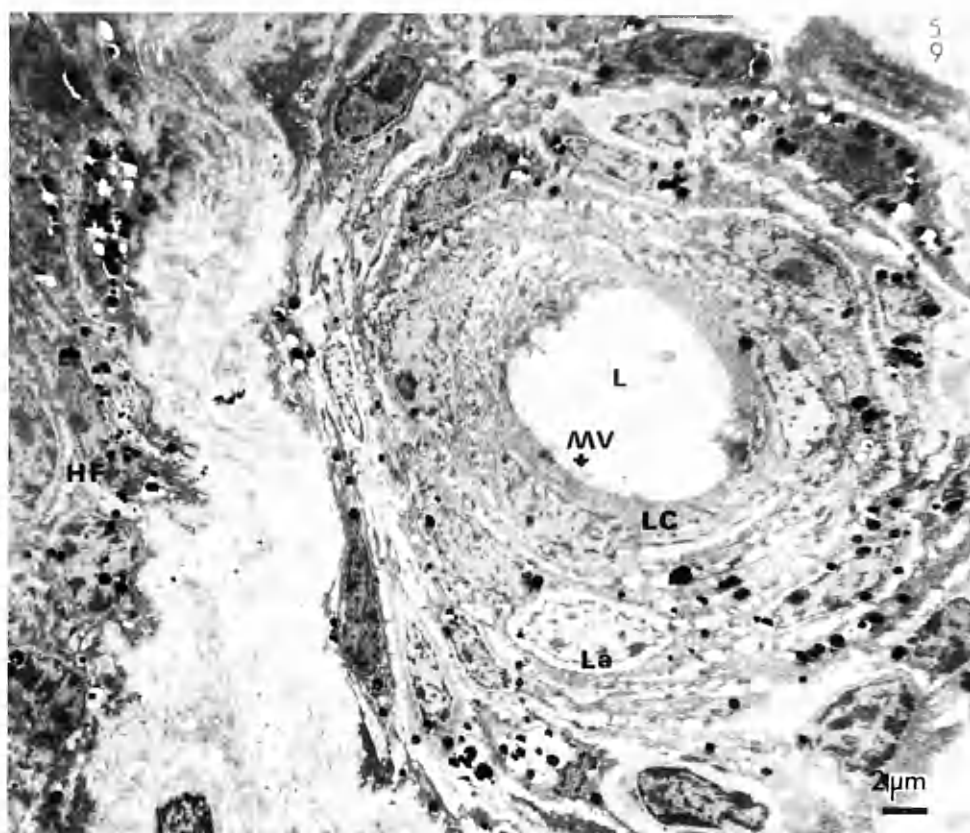
The lumen over the upper portion of the duct body was
again wider and ringed by 3-5 cuboidal cells with short
widely spaced microvilli at their luminal surface
(Fig.5.11). These luminal cells were interdigitated along
their lateral and basolateral surfaces. They were joined
to each other by apical junctional complexes and by
desmosomes to the underlying layer of now spindle shaped
cells. These ovoid basal cells were also attached by
desmosomes to their neighbours forming a complete outer
layer linked to the basement membrane by hemidesmosomes.
The outer connective tissue and fibrocyte sheath were now
prominent and apparently better developed. Throughout the
remainder of the duct body the overall structure was
basically similar. The lateral interdigitations at the
bases of the luminal cells were more prominent and
extended through the underlying layer of cells to the

Fig.5.11. The upper duct body. The wall is now only two
cells thick. The three cells forming the luminal layer are
interdigitated laterally, particularly at their bases.
They do not extend to the basement membrane as the
flattened basal cells, connected by desmosomes, form a
complete ring. The connective tissue within the fibrocyte
sheath is much thicker than in the perifollicular zone.
Langerhans cells are prominent between the outer cells.

basal membrane (Fig.5.12,13). There were, thus, fewer desmosomal connections between basal cells in the lower duct body although they were still firmly attached to the luminal cells and the basement membrane. The luminal cells contained a small Golgi apparatus, mitochondria and had short widely spaced microvilli. The lumen was now wider and sometimes contained necrotic secretory cells from the fundus (Fig.5.12).

As with the other species studied the duct/fundus transition zone of 5-6 cells had a more complete fibrocyte sheath. The basal cells contained prominent myofilaments, caveolae and were in all respects identical to the myoepithelium of the fundus although the cells surrounding the lumen were still identical to those of the lower duct body. They were low and cuboidal with short widely spaced microvilli, a small Golgi apparatus, mitochondria and the occasional small vesicle. Dovetailing at the serosal surfaces was still prominent and, as in the

---

Fig.5.12. Lower duct body. The lumen is now wider with the inner layer composed of about seven cells which have prominent lateral interdigitations. This dovetailing is particularly pronounced at the base where the cells now touch the basement membrane between the now smaller and flatter outer cells. In this section a necrotic fundus secretory cell is present within the lumen.

Fig.5.13. Lower duct body. This micrograph illustrates the basal interdigitations of the luminal cells which now extend to the basement membrane.

lower duct body, processes could reach the basement

membrane. At the junction with the fundus the luminal duct

cells were joined to neighbouring fundus secretory cells

by a junctional complex including a zonula occludens. Both

cell types can readily be distinguished, the fundus

secretory cell being packed with secretory vesicles and

having longer microvilli (Fig.5.14). Langerhans cells were

prominent between the basal cells and sometimes between

the luminal and basal cells of the duct throughout the

entire intradermal region. There was no evidence of a

nerve supply to the duct although nerves did accompany the

blood vessels regularly seen running close to it.

Effect of stimulation.

Fundus.

Heat-induced sweating had a dramatic effect on the

ultrastructure of the secretory cells of the fundus. From

the onset of sweating the cells varied in shape due to

secretory vesicle depletion. The secretory cells ranged

Fig.5.14. Duct/fundus transition zone. The fundus

secretory cell, with long microvilli and densely packed

vesicles is readily distinguished from its neighbouring

duct cell. The basal cells in this zone have a similar

appearance and resemble myoepithelial cells.

CT

LC

BC

L

MV

La

N

1 µm

BMV

L

V

LC

LMV

CT

1 µm
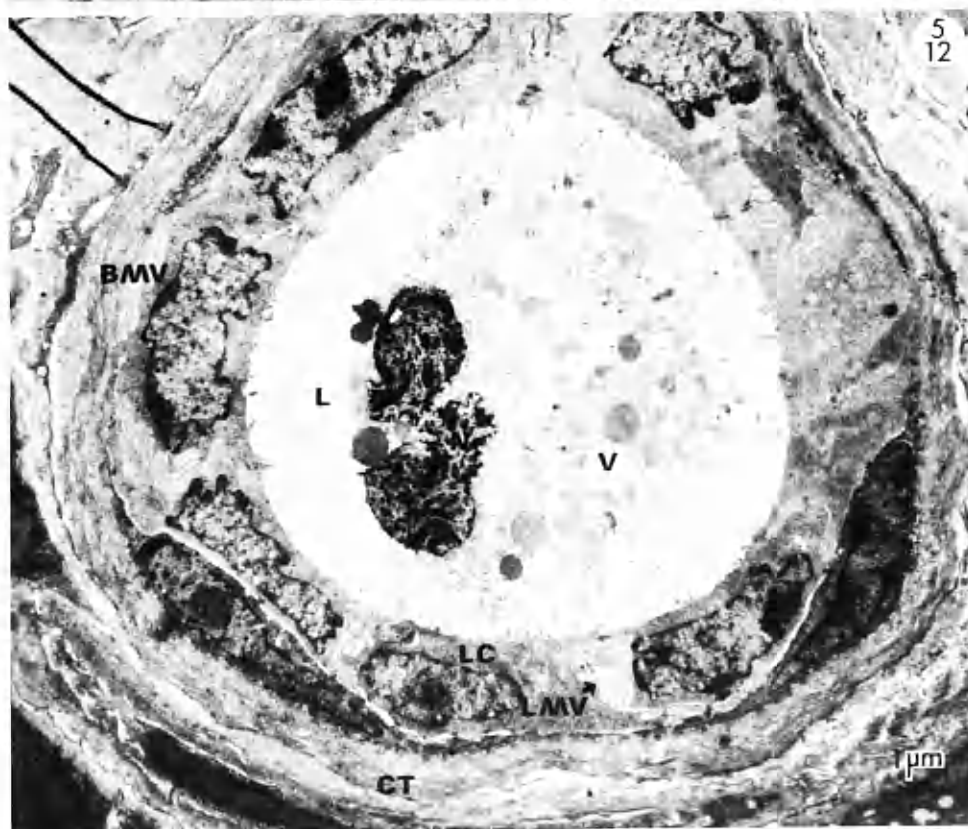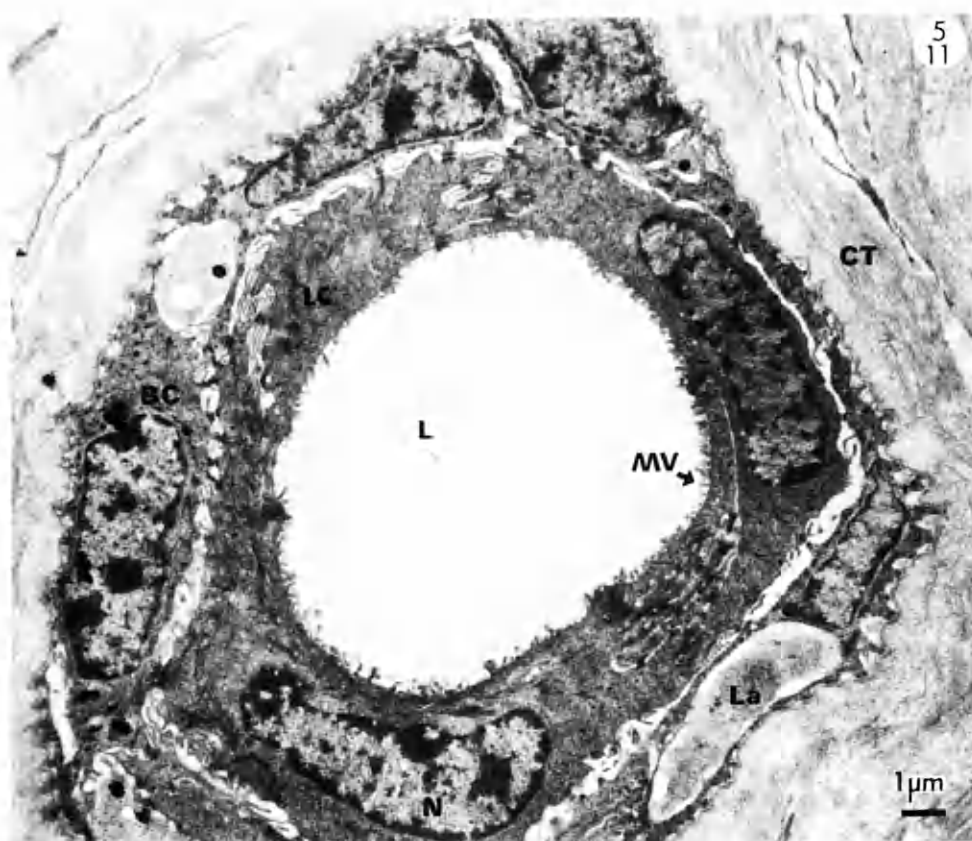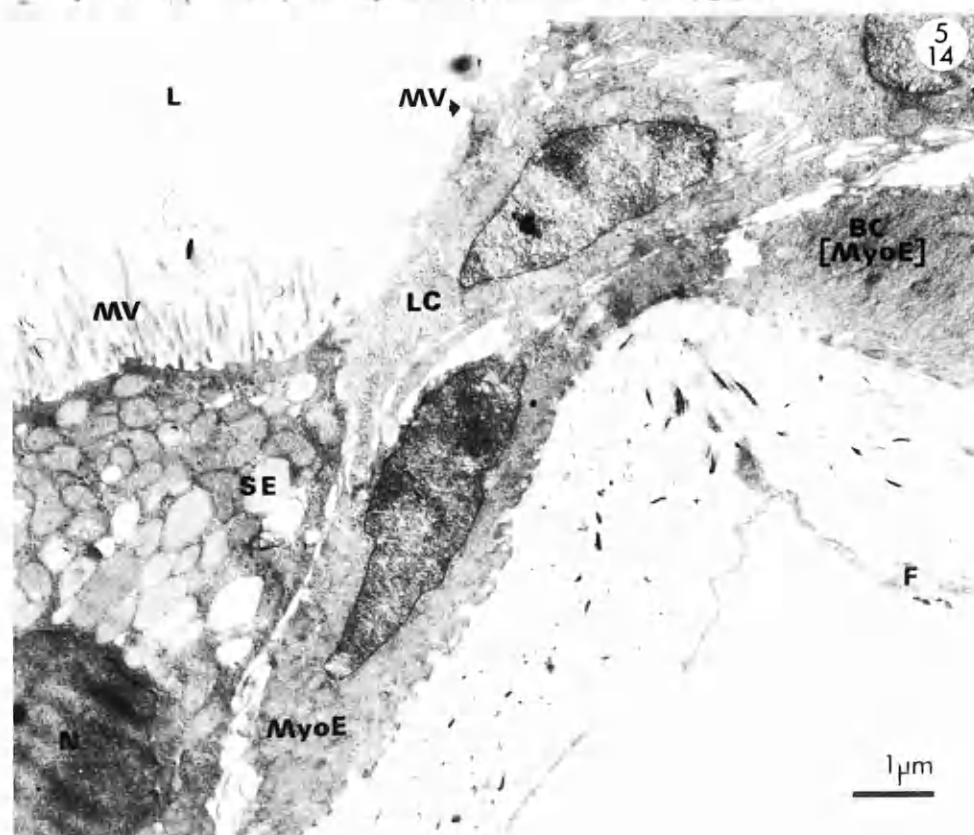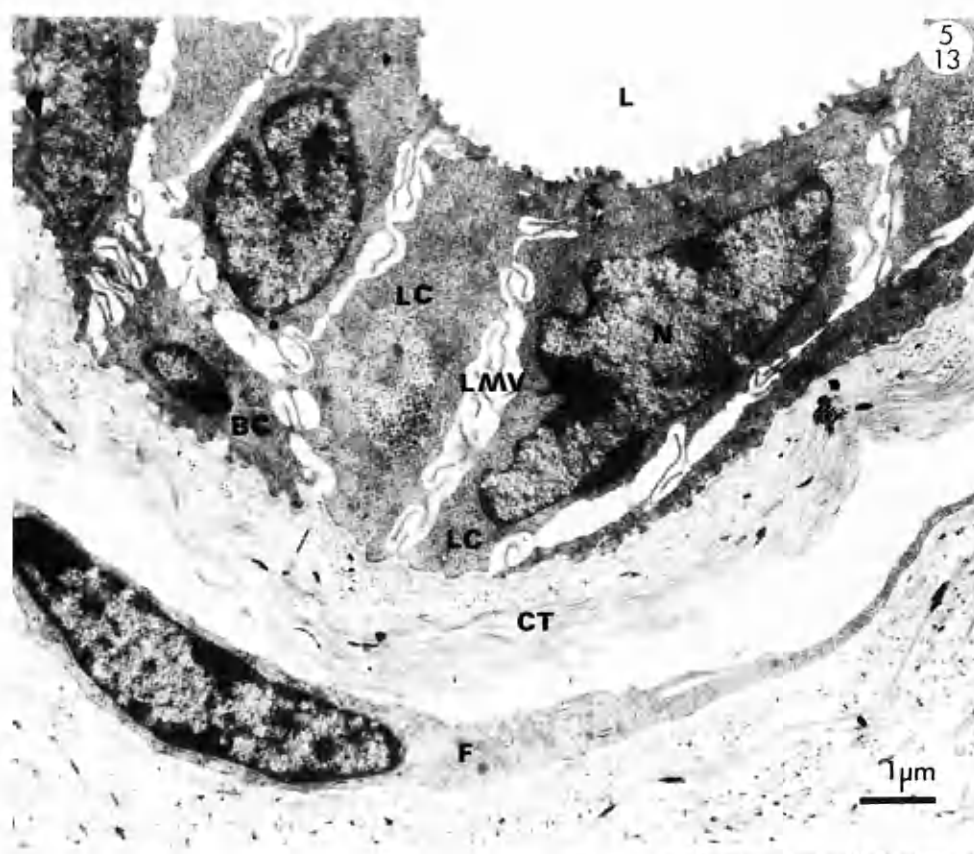
from low cuboidal (Fig.5.15) to squamous some of which were almost completely devoid of secretory vesicles (Fig.5.16). This difference in the number of secretory vesicles suggests different rates of secretory activity. Most cells appeared to be discharging their contents by exocytosis, however, membrane-bound vesicles complete with contents were also occasionally seen in the lumen (Fig.5.17). These membrane bound vesicles seem to be largely derived from disintegrating secretory cells as cytoplasmic organelles, including nuclei, can be found (Fig.5.17). The intercellular spaces were dilated and the gaps between the basal infoldings of the secretory cells had widened. The zonulae occludentes still appeared to be intact and the Golgi apparatus was still prominent within the cell. There was little evidence of

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Fig.5.15. A fundus shortly after the onset of sweating. The secretory cells are flatter and show evidence of vesicle depletion. The intercellular spaces seem to have increased in width and the crenated myoepithelium appears to have contracted.

Fig.5.16. A fundus shortly after the onset of sweating. The flatter secretory cells are now almost completely devoid of secretory vesicles. Intercellular dilatation is still evident.

Fig.5.17. A fundus after the onset of sweating. In this section the gland lumen is packed with debris from disintigrating secretory cells. Membrane-bound secretory vesicles, mitochondria and a nucleus can easily be seen amongst the luminal contents.

morphological change in the myoepithelial cells although they appeared to be more contracted (Fig.5.15).

After 3-4 hours of sweating the secretory cells lost their cuboidal appearance and became more flattened. They were generally almost completely devoid of vesicles (Fig.5.18) although a few containing some vesicles were still present. The Golgi apparatus was much less prominent and few vesicles were observed adjacent to it. The cytoplasm of the cell now contained few ribosomes or glycogen granules. The nucleus was more centrally positioned. The intercellular spaces were still dilated and the lumen was usually devoid of secretory vesicles. The myoepithelia still appeared to be contracted but otherwise exhibited little change from the resting state.

Duct.

Sweating induced by thermal stimulation had little effect on the structure of the duct. The intercellular spaces, particularly those in the lower portion of the luminal cells of the duct body appeared dilated. The only other evident change was in the lumen particularly in the perifollicular zone and upper duct body. In these zones it

Fig.5.18. A fundus after four hours of sweating. The secretory cells, with centrally placed nuclei, are now low cuboidal and almost completely devoid of vesicles. Intercellular spaces are dilated particularly at the basal region. The microvilli still appear normal and the lumen empty. The myoepithelium still appears to be contracted.

5
15

L

V

V

N

V

LMV

BMV

MyoE

2 μm

5
16

L

N

SE

M

M

CT

BMV

F

MyoE

2 μm
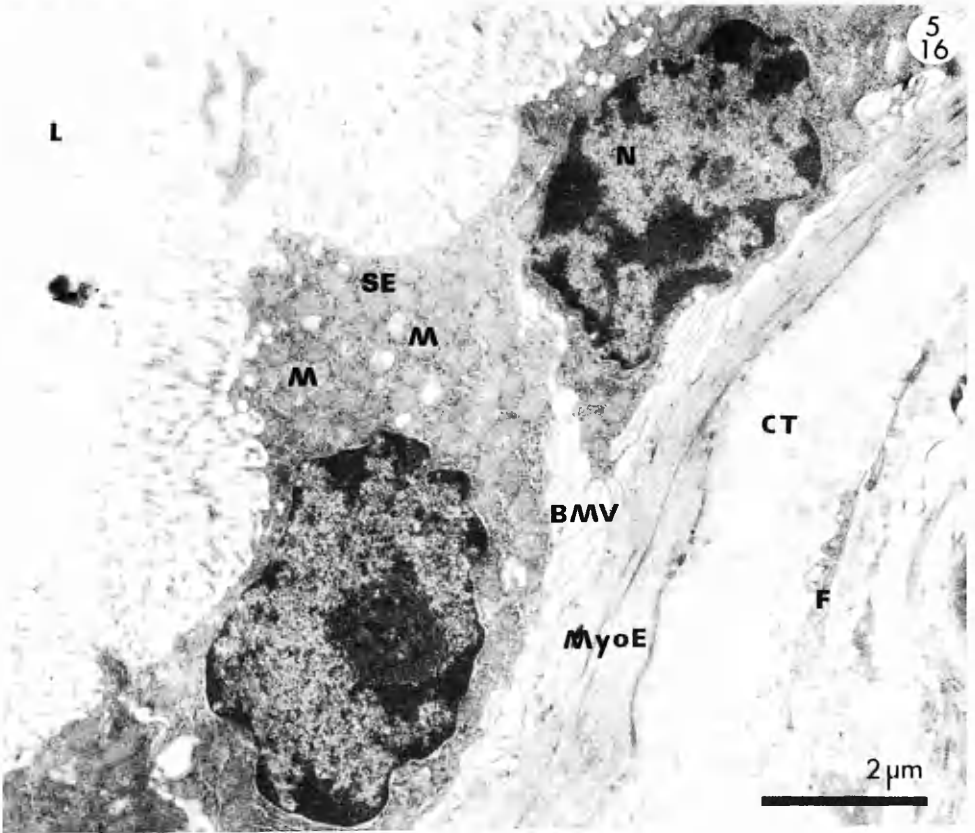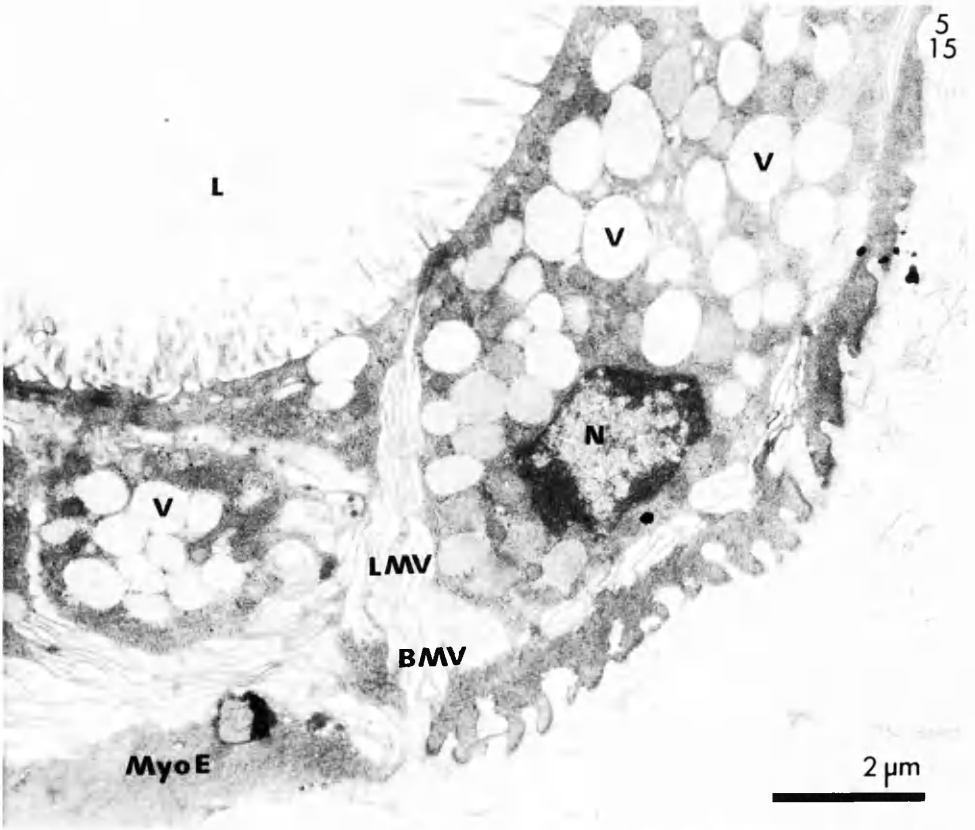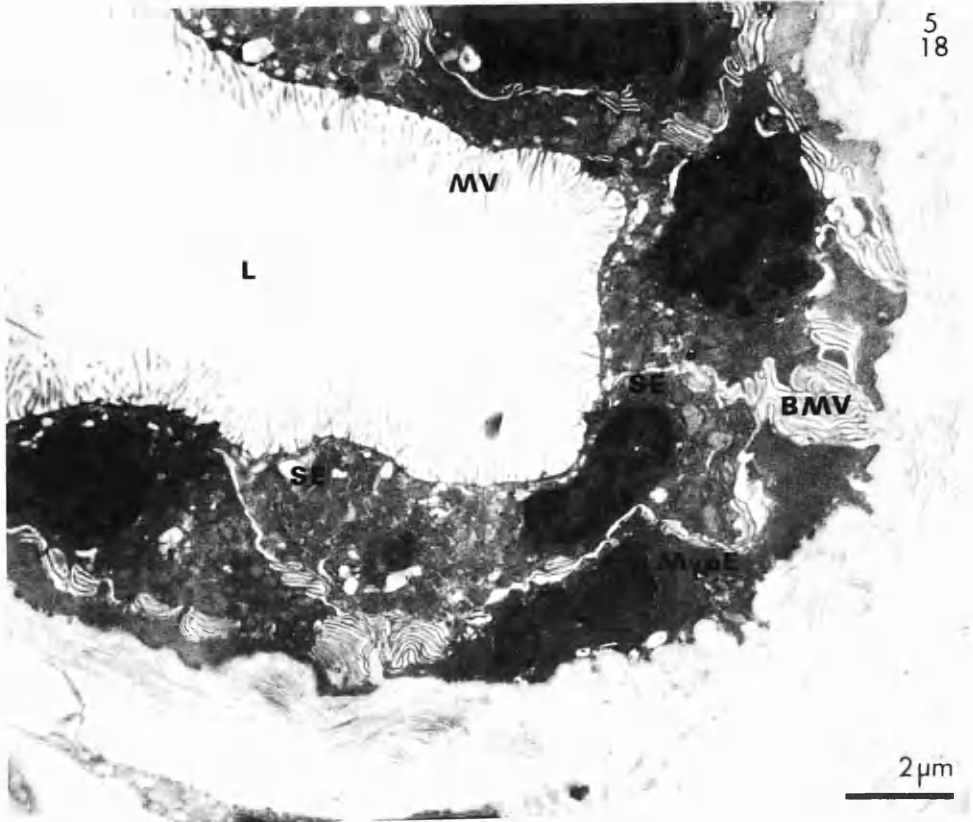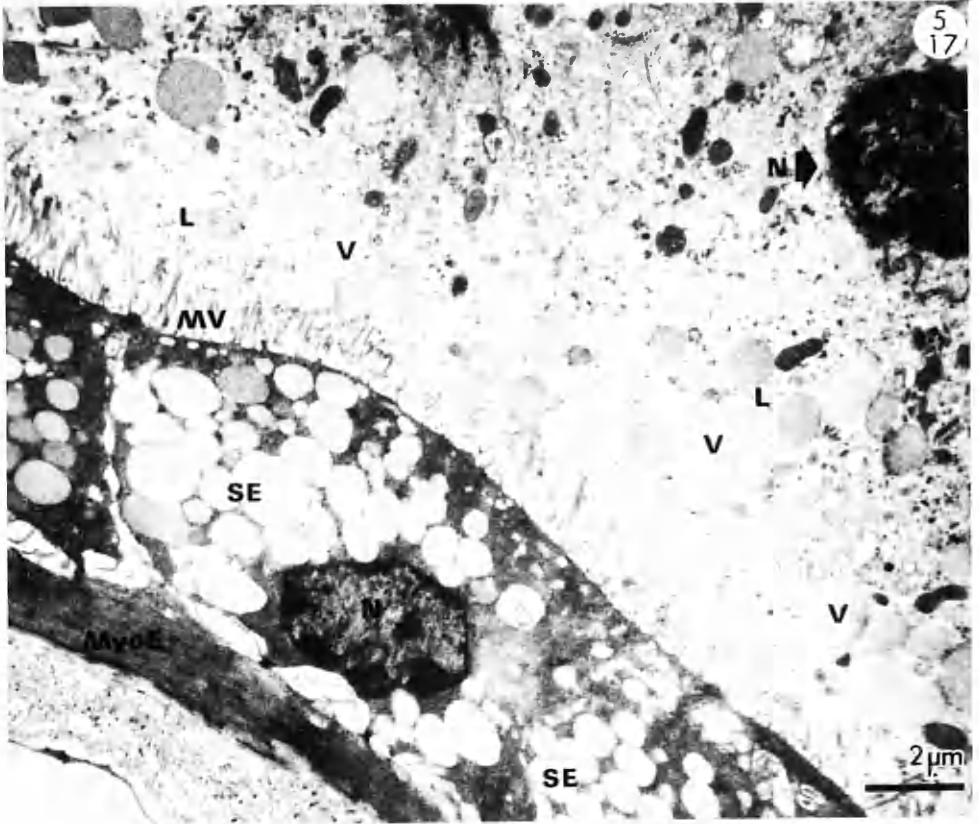
was wider and contained secretory granules and cytoplasmic organelles including nuclei.

In a later experiment corresponding samples were obtained from the same animals during exposure to high temperature and high humidity (40°C DB/35°C WB) in which the animals sweated visibly and profusely, although condensation of moisture on the skin prevented accurate measurement of sweat output with the ventilated capsule technique. In this environment it was observed visually that after about 4 hours exposure, sweating rate began to fall and that sweating had apparently almost ceased by the time the third skin sample was taken. The ultrastructural changes in the glands were identical to those observed in glands examined after exposure to the lower humidity.

DISCUSSION.

Sweating, as in the cow, sheep and goat (Chapter 3,4), had little effect on the ultrastructure of the equine sweat gland duct other than widening of the lumen. However the secretory cells of the fundus gradually lost their cytoplasmic vesicles as sweating progressed until, after four hours of activity, the now flattened cells were almost completely devoid of them. The release of vesicles was accompanied by a dilatation of the intercellular spaces, particularly the serosal lateral processes of the secretory cells. These basal interdigitations which resemble those of the human eccrine and apocrine glands (Hashimoto, 1978a,b), were more highly developed than those in cattle, sheep and goats, in which the secretory cells do not reach the basement membrane. They

are thus likely to be associated with the efficient fluid (ion) transport mechanisms in the horse and man. A high rate of fluid transport in the horse is emphasised by the fact that during sweating the fundus lumen was empty, suggesting that the contents of the vesicles are rapidly removed.

Most of the vesicles appeared to discharge their contents into the lumen by exocytosis; there was no evidence of the diacrine secretion described by Hashimoto (1978b). However, an appreciable number of the vesicles were found intact in the lumen of both fundus and duct, particularly in the unstimulated gland. These appear to be released by cell disintegration as cytoplasmic debris including nuclei and secretory vesicles were found within the fundus and duct. Occasionally vesicles were observed protruding from the luminal surface and, although none was observed to be 'pinching-off', the possibility that a micro-apocrine secretory process occurs in the horse cannot be eliminated. There was, however, no evidence in this study, either before or during secretion of the cytoplasmic protrusions described by Kurosumi et al., (1963) and Sorensen and Prasad (1973). Sorensen and Prasad (1973) describe them as fixation artifacts which agrees with the membrane blisters induced by fixation described by Shelton and Mowczko (1978). Sweat secretion in the horse therefore, as in the cow, sheep and goat, appears to involve fluid (ion) transport and exocytosis of vesicles but possibly also a micro-apocrine component.

The results show that equine sweat, as in other species, also contains the products of necrotic cells.

However, while in the sheep and goat (Chapter 4) secretory cell death occurs as a result of thermal stress, it is not certain, although very likely, that activity stimulates or accelerates cell death in the horse. The dead secretory cells, which were most frequently seen within the ductal lumen, and occur in the glands of anhidrotic equine skin (Lovatt Evans et al., 1957) could well characterize a specific response to activity. A few of the secretory cells incapable of responding to the stimulus for secretion, may die before discharging their contents by exocytosis. There was no evidence of cell loss after complete discharge of their contents. It is also possible that the necrotic cells, also observed in the duct of resting gland, could result from a mechanism of cell turnover, which need not be related to activity. There is no doubt, however, that cell debris is present in horse sweat. Sweat formation in the horse, therefore, as in the ungulates depends not only on secretion (fluid transport, exocytosis and possibly a micro-apocrine process) but also to some extent on the products of cell death.

Cellular variations in the rate of vesicle depletion, particularly during the onset of sweating, are evident and there is little doubt that during prolonged activity the rate of release exceeds that of vesicle formation. This results in a cell closely resembling the dense cells with poorly developed microvilli and devoid of secretory vacuoles occasionally detected by Kurosumi et al., (1963) in the unstimulated gland. These cells can now be interpreted as representing a different physiological state of the same secretory cell. The fundus of the horse

therefore appears to contain only one type of secretory cell and apparently only one type of secretory vesicle. The observed variations in the density of the contents of the secretory vesicles are likely to represent different stages of storage concentration but may be induced by fixation and chemical dehydration. Kurosumi et al., (1963) described them as secretory vacuoles while Sorensen and Prasad (1973) found only a flocculent material within them. Their composition has not been determined, but like the granules of the human axillary gland (Bell, 1974) they are PAS-positive and contain proteinaceous substances (Takagi & Tagawa, 1959; Talukdar et al., 1970). The decline in the protein content of sweat in the horse during prolonged sweating (Kerr et al., 1980) can thus be explained by the gradual fall in available secretory product from the vesicles.

The rate and method of vesicle replacement upon cessation of sweating has still to be determined although the most likely source is the prominent Golgi apparatus. The smaller vesicles of varying size closely associated with it are likely precursors of the larger ones. The variation in the appearance of their contents in the resting gland most likely represents different stages of development rather than activity, as suggested by Sorensen and Prasad (1973).

These results illustrate that the mode of secretion in the horse is basically the same as that found in the glands of cattle, sheep and goats. Sweat formation is composed of fluid transport, exocytosis, possible microapocrine secretion and cell death.

CHAPTER 6.

THE HUMAN GLAND.

INTRODUCTION.

Sweat glands from the body surface of some primates, including man, and the footpads of the dog and cat, arise from the epidermis and are not associated with hair follicles. These sweat glands were termed 'eccrine' by Schiefferdecker (1917) who considered them as physiologically different from the apocrine glands. This traditional concept and classification, although challenged (Dobson et al., 1958; Jenkinson, 1967, 1973) has been retained because it has never been proved that they are not eccrine.

However, histochemical and light microscopical studies of the human eccrine gland (Dobson et al., 1958) illustrate that, during activity, some cells vacuolate and retain glycogen, while others exhibit particle and glycogen depletion. The vacuolated cells are apparently damaged to the extent that they are unable to fuction normally. Although the authors at this time considered it unlikely that such a normal function as secretion of sweat would produce 'frank damage' to the secretory cells, it now seems possible in light of the evidence on the active apocrine gland that cell disruption and death can also occur in eccrine glands during secretion.

It is conceivable that the eccrine glands do not differ appreciably from the apocrine glands in their mode of secretion. Knowledge of the ultrastructure of the gland appears to be limited to the unstimulated gland (Ellis,

1962,1965; Montagna & Parakkal, 1974; Sato, 1977;

Hashimoto, 1978a; Kurosumi et al., 1982) apart from a

study of calcium pyroantimonate complex distribution in

the glands of the rat footpad (Sampson & Bowers, 1982).

The duct also has an important and active role in

sweat formation as the composition of the precursor sweat

produced in the fundus of the human eccrine gland is

altered as it passes along the duct. Water and ions are

reabsorbed, particularly in the coiled portion, where

vesicles and granules are also excreted by the luminal

cells (Sato, 1977; Hashimoto, 1978a; Morimoto, 1978). The

duct therefore, also has an important and active role in

sweat formation. Like the fundus, information on the fine

structure of the duct has been totally obtained from the

unstimulated gland (Hibbs, 1958; Ellis, 1962, 1967;

Hashimoto, Gross & Lever, 1966a; Shibasaki & Ito, 1967;

Breathnach, 1971; Montagna & Parakkal, 1974; Sato, 1977;

Kurosumi, 1977; Hashimoto, 1978a; Kurosumi et al., 1982).

Therefore the concept of physiologically distinct

sweat glands requires reappraisal. Consequently, the

ultrastructure of the human eccrine gland was examined

before and during thermally-induced sweating.

PROCEDURE.

Four males, aged between 20 and 22 years, kept

previously in an ambient environment of 16°C Dry Bulb

(DB)/9°C Wet Bulb (WB) for 1 hour, were exposed to 40°C

DB/30°C WB in the climatic chamber for up to four hours.

Cutaneous moisture loss was measured continuously from an

area on the loin and skin samples were taken with a 3mm

biopsy punch (Stiefel Laboratories Ltd.) from the

contralateral area immediately after the administration of
0.1ml of 2% xylocaine, before, at the onset of and during
sweating. Skin samples were processed for electron
microscopy as described in Appendix 1.

RESULTS.

Patterns of evaporative loss.

The patterns of evaporative loss, each to its own
baseline, obtained from the four humans during thermally
induced sweating are shown in Fig.6.1. The patterns
obtained, although differing in magnitude of response
between subjects were comparable. The output achieved
after the onset of sweating was maintained throughout the
period of exposure.

Sweat gland structure.

The human sweat gland (Fig.6.2) traverses from the
tightly coiled fundus through the dermis and penetrates
the cells of the epidermis opening onto the skin surface.
The duct can be divided into 2 regions as shown in
Fig.6.2.

1.) The intraepidermal region, which can be divided
into the upper and lower intraepidermal zones.

Fig.6.1. The patterns of cutaneous evaporative loss from
four humans in an environment of 40° C DB/23° C WB. Skin
samples were taken from the contralateral side at the
times shown (numbered arrows).

Fig.6.2. A diagram illustrating the fundus and different
zones of the duct from the human sweat gland.

Cutaneous evaporative loss ($\Delta°C$)

M.C.
118 $g\cdot m^{-2}h^{-1}$

D.C.
107 $g\cdot m^{-2}h^{-1}$

R.T.
118 $g\cdot m^{-2}h^{-1}$

A.D.
96 $g\cdot m^{-2}h^{-1}$

Time (h)

6
1

Intraepidermal region

Intradermal region

Ascending zone

Coiled zone

Fundus →

Transition zone

6
2
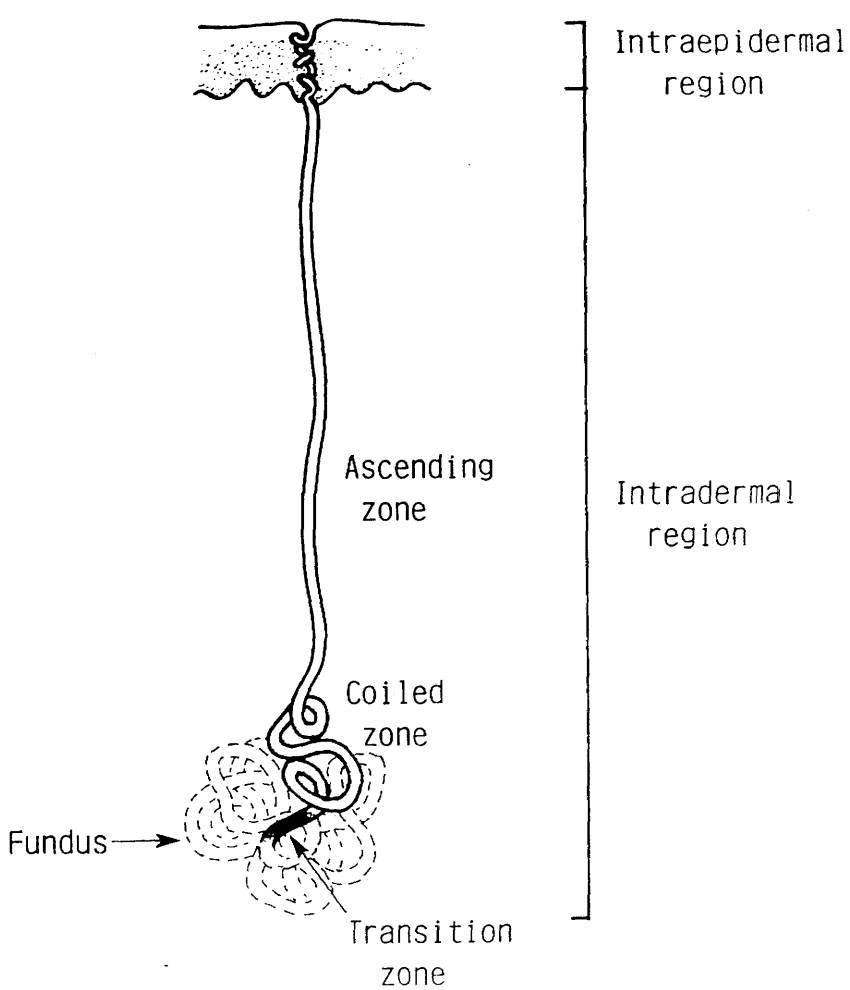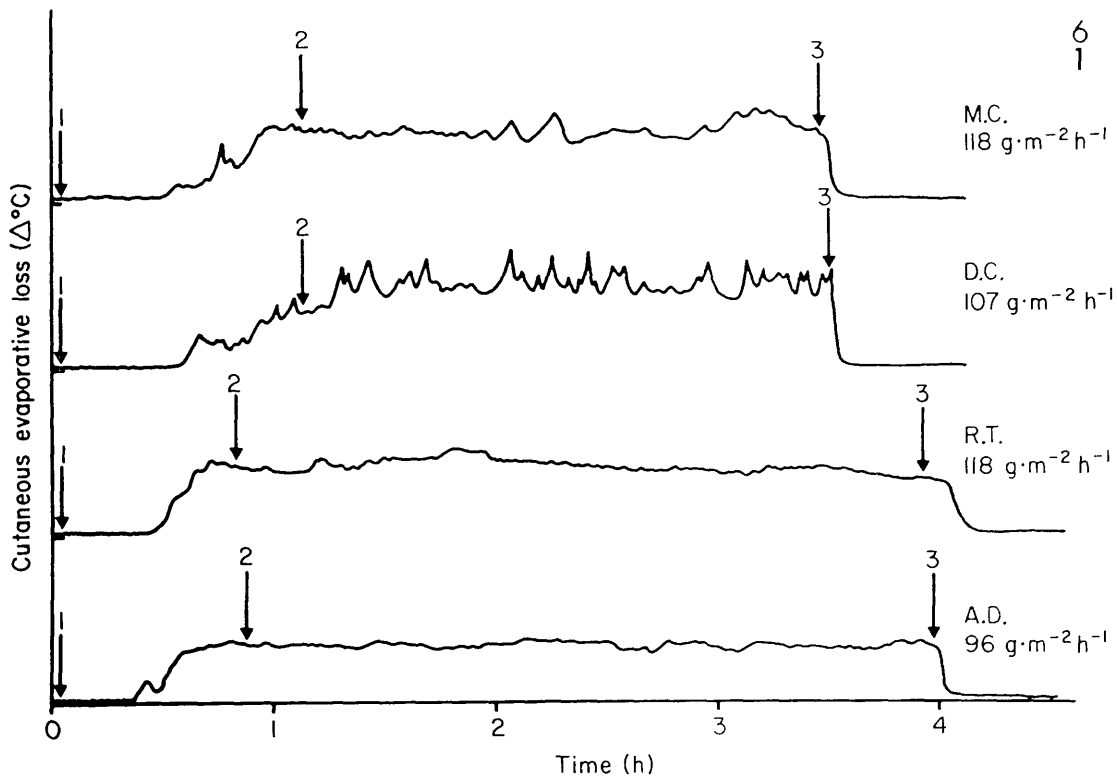
2.) The intradermal region, which again can be further divided. In this region there are three zones.

A.) Upper ascending duct.

B.) Lower ascending duct.

C.) Coiled duct. This portion of the duct was found amongst the coils of the fundus.

The unstimulated gland ultrastructure.

Fundus.

The fundus was composed of two cell layers surrounded by a fenestrated fibrocyte sheath (Fig.6.3). The outer layer of myoepithelial cells were elongated with a convex luminal surface and a generally flattened, although crenated, serosal surface attached by hemidesmosomes to a well developed basal lamina (Fig.6.4). They also contain dense bodies with a degree of alignment, which give, in some planes of section, a pseudo-stratified appearance to

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Fig.6.3. Electron micrograph of the fundus of the unstimulated atrichial sweat gland illustrating the secretory and myoepithelial layers and the surrounding connective tissue and fibrocyte sheath.

Fig.6.4. A transverse section through a myoepithelial cell illustrating the convex luminal surface and the flat crenated serosal surface attached by hemidesmosomes to the basal lamina.

63

F — CT — N — GC — N — NGC — BI — MyoE — GC — L — Ca — N — NGC — 4 µm

64

NGC — GC — BI — MyoE — N — CT — HD — HD — BL — NGC — 1µm
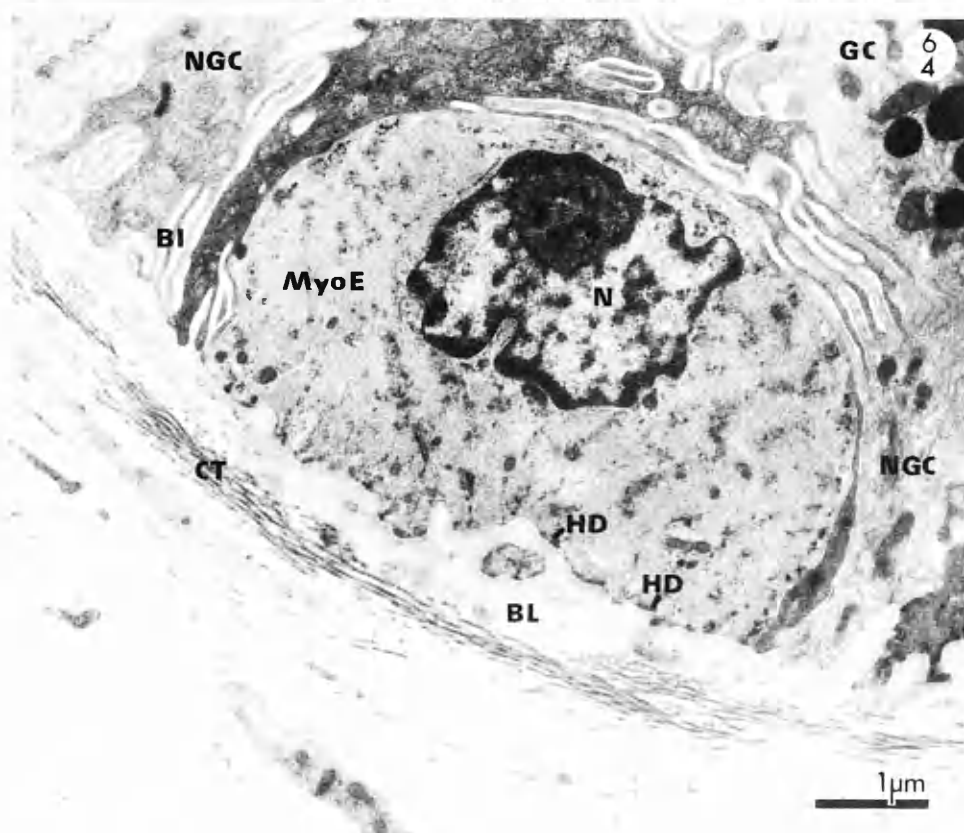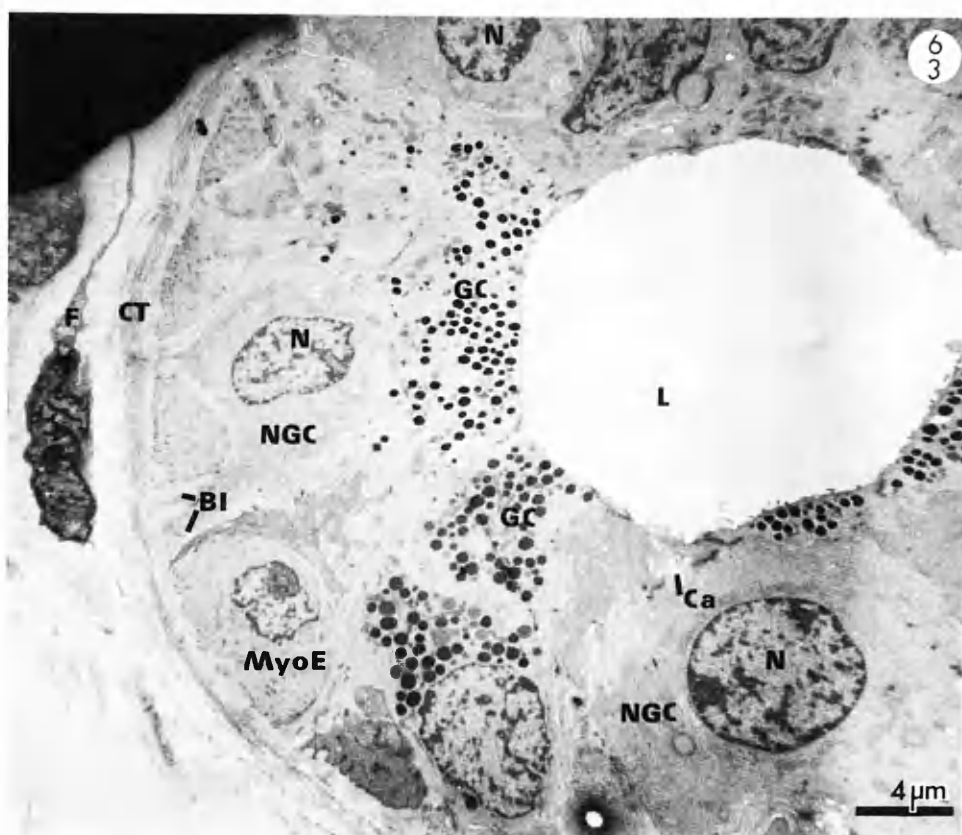
these smooth muscle cells (Fig.6.5). These cells formed an incomplete layer around the secretory cells giving the impression of a wide meshed myoepithelial net.

The inner secretory epithelium had two morphologically distinct types of secretory cell, granular and non-granular, which interdigitate giving the impression of a pseudo-stratified epithelium (Fig.6.3). Both cell types had small, sparse, stubby luminal microvilli. The most prominent feature of the more numerous non-granular cells were their complex basal infoldings, processes of which were found between the myoepithelium and resting on the basal lamina (Fig.6.6). Mitochondria were numerous adjacent to these basal and lateral interdigitations (Fig.6.7). Adjoining non-granular cells formed canaliculi which were open to the luminal surface and terminated among the basal infoldings (Fig.6.8). The lateral and

---

Fig.6.5. A longitudinal section through a myoepithelial cell illustrating the pseudo-stratified appearance of these smooth muscle cells. The myoepithelium is connected to the secretory cells by desmosomes and occasional gap junctions.

Fig.6.6. The basal portion of a non-granular cell illustrating the complex basal infoldings, processes of which rest on the basal lamina.

Fig.6.7. In this section of a non-granular cell numerous mitochondria are found adjacent to the basal and lateral infoldings.

Fig.6.8. A canaliculus between adjoining non-granular cells.

6
7

M

NGC

M

BI

M

BI

BL

MyoE

CT

0 5μm

6
8

GC

L

NGC

NGC

N

Ca

N

F

MyoE

2 μm
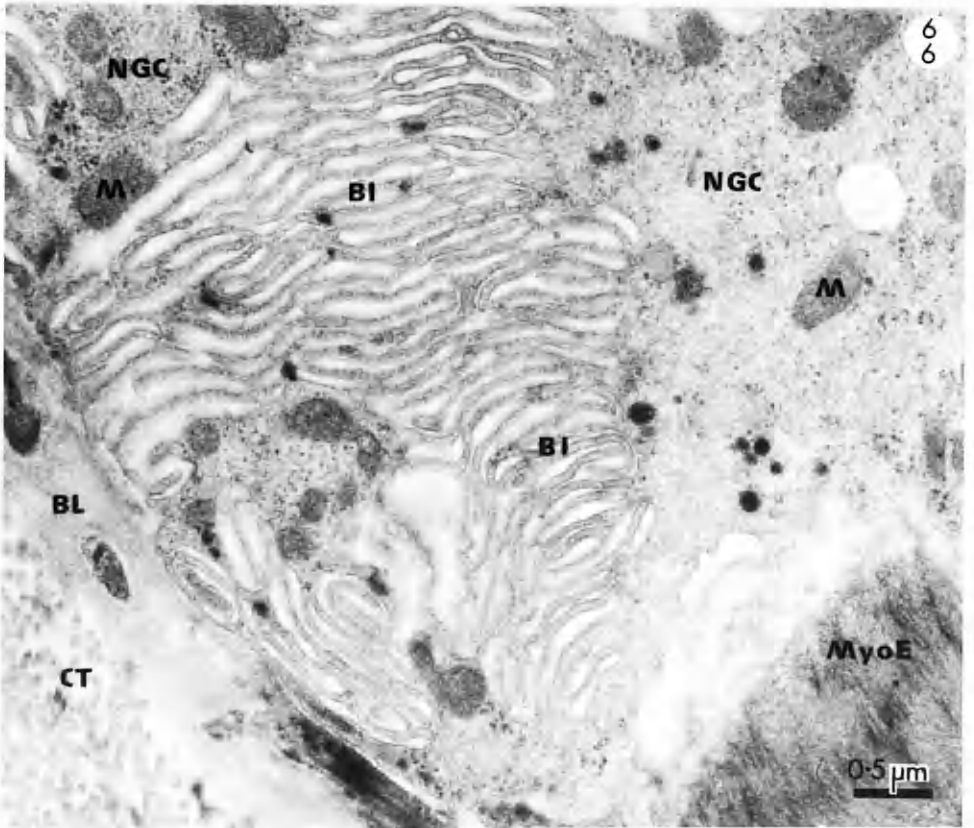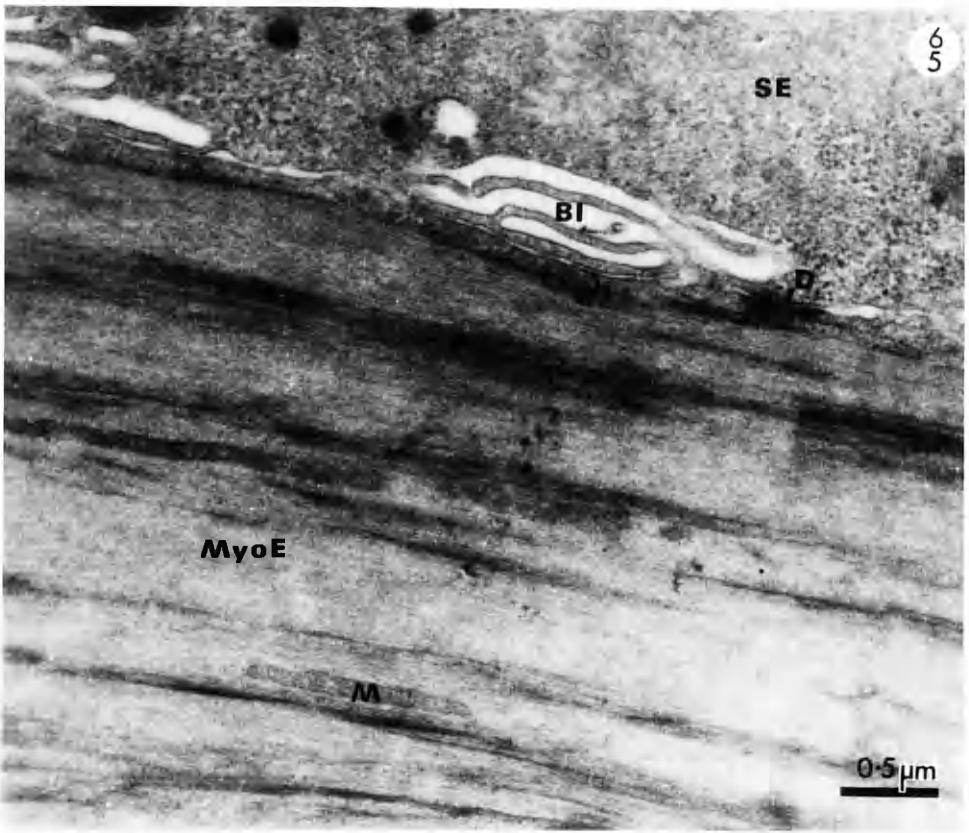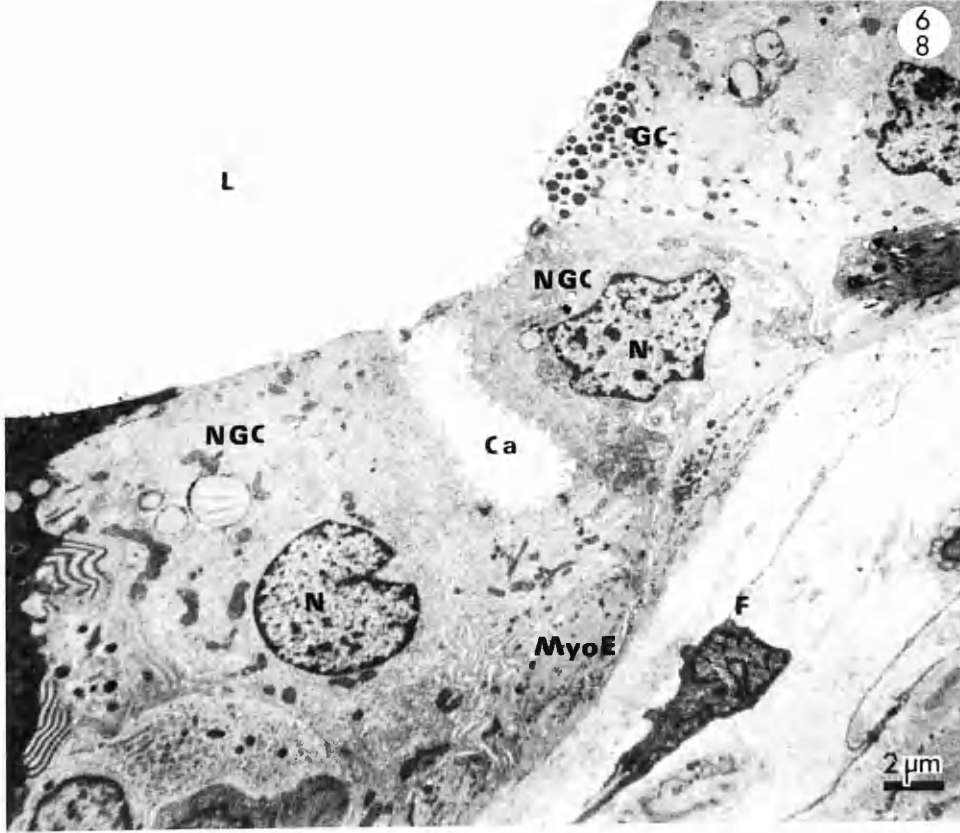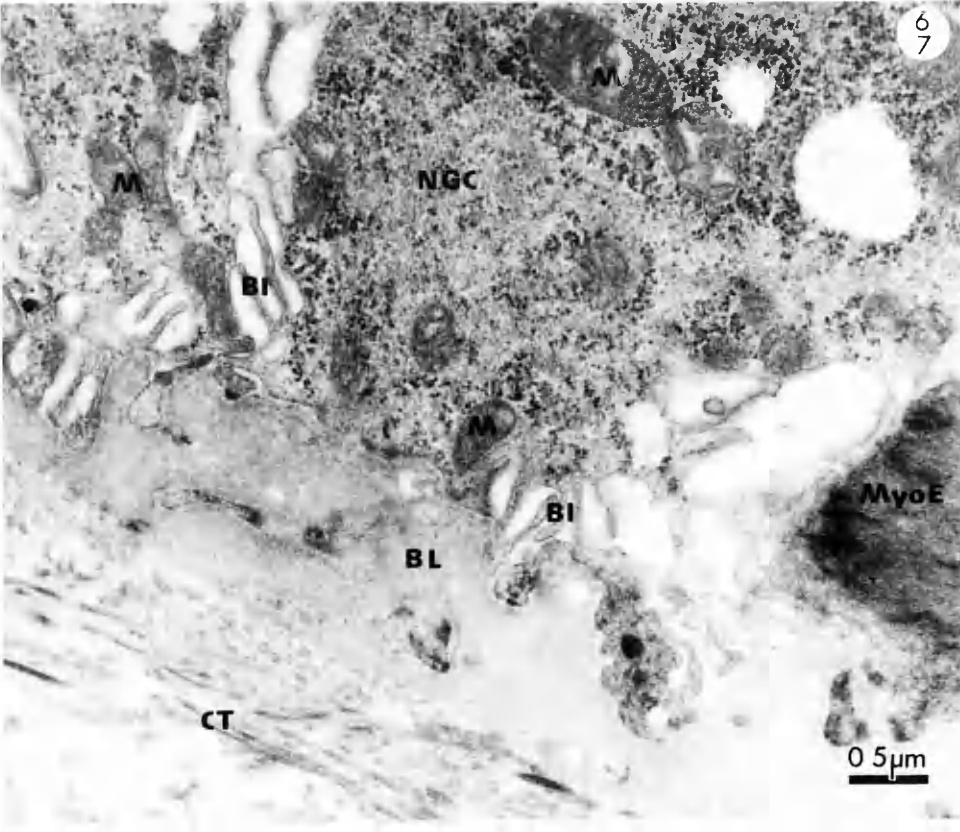
basal surfaces of these channels were sealed by junctional complexes, with <u>zonulae occludentes</u>, between cells, so that they could be considered as extensions of the luminal membrane. These canaliculi were not found between granular and non-granular cells or between adjacent granular cells. However, in both these locations neighbouring cells have a luminal junctional complex composed of desmosome and tight junction. Myoepithelial cells were also connected by desmosomes (Fig.6.5) and the occasional gap junction to secretory cells. Cell junctions between adjacent myoepithelial cells were rarely seen.

Granular cells have a shape similar to a wine glass with the nucleus of the cell situated towards the base of the bowl. The stem, occasionally seen in a favourable section showed the cell extending from the basal lamina to the lumen (Fig.6.9). The granules exhibited differences in the texture of their contents with those in some cells appearing much darker (Fig.6.10). Cells with a predominance of dark granules can easily be distinguished from those in which the majority of granules are much lighter (Fig.6.11).

<u>Fig.6.9</u>. A wine-glass-shaped granular cell extending from the basal lamina to the lumen.

<u>Fig.6.10</u>. A granular cell with a predominance of dark granules.

<u>Fig.6.11</u>. A granular cell with a majority of lighter granules.

L

DG

DG

0·5µm

PG

PG

0·25µm
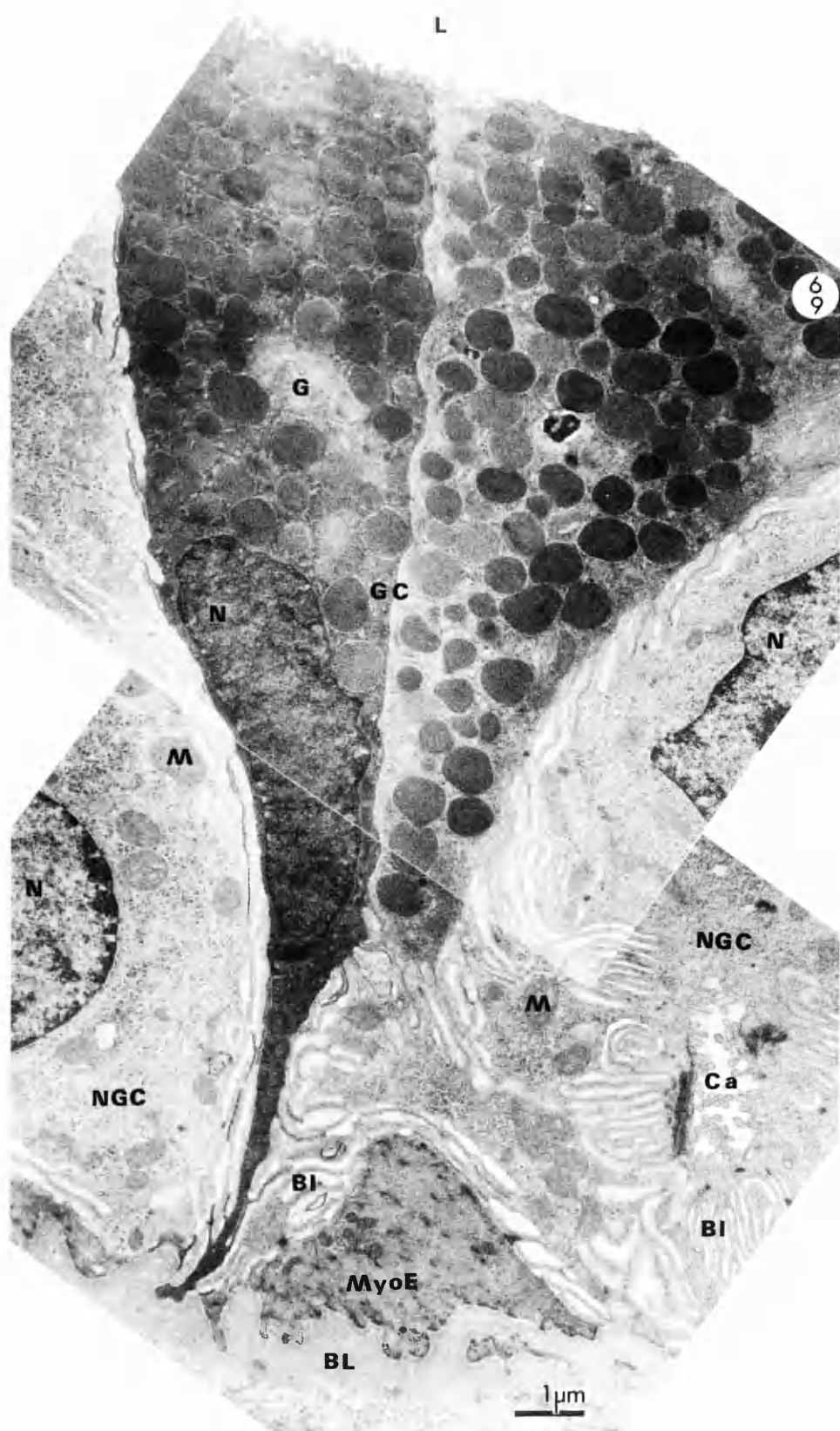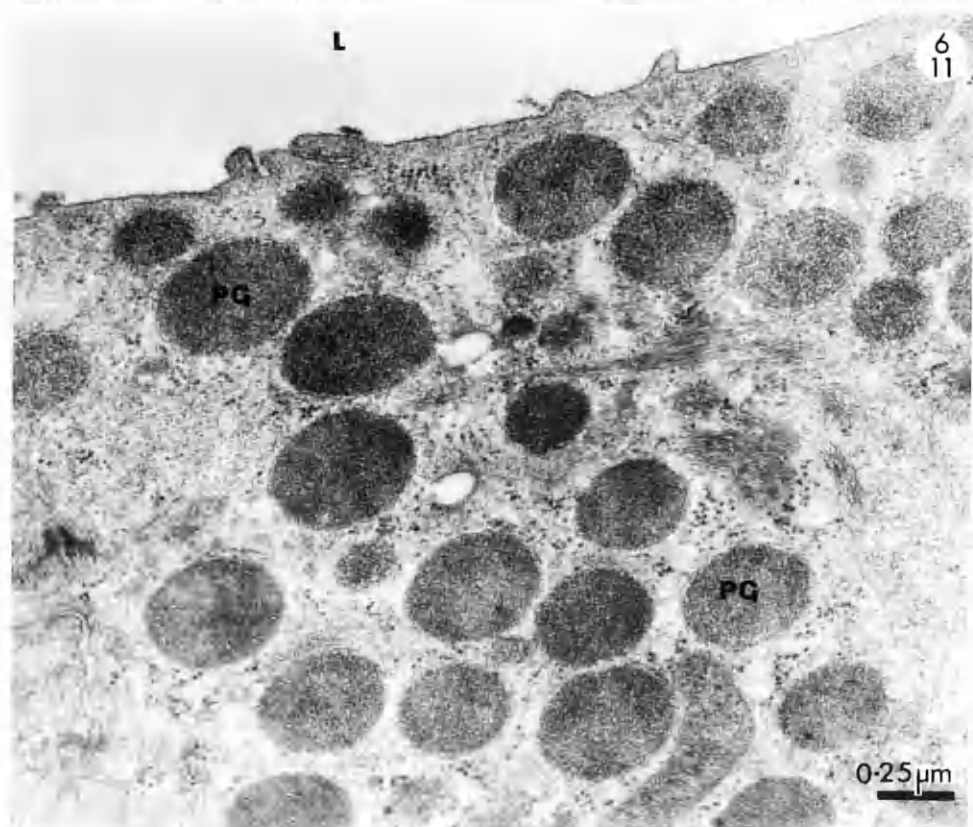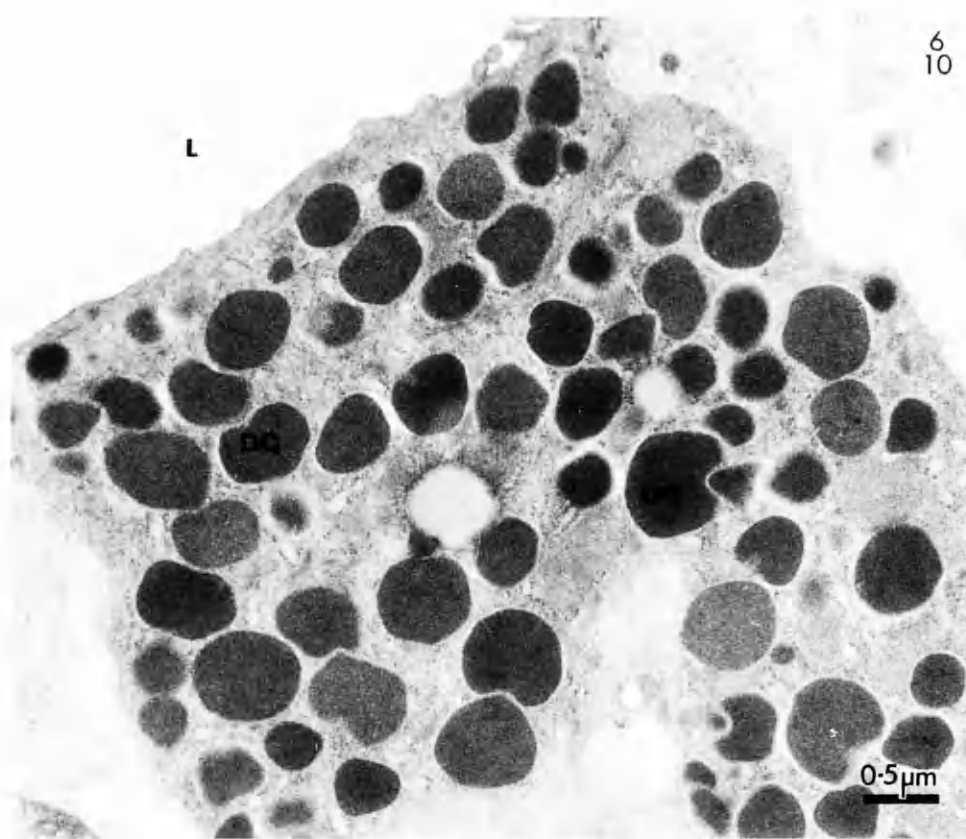
Duct.

Upper intraepidermal zone.

The spiral intraepidermal duct, just below the stratum
corneum, is composed of a ring of at least eight layers of
spindle-shaped granular cells with a cytoplasmic matrix
paler than that of the surrounding epithelial cells. The
tonofilament bundles in these ductal cells were located
mainly at the cell margins and not randomly as in the
epidermal cells. The epithelial wall of the duct was
stratified with a cornified layer lining the lumen.
Fingers of underlying, non-keratinized luminal cells,
possibly necrotic, were observed to extend upwards to
provide an inner lining (Fig.6.12).

Lower intraepidermal zone and upper ascending duct.

The duct wall deeper into the intraepidermal zone,
although still composed of about eight cell layers, no
longer showed signs of keratinization (Fig.6.13) and
resembles that of the upper half of the ascending portion
of the duct. The cells comprising the basal layers were

Fig.6.12. The upper intraepidermal duct of the
unstimulated sweat gland. The spindle-shaped granular
cells of the duct have a paler cytoplasmic matrix than
that of the surrounding epithelial cells. The lumen is
lined by keratinized cells and in this section by
processes from uncornified cells deeper in the duct.

Fig.6.13. The lower intraepidermal zone of the duct. The
duct is composed of about eight cell layers with those
comprising the basal layers having tonofilaments present
as a narrow circumferential layer.

71
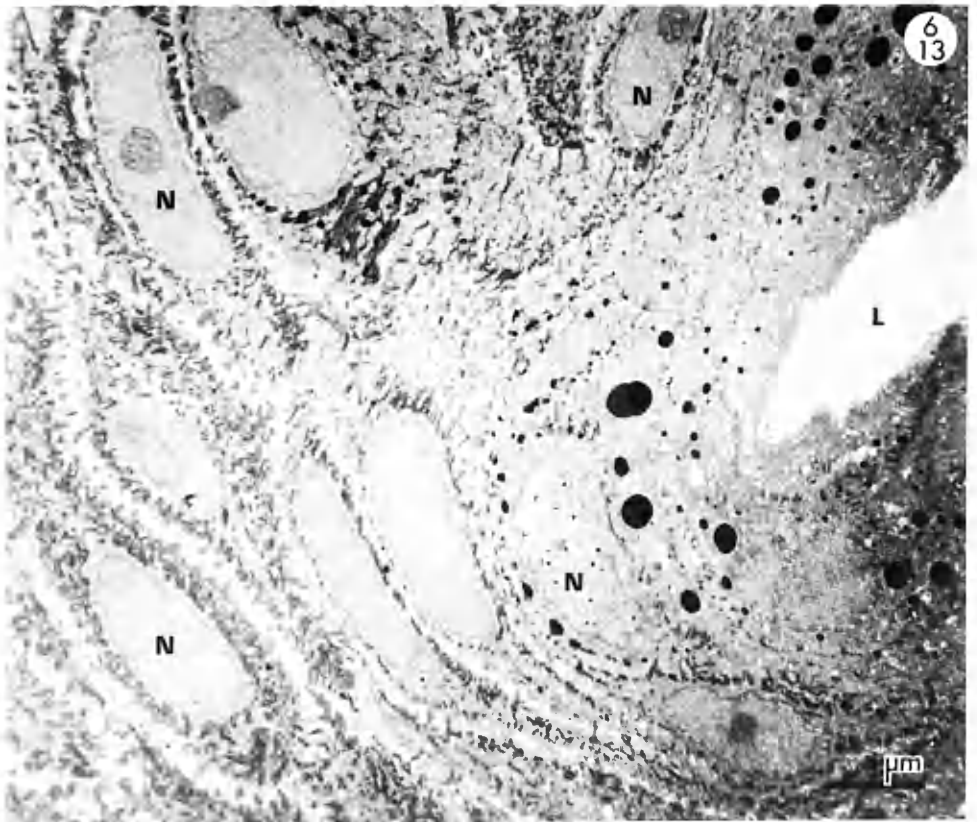
flattened and spindle shaped with tonofilaments present as a narrow circumferential layer in the peripheral cytoplasm. These basal cells were interconnected by desmosomes, the occasional gap junction and very occasionally cytoplasmic bridges (Fig.6.14). The three to five cells comprising the innermost ring are characterized by a prominent fine fibrillar network or terminal web situated apically and encircling the lumen. At the base of this web, on the luminal side of the nucleus, accumulations of multivesicular bodies are regularly observed (Fig.6.15). These multivesicular bodies are also present in the lower half of the ascending duct, although usually in smaller aggregations. The cytoplasm of the luminal cells contained a small Golgi apparatus, free ribosomes, a few mitochondria and the occasional lipid droplets. The region immediately surrounding the nucleus contained short lengths of RER and was devoid of tonofilament fibrils resulting in the appearance of a halo around it. Translucent, ovoid, membrane bound granules, 100-500nm in size and sometimes showing internal lamellar structure, probably membrane-coating granules, were also present in the cytoplasm. Numerous smaller and irregularly shaped, membrane bound vesicles were a characteristic feature within the lumen and were closely associated with

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Fig.6.14. A cytoplasmic bridge between two basal cells in the upper ascending duct. This example is from a duct at the onset of sweating.

Fig.6.15. Accumulations of multivesicular bodies at the luminal side of the nucleus from the upper ascending duct.

N

D

D

N

CB

BL

CT

F

0·5 µm

L

EF

LV

MV

D

MVB

MVB

N

0·5 µm
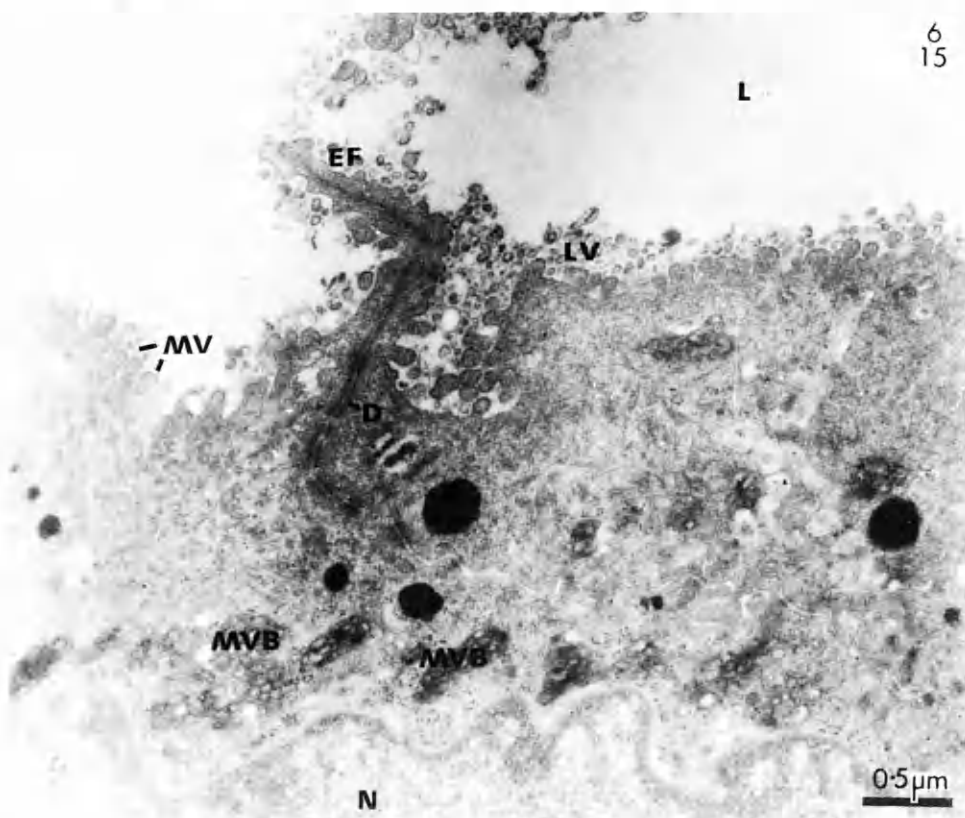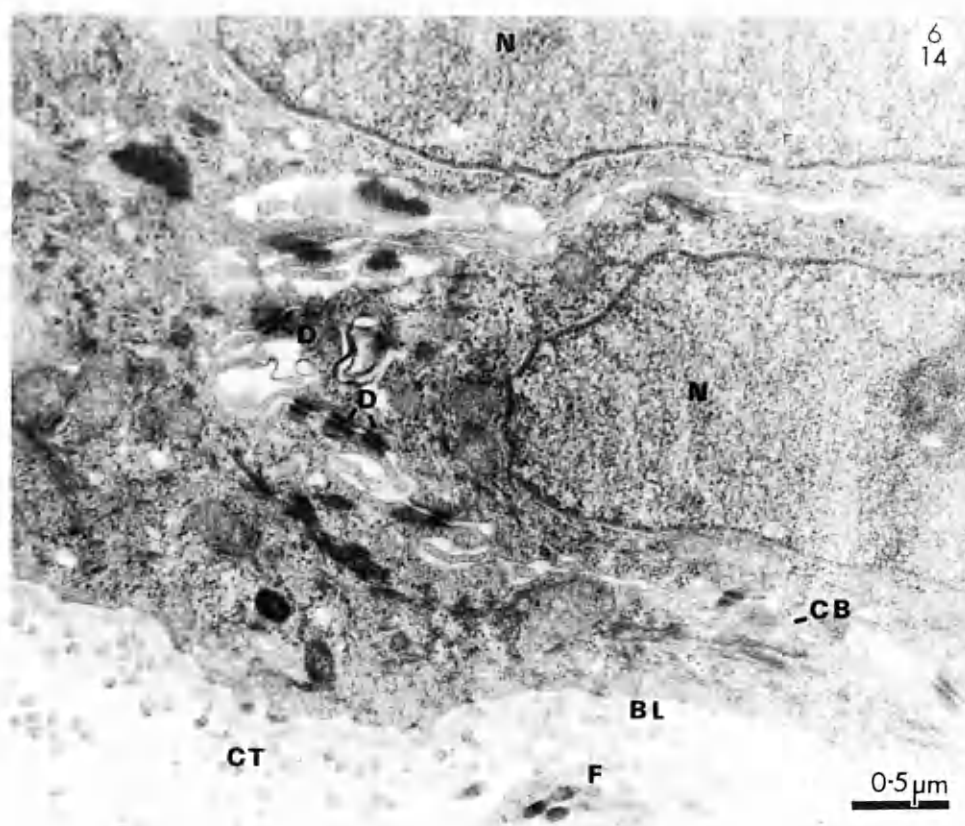
the short, densely packed apical microvilli (Fig.6.16).
Configurations commensurate with vesicle pinocytosis
(Fig.6.17) were regularly seen. Other configurations
suggestive of luminal vesicle formation by 'pinching-off'
from microvilli were also observed. Apical junctional
complexes with a zonula occludens are present between the
luminal cells (Fig.6.18). They also have extensive lateral
and basal interdigitations with numerous desmosomes and a
few gap junctions (Fig.6.19). Among these lateral and
basal interdigitations, zonulae occludens were also
present (Fig.6.20). The trilaminar structure of these
junctions, which were approximately 10nm in thickness,
were clearly visible (Fig.6.20 inset).

Lower ascending duct.
The wall of the ascending duct decrease from about eight
cells immediately beneath the epidermis to four to

Fig.6.16. Membrane-bound vesicles within the lumen and
closely associated with the microvili of the upper
ascending duct.

Fig.6.17. Membrane-bound cytoplasmic vesicles may be
formed from pinocytotic configurations between the bases
of the microvilli (arrows).

Fig.6.18. Apical junctional complex with a zonula
occludens between luminal cells.

Fig.6.19. Desmosomes and a gap junction between luminal
cells of the upper ascending duct.

Fig.6.20. Interdigitations between a luminal and basal
duct cell. In addition to the desmosome connections
between cells, a zonula occludens can be seen (inset).

L

LV   LV

MV

0·5 µm

L

LV

MV

0·5 µm

L

ZO

MV

D

D

0·5µm

6
19

6
20

0·25 µm

0·25 µm

0·1 µm
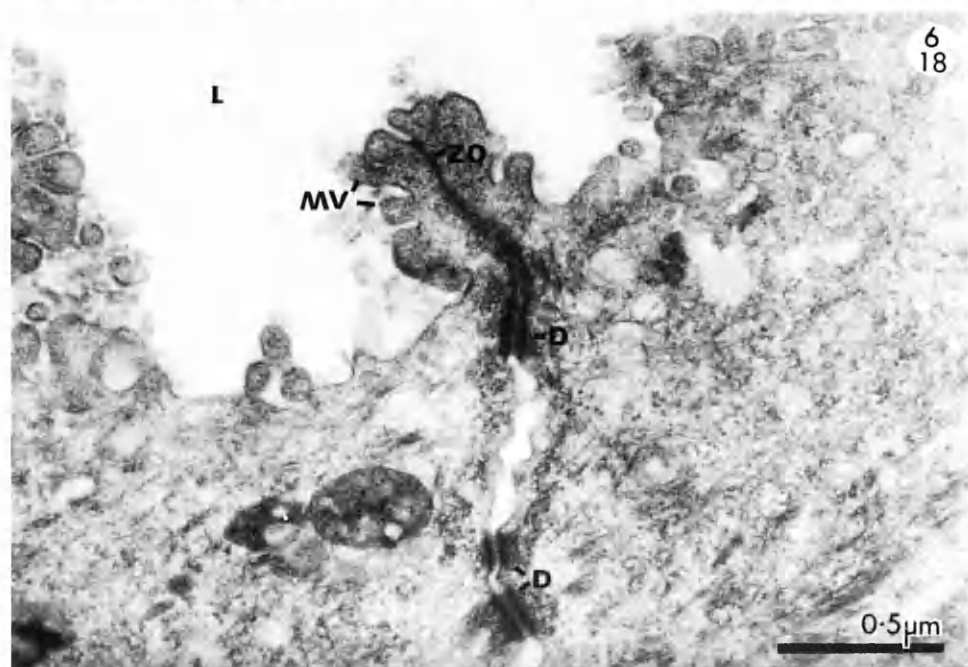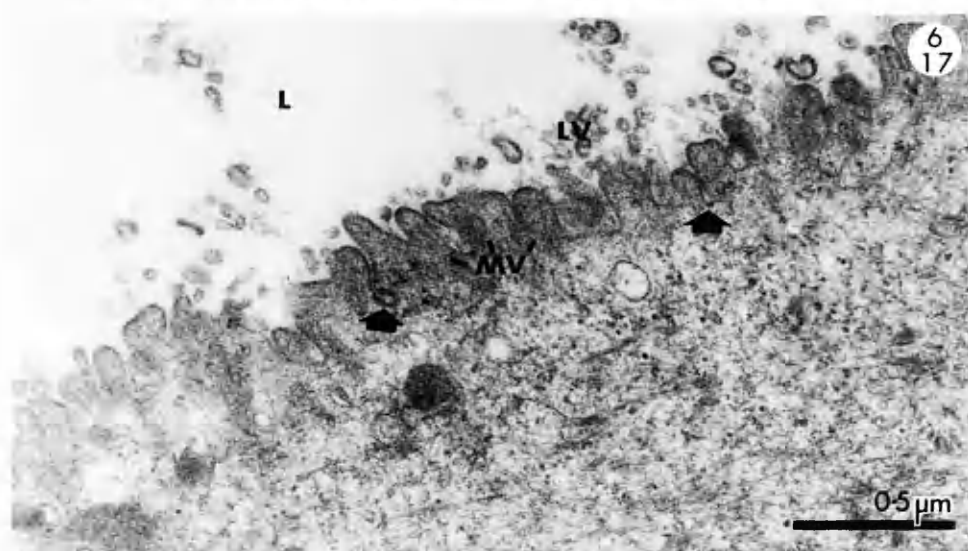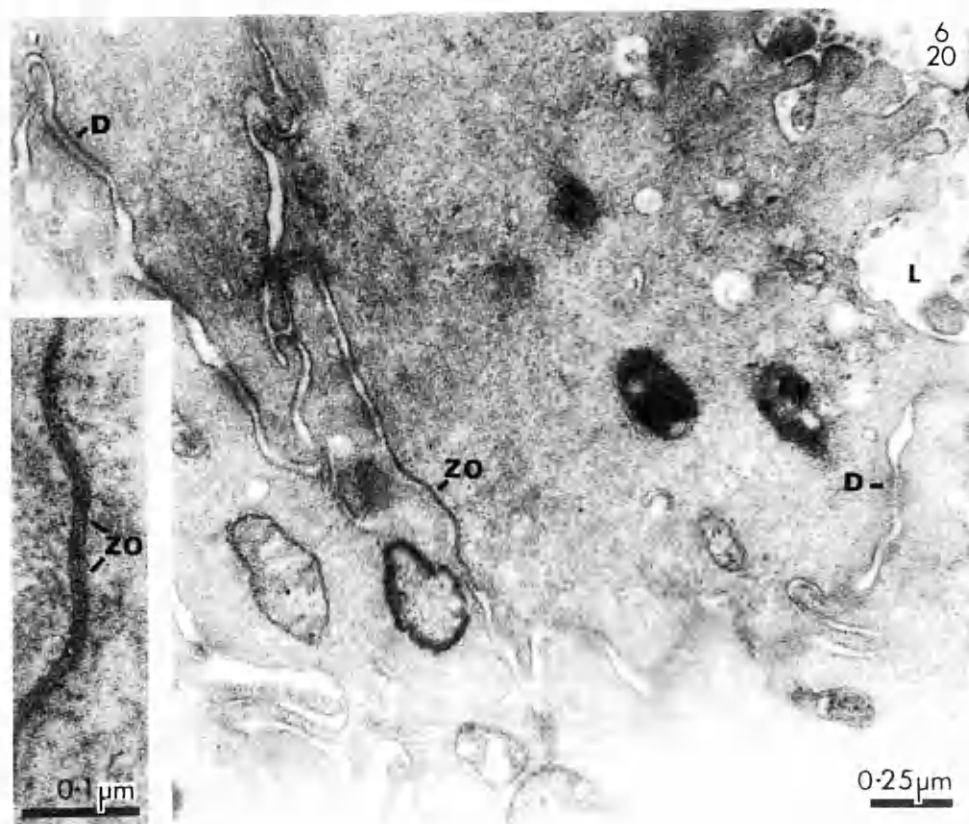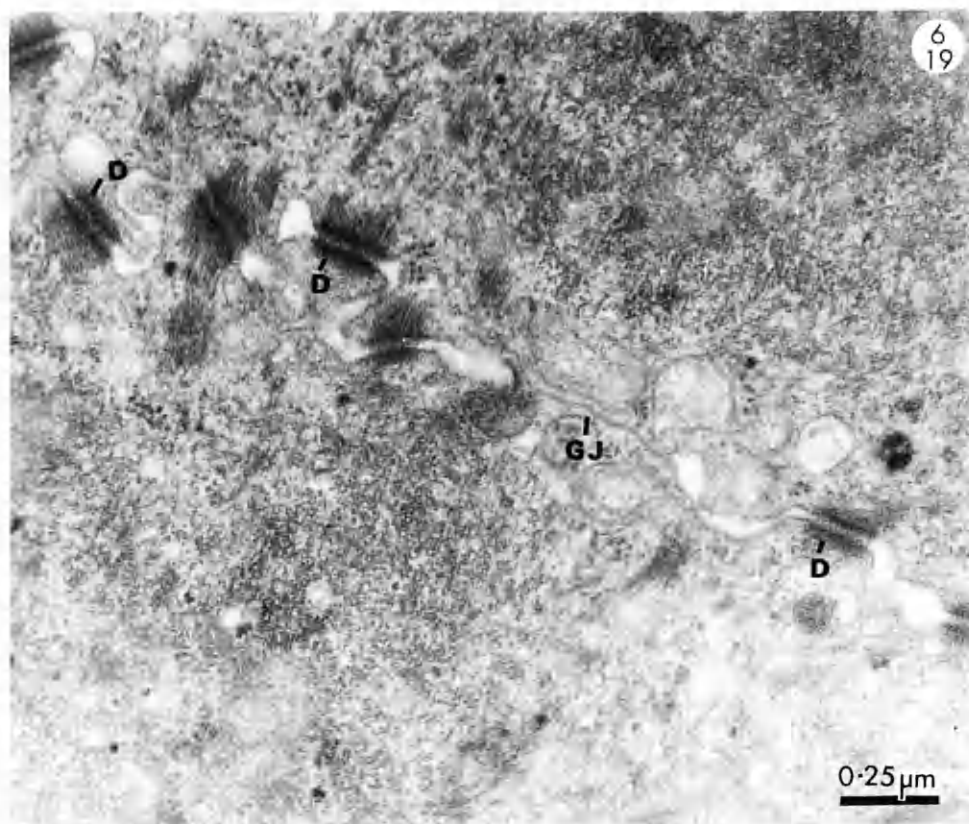
six layers adjacent to the coiled duct (Fig.6.21). At this
level the connective tissue surrounding the duct was more
dense with a more highly developed fibrocyte sheath.
Nerves were never found within 20µm of the fibrocyte
sheath surrounding the duct. Within the deeper region of
the ascending duct the luminal cells still exhibit a
pronounced terminal web and stubby microvilli with a
filamentous core but fewer of the associated luminal
vesicles. The nuclei of these luminal cells contain more
euchromatin. The cytoplasm of the cells have increased
numbers of now larger mitochondria, free ribosomes and
electron-translucent vesicles. An apical junctional
complex with zonula occludens was present but was not
detected on the lateral and basal membranes. However, the
lateral dovetailing between these luminal cells was much
more pronounced. Basal cells are still flat and
interdigitated with numerous gap junctions between them.
Throughout the duct Langerhans cells, with Birbeck
granules, were observed between the layers of epithelial
cells (Fig.6.22).

Fig.6.21. The lower portion of the ascending duct. The
wall is still approximately six layers thick. A terminal
web is prominent in the luminal cells.
Fig.6.22. A Langerhans cell between epithelial cells of
the lower ascending duct.
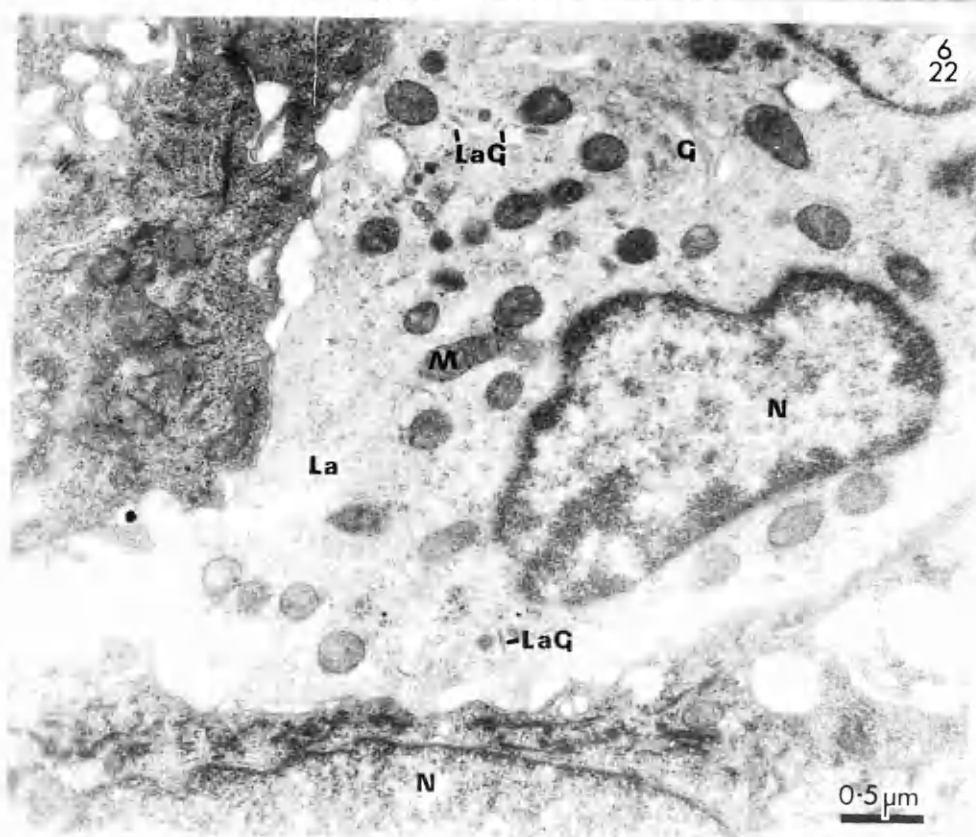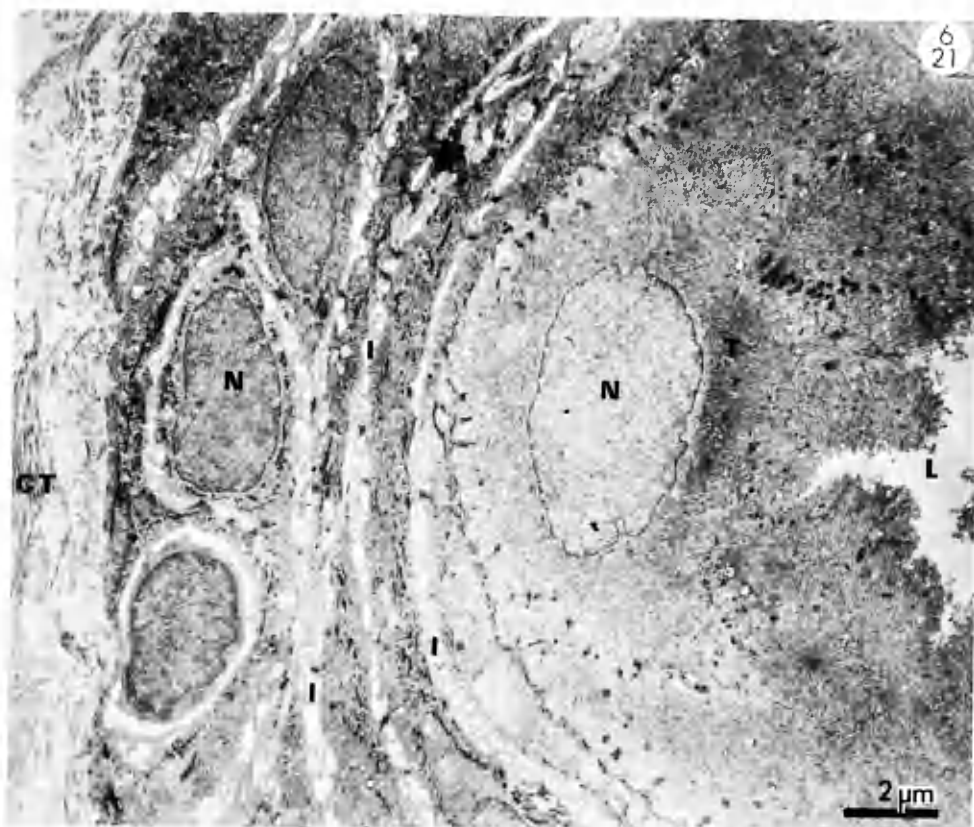
Coiled duct.

The epithelial wall of the coiled duct generally consisted of an inner ring of luminal cells surrounded by two layers of flat basal cells (Fig.6.23), with dense connective tissue within the external fibrocyte sheath. The now columnar luminal cells, like those of the ascending duct, had a well developed terminal web of fine tonofilaments and containing numerous mitochondria, accumulations of which are often found in the preinuclear region (Fig.6.24). These cells are rich in ribosomes and contained occasional lipid droplets. Membrane bound vesicles were still present within the lumen in close association with the surface microvilli but were observed more rarely. The luminal cells are joined by apical junctional complexes. The lateral dovetailing between them was even more complex than found in the ascending duct. These tortuous interdigitations were connected by numerous desmosomes and gap junctions, which were also found extensively among the interdigitations between luminal and basal cells. _Zonulae occludentes_, other than those in the apical junctional complexes were not identified between

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Fig.6.23. The coiled portion of an unstimulated duct. The duct wall is still three layers thick. Luminal and basal cells are interconnected by complex interdigitations with numerous desmosomes.

Fig.6.24. High power view of the transition zone of an unstimulated duct. The perinuclear region of the luminal cell has a terminal web of fine tonofilaments and numerous mitochondria.

6
23

6
24
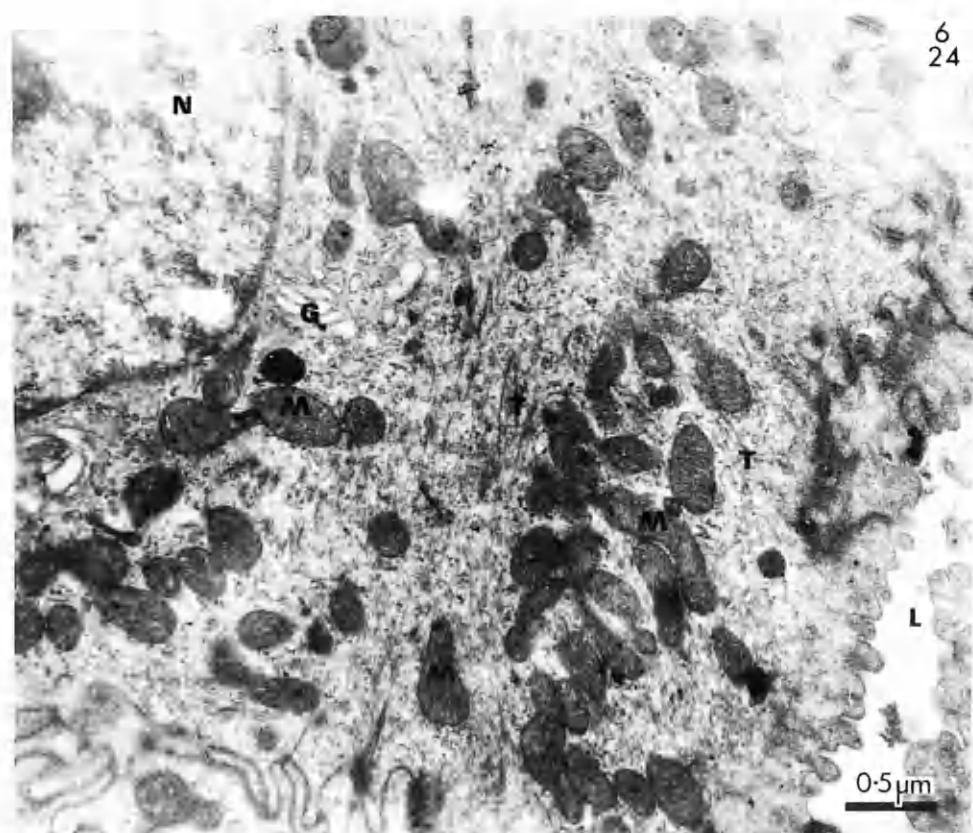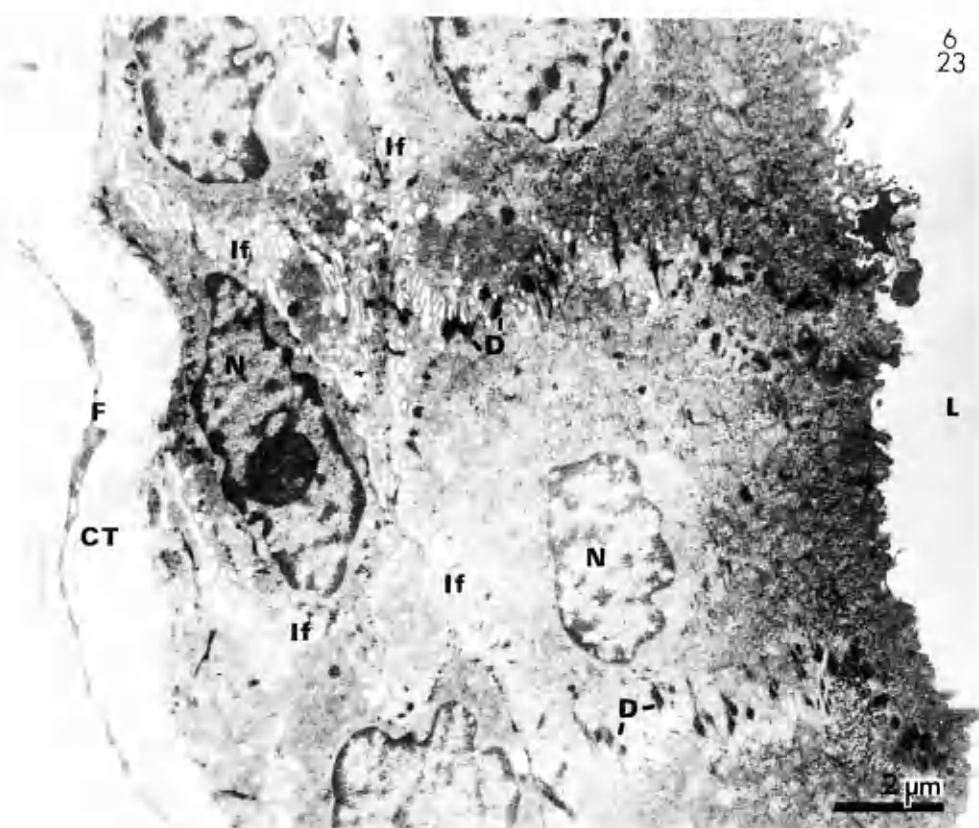
cells in this ductal region.

Transition zone.

The short transition zone was generally three to six cells in length. The wall of the duct was usually two cell layers in thickness (Fig.6.25) although in places luminal cells were observed reaching and touching the basal lamina (Fig.6.26). The basal lamina was more prominent than that of the remainder of the duct and comparable in thickness to that of the fundus. The luminal cells had sparse microvilli, contained fewer mitochondria and were flatter than those of the coiled duct. The cells were more reminiscent of non granular fundus cells than of duct cells. The interdigitations between cells were not as prominent as the basal infoldings of the fundus or the interdigitations of the luminal cells of the coiled duct. Myoepithelial cells now formed the basal layer (Fig.6.26).

Fig.6.25. Transition zone of the the unstimulated duct. The wall·of the duct is now two cell layers in thickness. The basal layer now formed by myoepithelial cells.

Fig.6.26. Luminal cell of the transition zone reaching and touching the prominent basal lamina (arrows). The luminal cells have few microvilli and contained fewer mitochondria than the neighbouring coiled duct. Basal interdigitations in this zone are not as prominent as the basal infoldings of the fundus or interdigitations of the coiled duct.

2 μm

1 μm
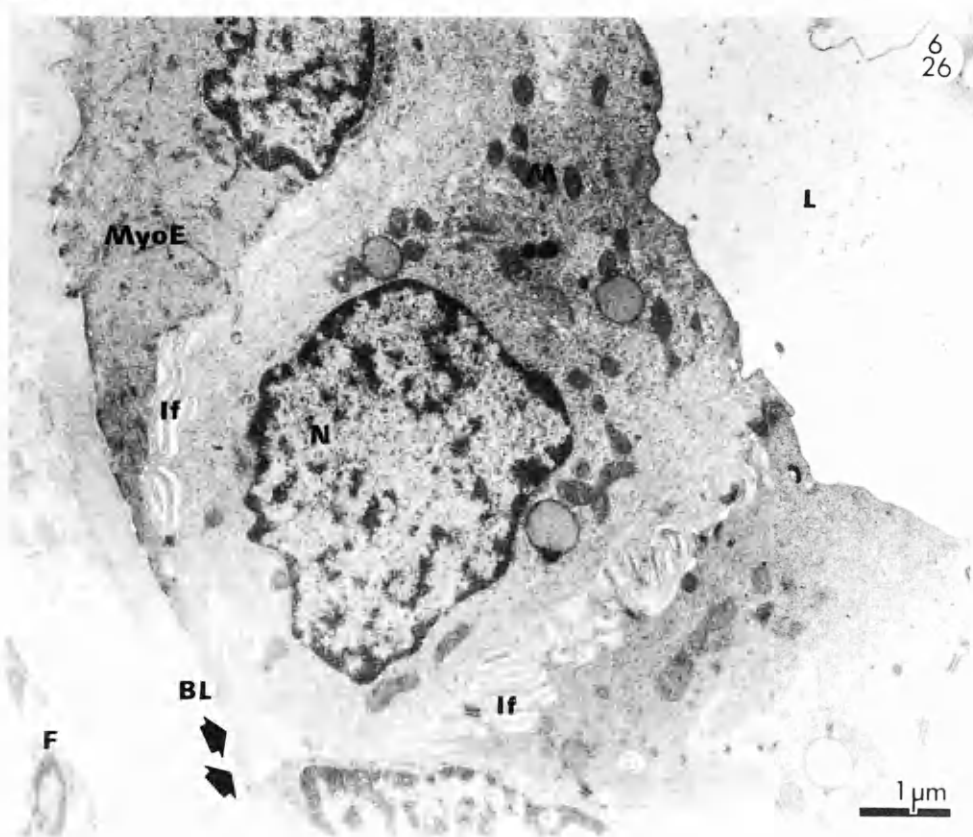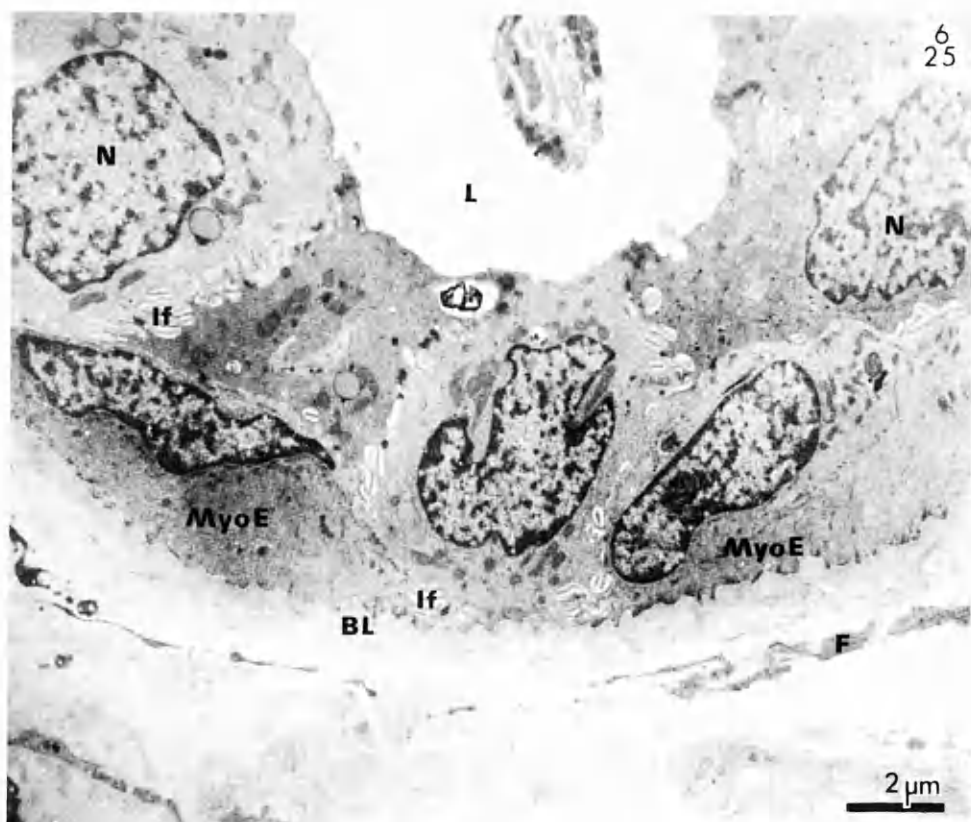
Effects of stimulation.

Onset of sweating.

Fundus.

The fundus of the active gland (Fig.6.27) differed in appearance from the unstimulated gland even when examined by light microscopy. Soon after the onset of sweating there was an apparent thickening of the basal lamina and vacuoles were seen within the secretory epithelium, usually associated with a decrease in the size of the lumen.

Electron microscopy illustrated that the smaller lumen at onset contained a fine filamentous material (Fig.6.27) and cell debris. The myoepithelial cells were taller and, judging by the puckering of their serosal surfaces, appeared to be under tension (Fig.6.28). A prominent feature of the secretory epithelium was the dilatation of the intercellular spaces, particularly the

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Fig.6.27. The appearance of the fundus at the onset of sweating. Some granular cells exhibit evidence of granule depletion and the dilatation of the basal infoldings between non-granular cells is striking.

Fig.6.28. High power view of the base of the fundus at the onset of sweating. The taller myoepithelial cells have puckering on the serosal surface indicative of tension. In this section the basal infoldings, although wider than in control sections, are not greatly distended.

6
27

L

GC

GC

L

N

NGC

BI

BI

MyoE

BL

4 μm

6
28

GC

N

MyoE

BI

BL

MyoE

BI

CT

2 μm
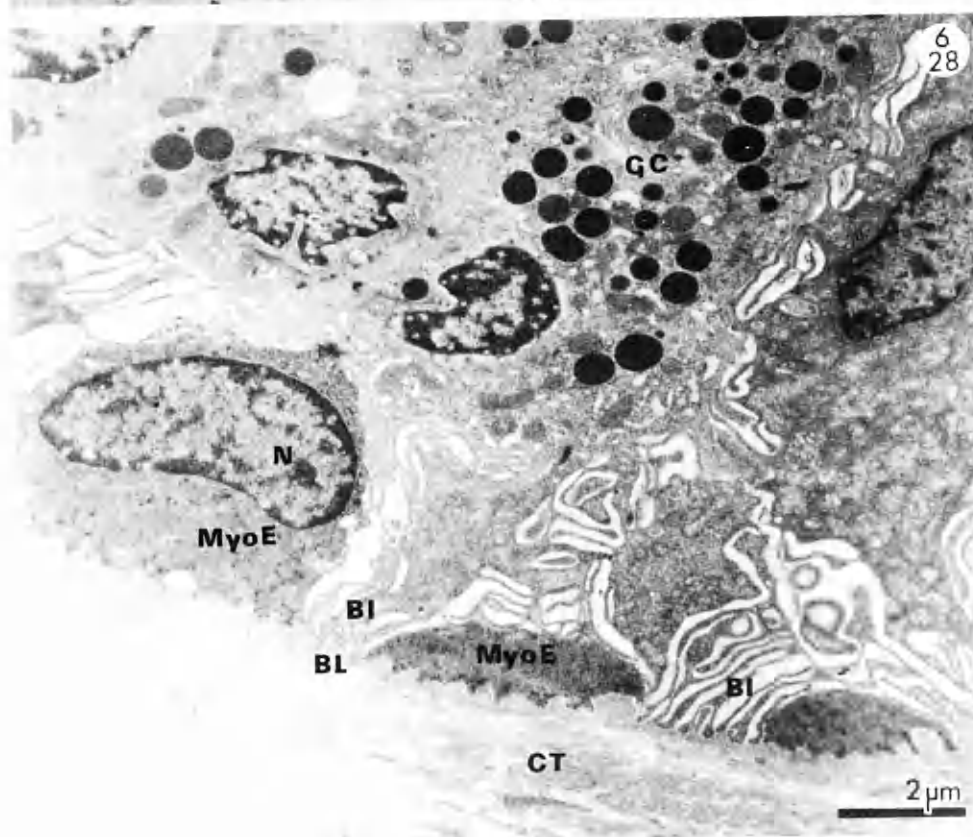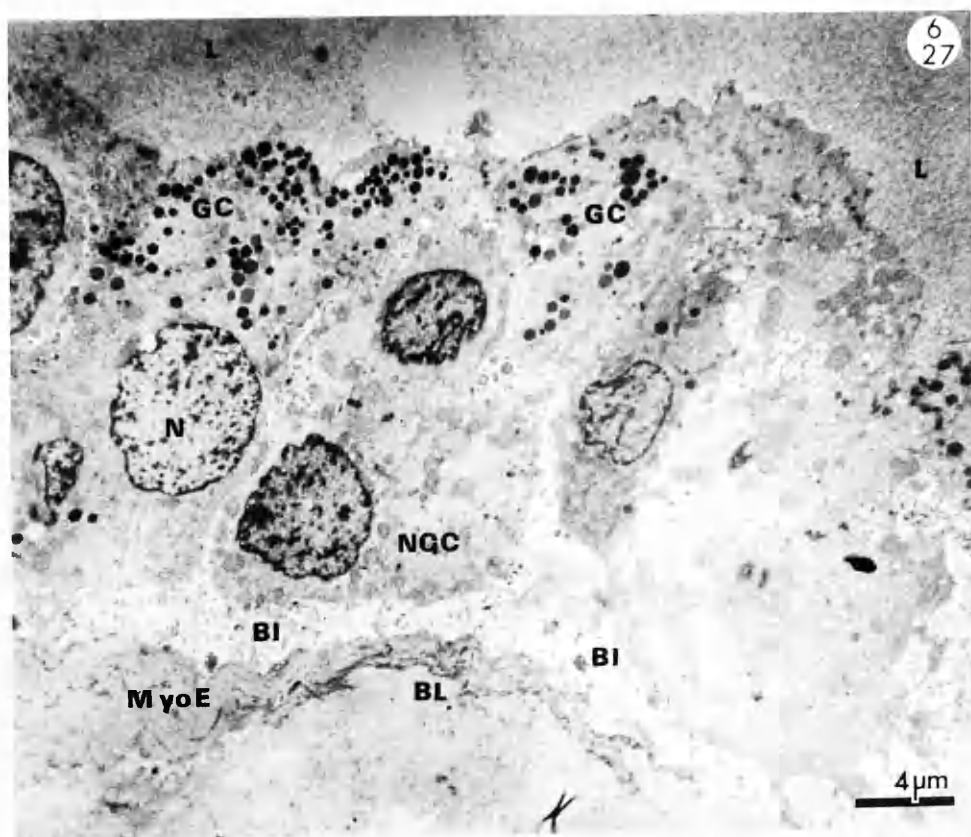
basolateral infoldings (Fig.6.29) and canaliculi between
non-granular cells. The basal infoldings were generally,
but not always, markedly distended. The cytoplasm of the
now more cuboidal non-granular cells contained less
glycogen and had a foamy appearance as a result of SER
distension. Cytoplasmic vesiculation was particularly
pronounced towards the lumen and adjacent to canaliculi.
Mitochondria exhibited translucent zones in their
matrices. There were some non-granular cells within the
secretory epithelium, identified by their characteristic
mitochondria and proximity to canaliculi with large
deposits of glycogen in their cytoplasm (Fig.6.30). Cells
with this appearance which still contained vesicles and
lipid droplets were also occasionally found in the
unstimulated gland.

The intercellular spaces between the now columnar
granular cells, although also distended, were not as
prominent. The short lengths of RER within the cytoplasm
of these cells had dilated but the Golgi apparatus was

Fig.6.29. This portion of the basal epithelium at onset
illustrates marked dilatation of the basal infoldings and
the presence of mitochondria in close proximity to the
projections.

Fig.6.30. A dark cell containing lipid droplets, small
vesicles and abundant glycogen, adjoining a canaliculus in
the secretory epithelium of a fundus at the onset of
sweating. Cells of this type were also present in samples
taken after 3 hours of continuous sweating and
occasionally detected in unstimulated glands.

6
29

NGC

N

BL

Bl

Bl

CT

MyoE

2 µm

6
30

V

Ca

GC

BL

Ca

MyoE

V

Gly

2 µm
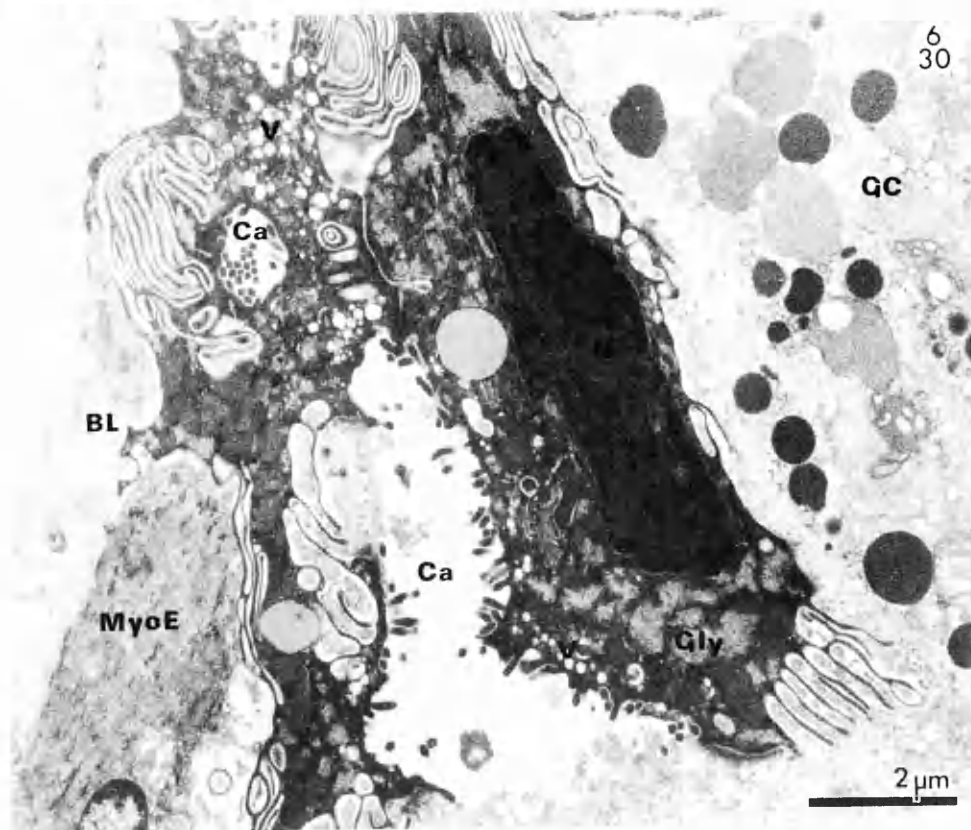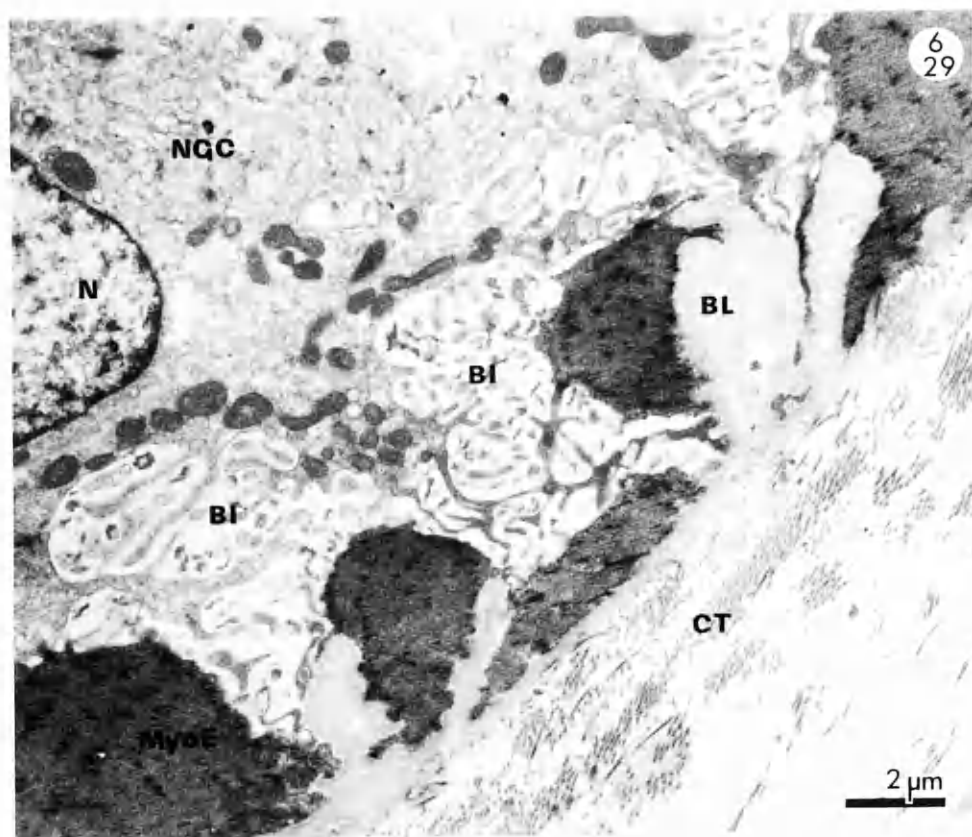
still not prominent. In all of these cells there was an accumulation of the now more spherical granules at the apical surface (Fig.6.31) and in some there was evidence of granule depletion. A limiting membrane was observed more easily around the paler granules than around the darker ones in neighbouring cells. Granule release to the lumen appeared to be mainly by a process of exocytosis. Although no granules were caught at the exact moment of liberation, frequent examples of configurations consistent with this secretory process were seen (Fig.6.32 inset). Evidence of granule loss by a microapocrine process were also seen (Fig.6.32) and some were released into the lumen along with other cytoplasmic constituents after complete

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Fig.6.31. Granular cell at the onset of sweating illustrating accumulations of the now more spherical granules at the apical surface.

Fig.6.32. The luminal surface of a fundus at the onset of sweating illustrating the probable modes of granule transport to the lumen. The inset shows a commonly observed appearance where the luminal membrane forms an open cup, most likely the result of exocytotic granule release. However, granules were in some instances, as shown, exuded from disrupted cells along with the cytoplasmic contents. Configurations reminiscent of a microapocrine process were also occasionally observed.
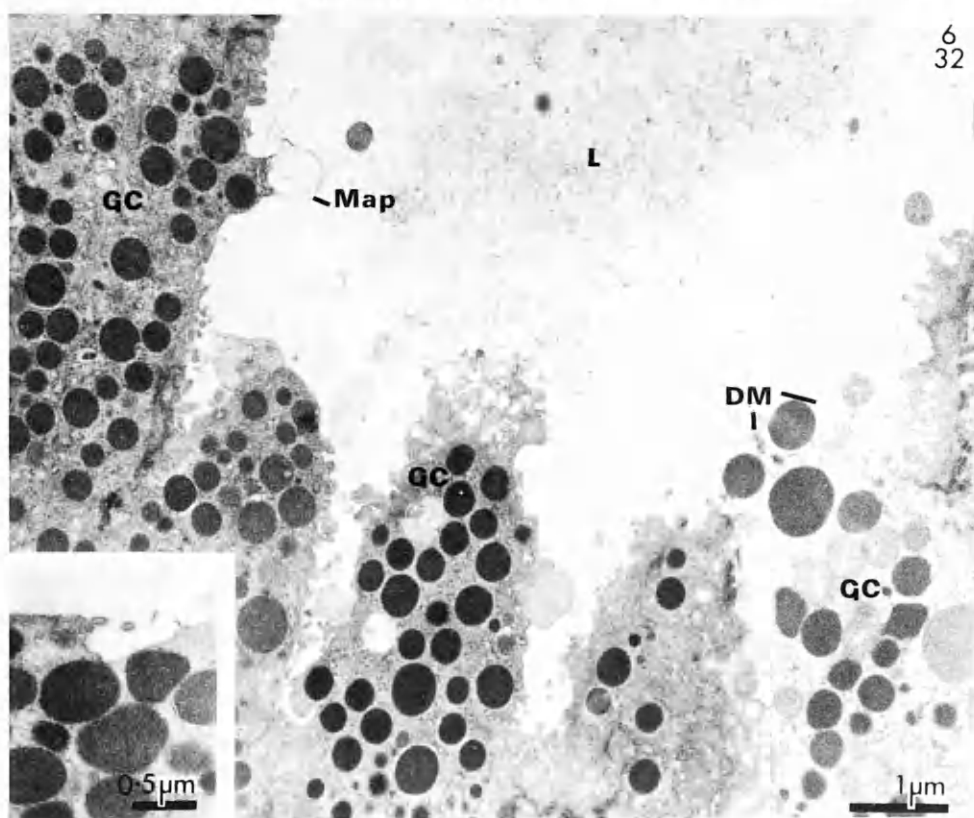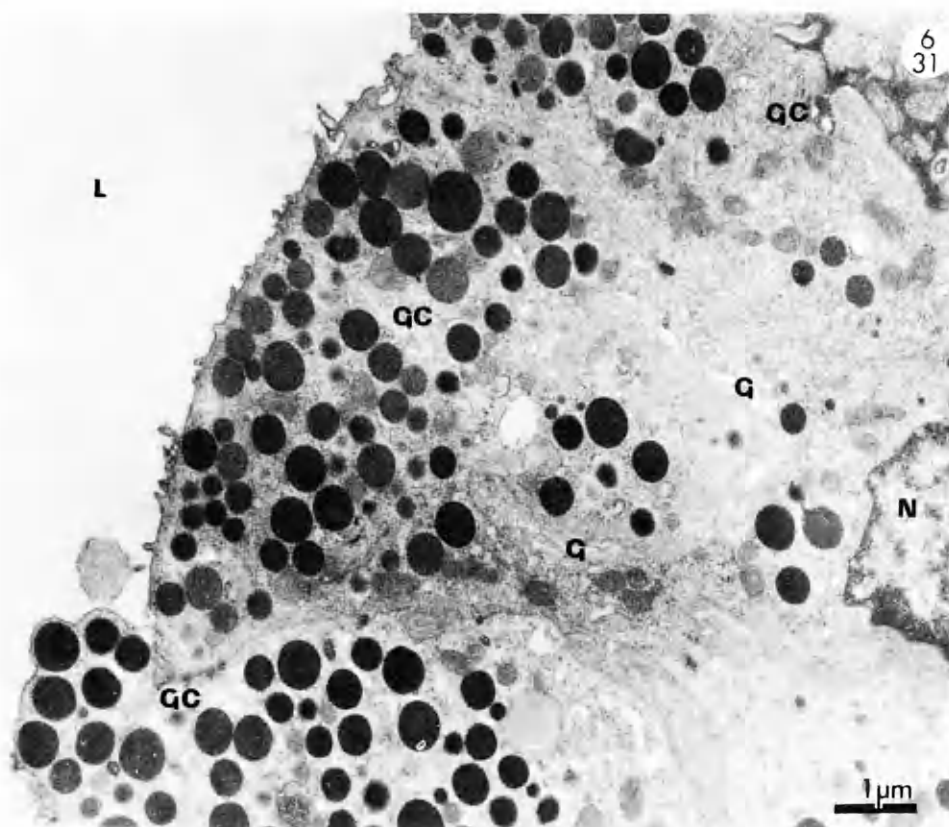
disruption of the luminal membrane (Fig.6.32,33).
Cytoplasmic protruberances at the luminal surface of these
cells were also occasionally observed (Fig.6.34).

Prolonged sweating.

After more than three hours of continued sweating the
glands appeared much less contracted. The lumen
considerably larger and cell vacuolization was more
pronounced. The myoepithelium, although still taller than
in the unstimulated glands lacked the basal crenations,
but still contained glycogen. The secretory epithelium was
apparently less stratified and the cells more cuboidal.
The now wider lumen contained cellular debris including
lengths of membrane. The non-granular cells were almost
completely devoid of glycogen and had pale mitochondria.
The spaces and canaliculi between them were visually
narrower than at the onset of sweating, although they were
still wider than the unstimulated gland. These cells
maintained their contact with each other and the basal
lamina and still appeared to be actively secreting. The
cells, rich in glycogen, adjoining canaliculi were also
found in sections taken at this stage of activity. The RER
of the now cuboidal granular cells was less dilated and
the Golgi apparatus was not prominent. The cells still
showed signs of granule transport to the lumen by

Fig.6.33. Disruption of the luminal membrane of a granular
cell at the onset of sweating. The lumen contains
cytoplasmic debris.

Fig.6.34. Cytoplasmic projections at the luminal surface
of granular cells during sweating.
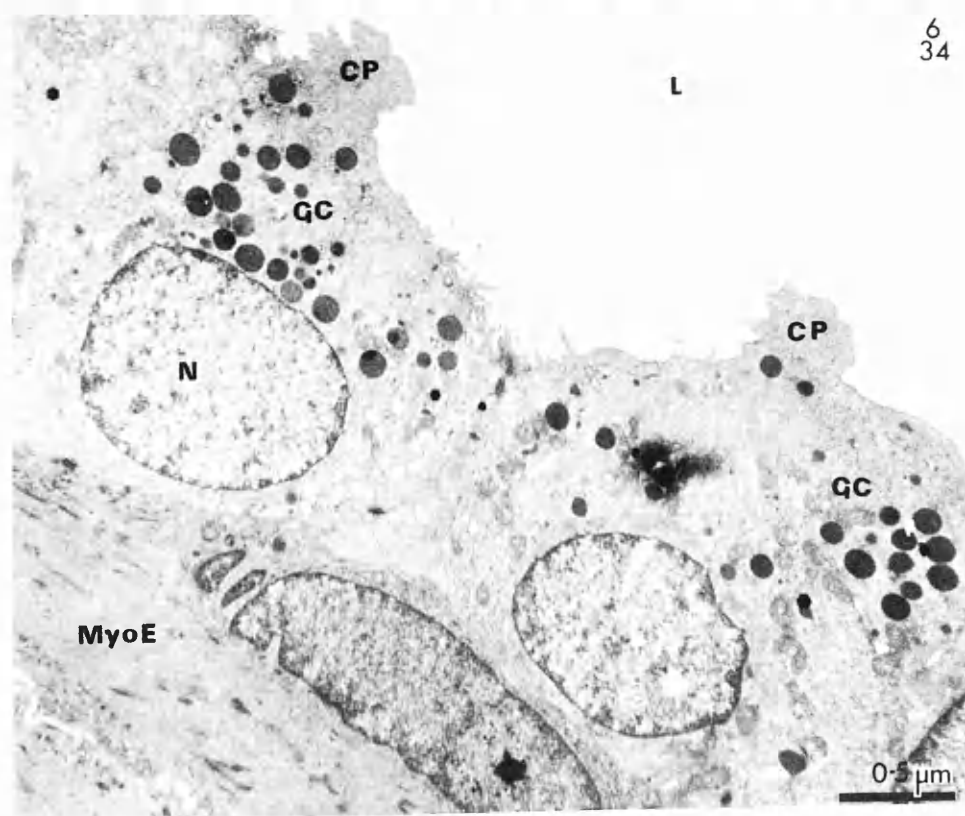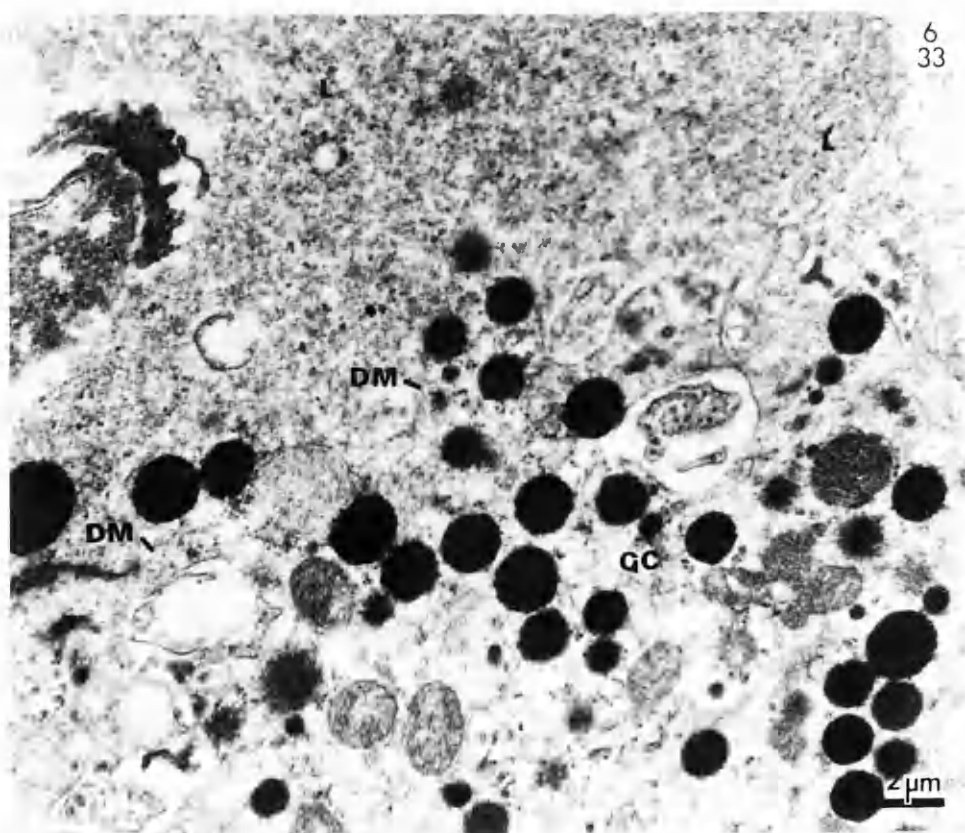
exocytosis and occasionally by microapocrine secretion. The granules were concentrated at the luminal portion of the cells and although the number in some cells was reduced there was no evidence of complete degranulation. Cells with dark granules and others with pale ones were still observed.

Within the secretory epithelium, interspersed between the different cell types at irregular intervals were dying and dead cells or their vestiges. These resulted in gaps in portions of the epithelium extending from the lumen to the basal lamina (Fig.6.35,36). It was impossible to determine with certainty whether these resulted from the death of granular or non-granular cells or both.

Duct.

Active intraepidermal zone.

In this zone the spaces between the cells were dilated when compared with the unstimulated ducts. The most noticeable changes in the upper portion of the intraepidermal zone of the duct were an increase in the number of membrane bound vesicles in the lumen. These vesicles were associated with the microvilli of the

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Fig.6.35. In this section from the fundus after 3 hours of sweating a noticeable feature is the presence of atretic cells. These atretic cells disintegrate and lose their apical zonulae occludentes and luminal cell membranes. Fig.6.36. Disintegration of the atretic cells causes gaps in the secretory epithelium, where the basal lamina is exposed to the lumen and hence a loss of epithelial integrity results.

81

6
35

GC

Ca

MyoE

L

CR

N

GC

CR

2 μm

L

6
36

MyoE

BL

0·5 μm
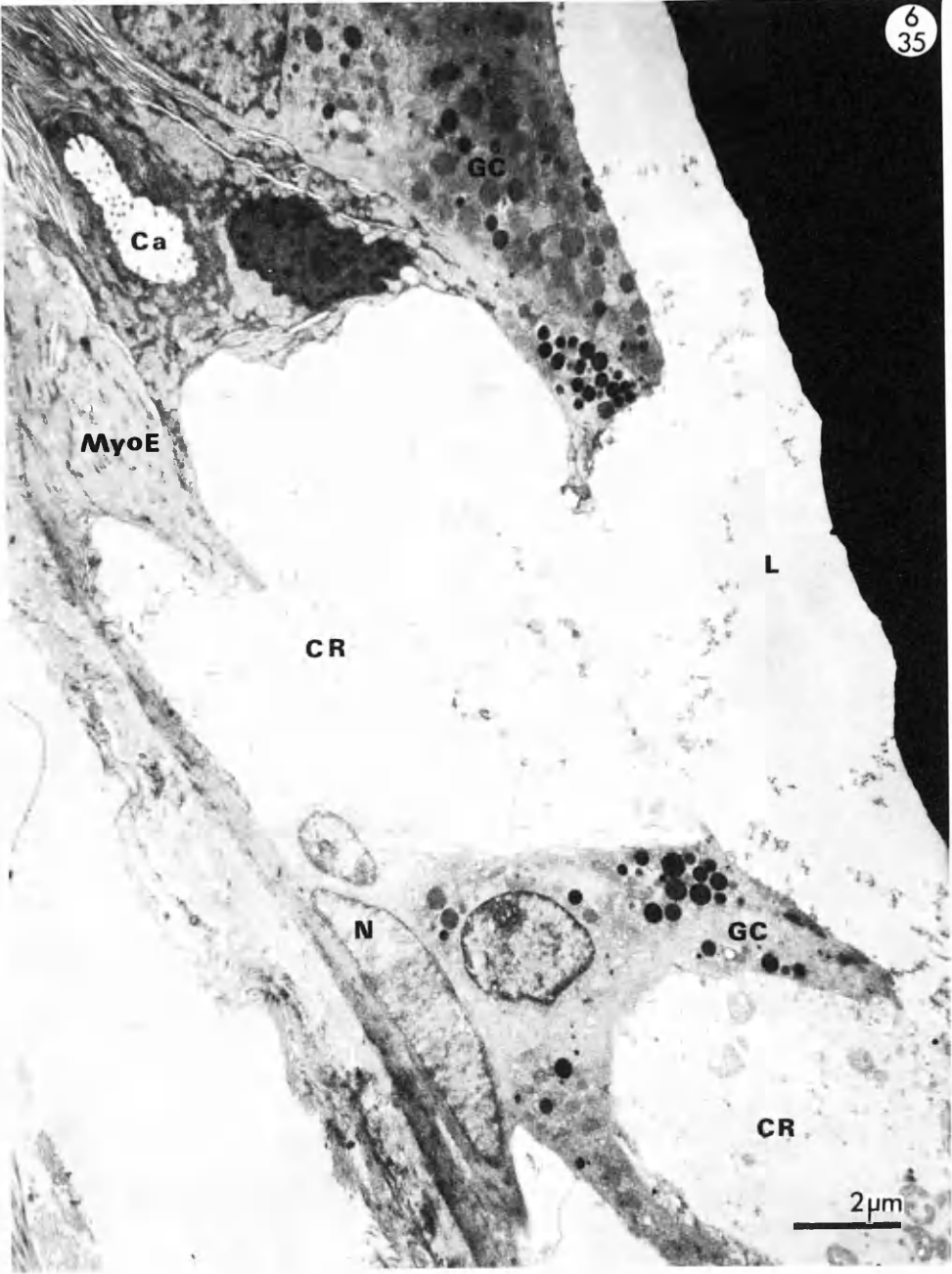
fingers of the non-keratinized cells deeper within the
duct and also in the quantity of debris in the lumen.

Active upper ascending zone.

During sweating there was dilatation of the
intercellular spaces (Fig.6.37) and a narrowing of the
lumen, which is often packed with electron-opaque material
including cell debris (Fig.6.38). Large, rounded,
electron-opaque bodies were sometimes observed associated
with the tonofilaments of the terminal web (Fig.6.38).
There was also an increased number of the membrane-bound
vesicles adjacent to the surface microvilli and the
vesicles within the cytoplasm of the luminal cells were
more numerous and tended to be larger (Fig.6.39). Small
vesicles were also occasionally observed within the basal
cells adjacent to the intercellular spaces.

Fig.6.37. The upper ascending duct during sweating. The
intercellular spaces are dilated and a terminal web is
present in the luminal cells.

Fig.6.38. Electron-opaque body at the base of the terminal
web of a luminal cell. This structure, which has no
limiting membrane, was observed only during sweating.

Fig.6.39. Vesicles within the cytoplasm of luminal cells
were more numerous and larger than in the unstimulated
duct. There was also an increased number of membrane-bound
vesicles adjacent to the surface microvilli.

<u>Active lower ascending duct</u>.

The response to sweating was similar to that found in the upper half of the ascending duct (Fig.6.40) except that the vesiculation at the basal cell margins was more pronounced.

<u>Active coiled duct</u>.

The appearance of the apical portion of the luminal cell changed considerably during sweating. The microvillous cytoskeletal architecture of the unstimulated gland (Fig.6.41) was no longer uniformly present and there was a condensation of the terminal web. The mitochondria, although apparently larger and more numerous, were generally not present within the web during sweating; they were still mainly perinuclear but with considerable concentrations at the base of the terminal web (Fig.6.42). Lysosomes and small electron-opaque granules were frequently observed in the cytoplasm (Fig.6.43).

The plasma membrane and microvilli of the luminal cells showed varying degrees of distortion. The microvilli of some cells were still present in places although the

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<u>Fig.6.40</u>. Active luminal cells from the ascending duct. Note the marked vesiculation in the cytoplasm.

<u>Fig.6.41</u>. Luminal cells from the unstimulated coiled duct. Microvilli are short and stubby. Vesicles, although present in the cytoplasm, are not prominent. The cell is rich in mitochondria.

<u>Fig.6.42</u>. The terminal web of this cell, from an active duct, is more electron-opaque. The luminal microvilli are in places (arrows) either distorted or absent.

L

V

LV

V

V

D

D

MV

V

0·5 µm

T

T

V

L

V

V

V

T

N

3 µm

6
41

6
42
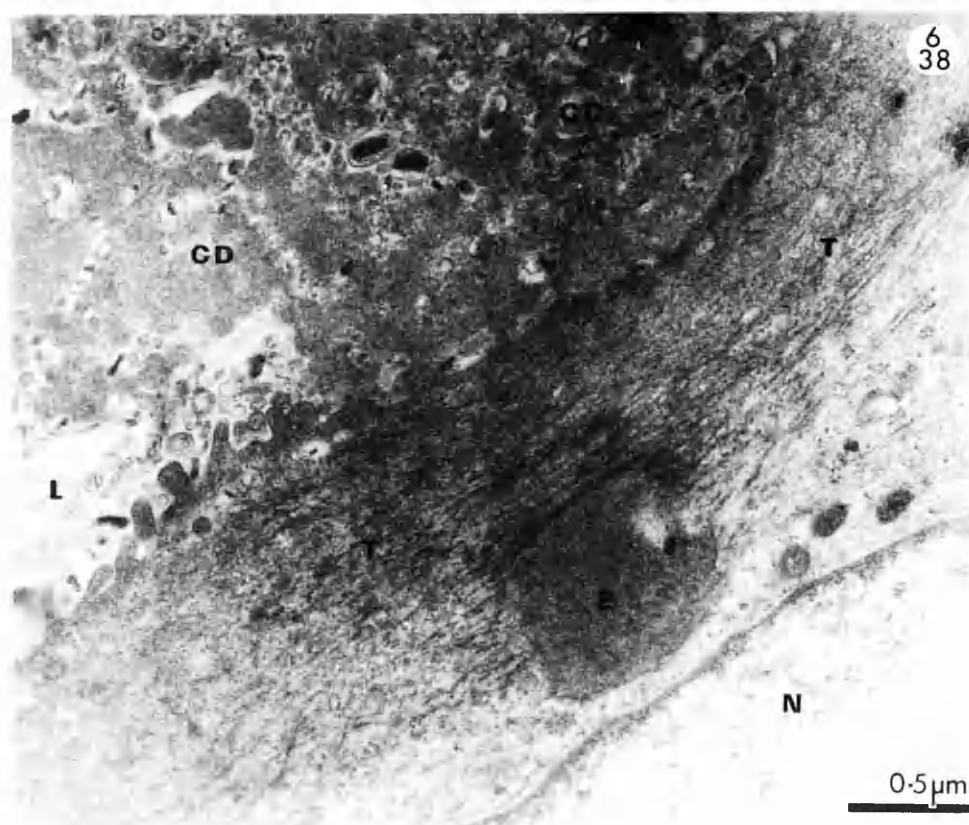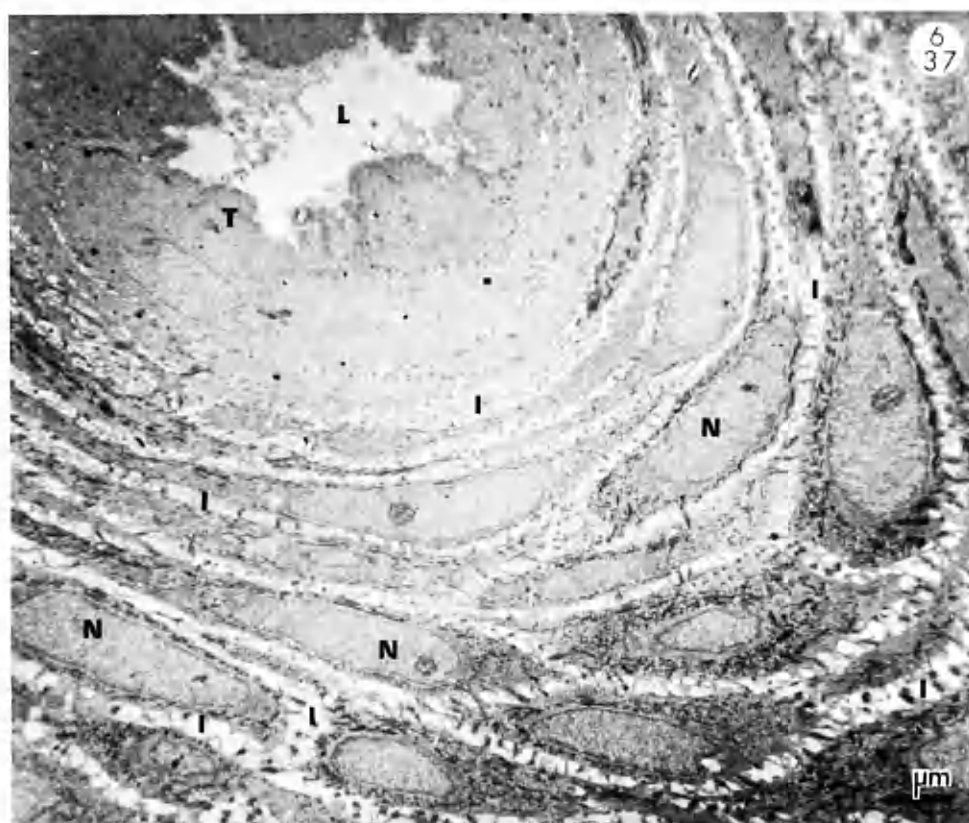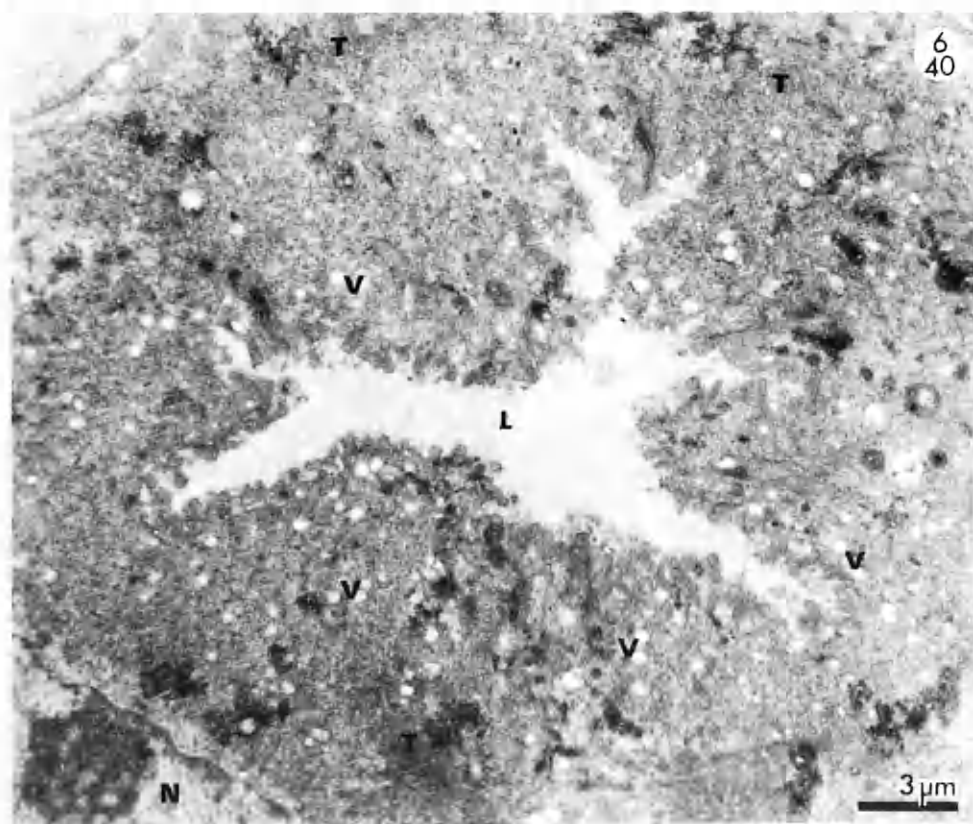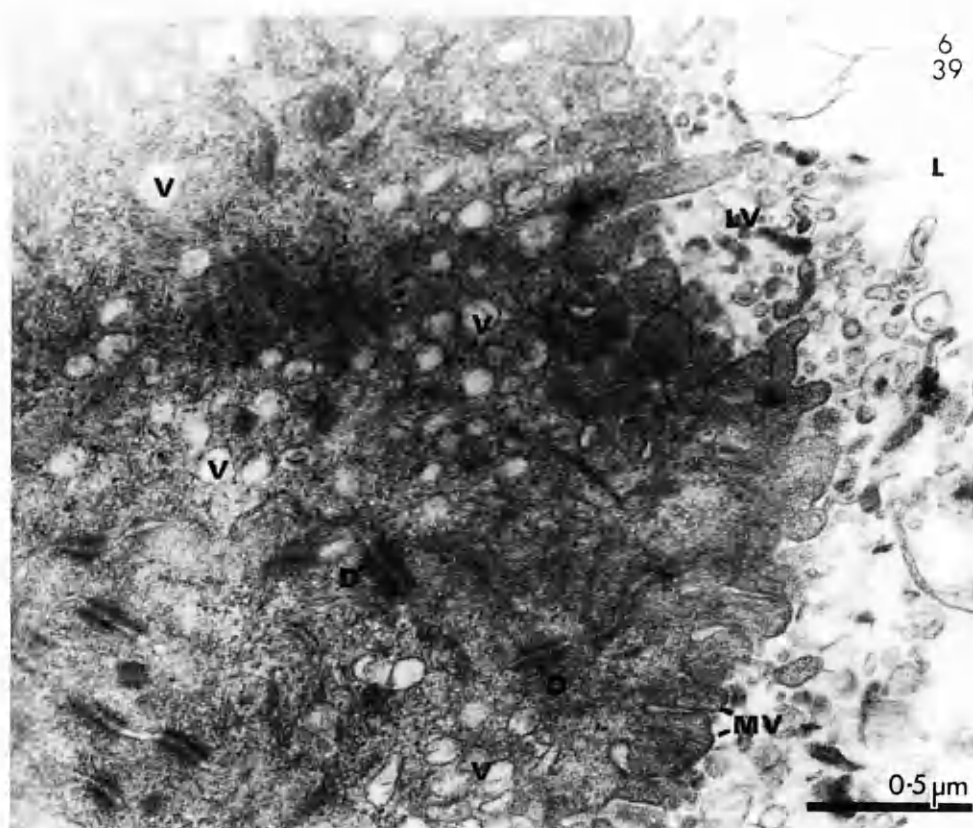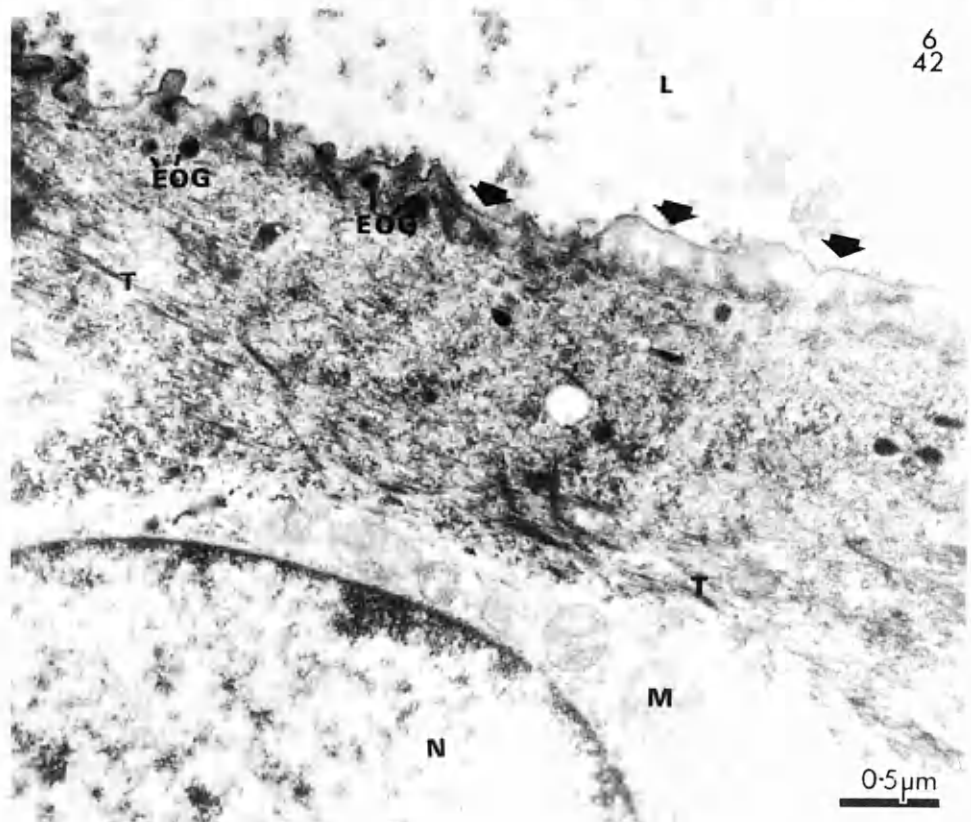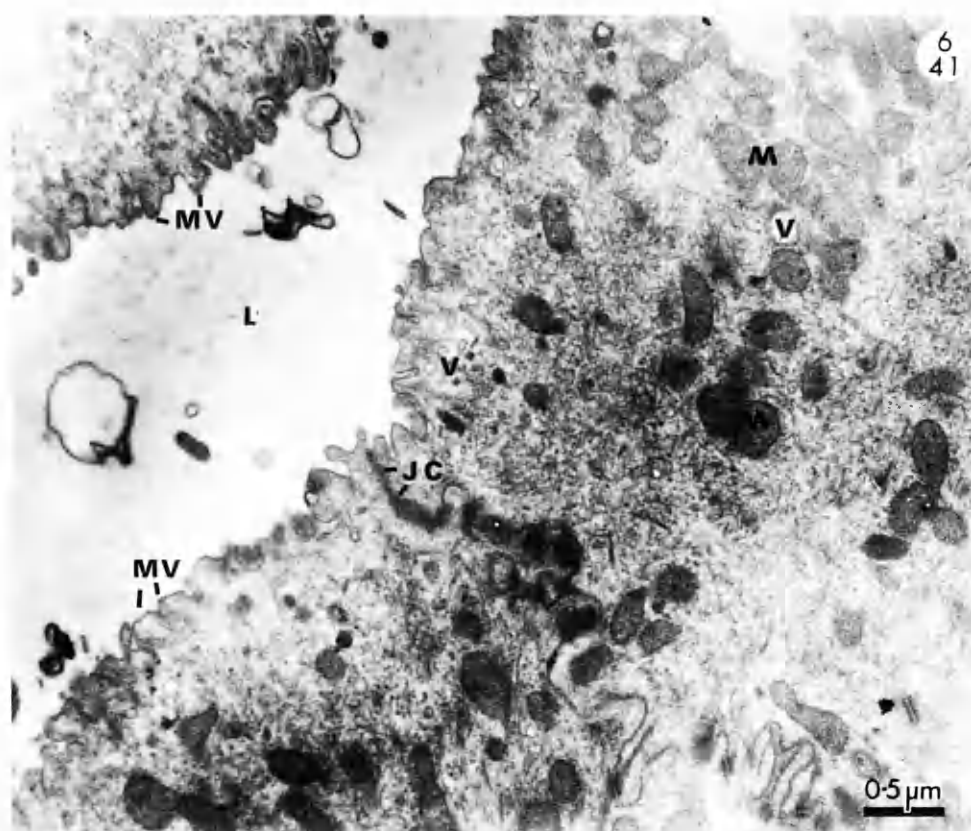
luminal surface was more undulating. Microvilli were absent from other regions. In other cells microvilli were no longer present along extensive portions of the membrane (Fig.6.42), which had become distended to form cytoplasmic protruberances (Fig.6.43). These surface swellings were often so large that they occupied much of the luminal space (Fig.6.44). The cytoplasm of these luminal cells contained structures consisting of one to four small, finely textured, electron-opaque granules surrounded by a common membrane, which were not observed in the unstimulated duct (Fig.6.43,inset). They were, however, not detected after prolonged sweating, although regularly found at onset. The number of vesicles in the basal cells increased during activity and in the dilated spaces between luminal and basal cells membrane-bound debris could be found.

Fig.6.43. In this cell, also from a duct during sweating, the mitochondria are again perinuclear. A portion of the luminal membrane is now greatly distorted and apical blebbing has occurred (arrows). Examples of the granule-containing organelles comprising 2-4 electron-opaque granules surrounded by a common membrane can be seen. Such structures were traced only in the stimulated glands.

Fig.6.44. In this instance sweating has resulted in distortion of the apical membrane to the extent that the blebs occupy a large proportion of the lumen. The terminal webs of the cells are prominent and the luminal cells are vesiculated.

6
43

6
44
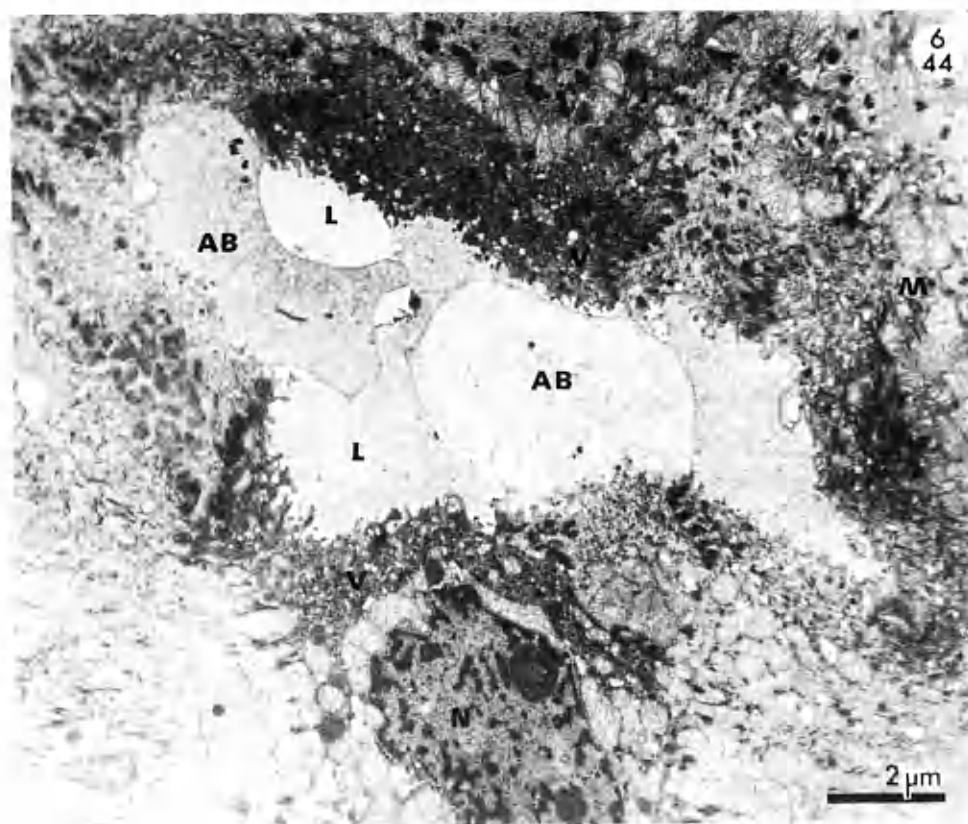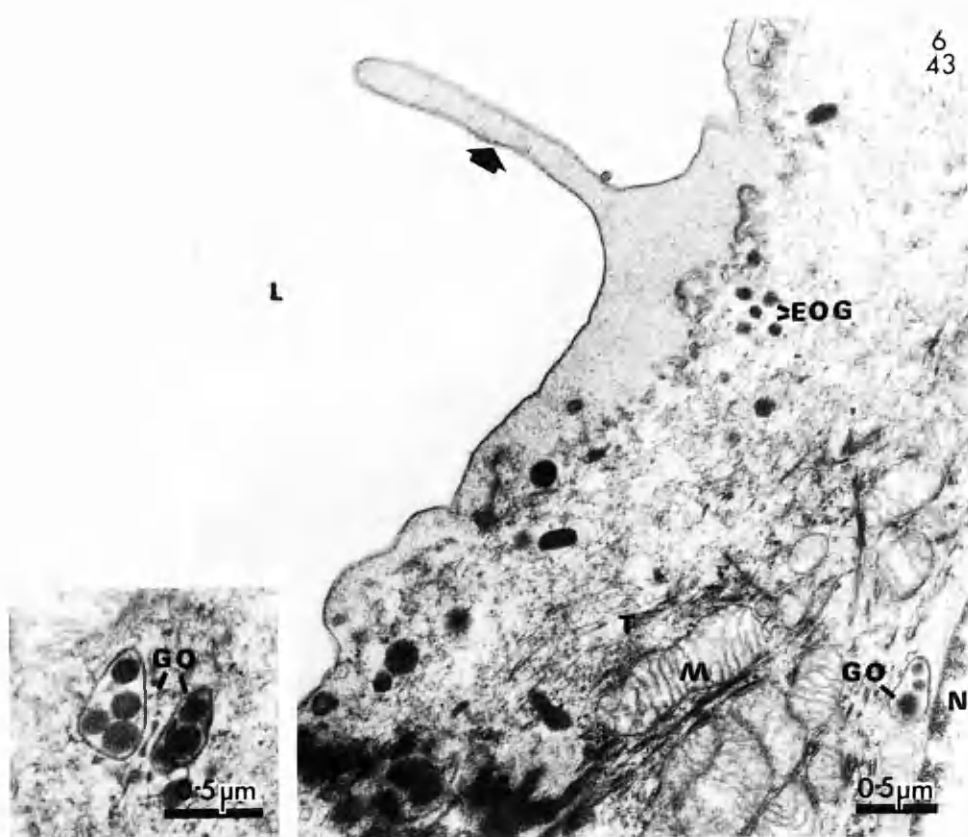
<u>Active transition zone</u>.

There was little visible change in appearance in the transition zone during sweating other than dilation of the intercellular spaces and increased numbers of membrane-bound vesicles in the lumen.

<u>DISCUSSION</u>.

The structure of the unstimulated eccrine sweat gland fundus as seen using light and electron microscopy , was in accord with previous findings (Dobson, et al., 1958; Ellis, 1962, 1965; Hashimoto et al.,1966a,b; Montagna & Parakkal, 1974; Hashimoto, 1978a; Kurosumi et al., 1982). Light microscopy also showed heat-induced changes identical to those described by Dobson et al., (1958) even although the skin samples for the present study were taken immediately after local anaesthesia. The human gland differed in the respect that in the cow, sheep, goat and horse, the fundus appeared to remain contracted during sweating (Chapter 3,4,5). Electron microscopy revealed that the sweat glands were seemingly contracted in all specimens examined during the early period of sweating and later were always dilated. The results show that human sweat like that of domestic animals is formed not only by secretion but also from the products of cell death. Two of the secretory processes displayed by the human gland, fluid transport and vesicle exocytosis are common to all the animal species studied and the third, microapocrine secretion, also occurs in the horse. The data illustrate that the fundamental processes of sweat formation in the human sweat gland are the same as those in haired animals

and it can thus be proposed that sweat is formed from the products of:

A.) Secretion. This mainly by fluid transport, vesicle exocytosis and a possible subsidiary microapocrine process.

B.) Cell death.

The contribution and efficiency of the different secretory processes clearly vary between species and in man secretory cell specialization appears to have evolved. Estimates of the contribution that degenerate cells make to sweat composition awaits comparative information on secretory cell production and turnover in both the unstimulated and active gland. However, the morphological evidence, while again showing species variability, indicates that in some instances at least cell death is either induced or accelerated upon glandular stimulation. The resulting influence on sweat composition is likely to be significant particularly in man, in view of the effects of disruption on the secretory epithelium.

The intercellular spaces between non-granular cells and the blind ended canaliculi which may well, as in many other epithelia, generate a standing ionic gradient (Diamond & Bossert, 1967) were, at the onset of sweating, more distended in some areas of the epithelium than in others, suggesting variability in the response to stimulation. When sweating continued for more than three hours there was no decline in the fluid output but there was a narrowing of the intercellular spaces between non-granular cells. These morphological changes may foretell the eventual secretory failure which Dobson et

al., (1958) described in instances of profuse sweating continued for longer periods than in the present study. A small number of the cells adjacent to canaliculi, which have mitochondria typical of the non-granular cells, contain accumulations of glycogen and appear to be less active. The role of these cells, if any, in the epithelium is unknown. They could represent the non-granular cells in an early stage of degeneration having failed to respond to stimulation. However, their nuclei were not degenerate and their membranes were still intact. They have vesicles, lipid droplets and dilated RER in their cytoplasm suggestive of activity, therefore, it is possible that they are specialized secretory cells since they are also found in the unstimulated gland.

The granular cells show less intercellular dilatation and are possibly more concerned with granule production than with fluid transport. The processes, which in domestic animals are carried out by one type of secretory cell, may well therefore have been devolved to specialized cells in the human eccrine gland. The response of the granular cells to stimulation was not uniform as some exhibited a greater degree of degranulation than others. However, they all demonstrated granule accumulation at the luminal membrane of the cell. The most likely source of the secretory granules is the Golgi apparatus (Kurosumi et al., 1982). The paler granules had a density comparable to their associated mitochondria, but evidence of mitochondrial transformation, as found in cattle, was not seen. The evidence particularly from the active gland supports the conclusion (Inque, 1979;

Kurosumi et al., 1982) that granule transport to the lumen
of the human sweat gland is by exocytosis; diacrine
secretion described by Hashimoto (1978a) was not observed.
The cytoplasmic protrusions, occasionally found at the
luminal surface of active cells possibly indicate
cytoplasmic extrusion by a microapocrine process but
another possibility in this instance is blistering of the
luminal membrane by fixation (Shelton & Mowezko, 1978).

After three hours of continued sweating, cell
remnants and even gaps were regularly found within the
secretory epithelium. It was impossible to identify with
certainty the cellular origin of these remnants since the
remaining organelles were disorganised and distorted.
These remains are unlikely to be solely from disrupted
granular cells as Dobson (1960), using light microscopy,
observed atrophy of the large pale cells after repeated
episodes of profuse sweating and the present study does
not exclude this possibility. The vacuolation observed by
Dobson et al., (1958) is most likely caused by disruption
and loss of both granular and non-granular secretory
cells.

Using electron microscopy it is clear that during a
single exposure to heat, portions of the secrtory
epithelium are breached, exposing the basal lamina. In
such regions the apical junctional complexes have been
eliminated leaving no visible barrier to fluid exchange
with the surrounding connective tissue. Some general
distortion of the secretory coil and cellular atrophy can
still be found by light microscopy 24 hours after
thermally induced sweating (Dobson & Lobitz, 1958).

Therefore epithelial cell replacement is likely to be slow with cell contacts not being rapidly re-established. In this respect human eccrine sweat glands appear to differ from those of domestic animals, in which the neighbouring cells rapidly interconnect after cell death during sweating preventing the passage of intercellular lanthanum into the lumen (Jenkinson et al., 1983).

The results, in the human loin, do not confirm the concept of a uniformly structured duct wall of two or three cell layers extending from the coiled portion to the intraepidermal zone (Hibbs, 1958; Ellis, 1962; Hashimoto et al., 1966a; Shibasaki & Ito, 1967; Hashimoto, 1978a; Kurosumi et al., 1982). Kurosumi (1977) sometimes found more than two cell layers in the duct of glands from the external auditory meatus or retroauricular region. It therefore seems likely that either serial sectioning has revealed a more complex ductal structure than previously found, or there are regional variations in the morphology of the duct. As in other species (Chapter 3,4,5) Langerhans cells were found between ductal cells, particularly in the intraepidermal zone and upper ascending duct. The importance to the ascending zone of specialized intercellular connections is still unknown; if present elsewhere in the duct, they are not readily found. It is possible that these connections might be involved in the intercellular passage of fluids, particularly if, as suggested by Hashimoto (1978a), the projecting luminal intraepithelial fold acts as a valve controlling the passage of preformed sweat. The function of the cytoplasmic bridges between the basal cells of the duct,

also noted by Ito, Enjo and Uchida (1954) using the light microscope, is still unknown.

The finding that myoepithelial cells are absent from the short transition zone is supported by Shibasaki and Ito (1967) and Hashimoto (1978a). The specialized sphincter described by Kurosumi et al., (1982) in the initial segment and the bundles of filamentous material in the luminal cells, resembling dragonfly wings were not found.

Sweating brought about three changes along the entire duct.

1.) A widening of the intercellular spaces, except at the junctional complexes.

2.) Increased cytoplasmic vesiculation with an accompanying rise in intraluminal vesicles.

3.) The presence in the often narrow lumen of particulate matter.

These were the only detectable differences also found in the short transitional and lower intraepidermal zones, which are therefore unlikely to be major regions of electrolyte and water reabsorption (Kurosumi et al., 1982)

The greatest change in ductal morphology upon thermal stimulation occurred in the coiled duct, where some of the luminal cells displayed varying degrees of apical distension. These blebs were not seen in the unstimulated duct. Although the appearance may have been influenced by the glutaraldehyde fixation, which can induce blistering of cell membranes (Shelton & Mowczko, 1978; Sletyr & Robards, 1982), including those of the sweat gland (Kurosumi et al., 1982; and the present study), there is

little doubt that a change in the morphology of the luminal membrane and apical cytoplasm occurs during sweating. The impression obtained is of distension of the plasma membrane and distortion of the microvilli by cell fluid filtering through the apical terminal web of microfilaments. The presence among the luminal contents of isolated membranous configurations similar in appearance, suggests that they are the expression of a secretory process. The luminal blisters may well give rise to the vacuolization of the active epithelium of the coiled duct seen by light microscopy (Dobson et al., 1958).

The dark and clear vesicles observed by Shibasaki and Ito (1967), amongst the tonofilaments of the luminal cells of the coiled duct, were also seen and found to be more numerous in the active coil. The glands examined by Shibasaki and Ito (1967) were probably active, at a low level, as the authors found occasional intercellular dilations. The coiled duct, a region of electrolyte absorption, is probably also an area of secretion due to its mitochondria rich cells and the blebbing process. The origin of the debris in the dilated areas between the cells of this zone is not known. It is probable that, as found in the fundus of sheep and goat (Chapter 4), the enclosed cell debris will be phagocytosed by the duct epithelium. Interpretation of the method of cell replacement in the sweat gland fundus and duct together with the contribution to the sweat constituents of cell disruption requires data on cell kinetics under varying physiological conditions.

CHAPTER 7.

CYTOCHEMISTRY.

INTRODUCTION.

The ultrastructural studies illustrate that the mode of sweat secretion is basically the same in all the species examined. Sweat is formed by secretion (fluid transport, exocytosis and possible microapocrine secretion) and cell death. In order to explain the differences between the patterns of sweat output (Figs.3.1.,4.1.,5.1.,6.1.) it is necessary to examine functional aspects of sweat secretion. Fluid transport involving the movement of electrolytes and water by active transport across epithelial boundaries is now regarded as being driven in most instances by a ouabain sensitive, sodium and potassium dependent adenosine triphosphatase (Na,K-ATPase) (Skou, 1965). The ultrastructural localization of this enzyme in sweat glands at rest and during thermal stimulation is of importance in establishing the cellular route of electrolytes and water across the secretory epithelium. Na,K-ATPase has been localized by immunocytochemistry in kidney (Kyte, 1976a,b) and retina (Stahl & Baskin, 1984) and by autoradiography using tritiated ouabain on intestine (Stirling, 1972). Quinton and Tormey (1976) using tritiated ouabain on isolated sweat glands from the volar surface of the male human forearm, localized the enzyme on the basal and lateral membranes of the secretory epithelium of the fundus.

The Wachstein and Meisel (1957) technique for the localization of ATPases has fallen out of favour because of two inherent weaknesses. The capture agent, lead, is toxic and ATP as a substrate offers no selectivity for Na,K-ATPase (reviewed by Firth, 1978; Ernst & Hootman, 1981). Ernst (1972a,b) using the avian salt gland developed a cytochemical technique for the localization of the enzyme. This technique, using strontium as the capture agent and p-nitrophenyl phosphate as substrate, demonstrates the K-dependent p- nitrophenylphosphatase (K-NPPase) activity of the Na,K-ATPase complex and it shows the appropriate ion dependence and inhibitor sensitivity. It also has the benefit that the specimens can be chemically fixed then processed after the completion of the experiment (Ernst & Philpott, 1970). The technique has been used successfully on a range of tissues, including, kidney cortex (Ernst, 1975; Ernst & Schreiber, 1981), placenta (Firth, Farr & Koppel, 1979) blood platelets (Cutler, Feinstein & Christian, 1980) and salivary glands (Cossu, Lantini, Puxeddu & Riva, 1984; Speight & Chisholm, 1984). When a positive reaction was obtained, this technique satisfied the criteria suggested by Ernst and Hootman (1981) that:-

1.) Deposition of the cytochemical reaction was potassium dependent and inhibited by ouabain.

2.) The reaction product was still present when the alkaline phosphatase inhibitor, levamisole, was present in the incubation substrate (Firth, 1974; Hootman & Philpott, 1979).

3.) The reaction products attributable to K-NPPase should be localized to the cytoplasmic side of the plasma membrane (Sen & Post, 1964).

4.) The reaction product should be localized to membranes and not be confused with non specific binding on other tissue components.

5.) Tissue ultrastructure should be preserved without an excessive loss of K-NPPase activity.

GLANDULAR Na,K-ATPase.

   Trials.

   Before experiments were attempted in the environmental chamber a series of trials was undertaken to determine the efficacy of the Na,K-ATPase technique (Ernst 1972a,b) described in appendix 2.

   Rat Kidney and Footpad Sweat Gland.

   The initial trial was carried out on kidney as the result was already known (Ernst, 1975) and the footpad sweat gland as it is an available model for other experimental animals.

   Result.

   The enzyme distribution in the kidney cortex was similar to that found by Ernst (1975). The distal convoluted segment reacted intensely on the cytoplasmic side of the highly folded basal membranes (Fig.7.1), whereas the proximal convoluted segments (Fig.7.2) were barely reactive. In the sweat gland fundus the reaction

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Fig.7.1. Rat kidney cortex: Distal convoluted tubule with intense reaction product on the cytoplasmic side of the highly folded basal membrane.

94

product was localized on lateral and basolateral membranes
of the secretory cells (Fig.7.3,4,5). No reaction product
was found on the luminal microvilli of the secretory cells
or the membranes of the myoepithelial cells (Fig.7.3). In
concomitant controls the reaction product in the footpad
sweat gland was potassium dependent and inhibited by
ouabain. The results of the control kidney samples again
agreed with Ernst (1975), rat kidney K-NPPase was
potassium dependent and sensitive to ouabain. The
technique was repeated on the kidneys and footpad sweat
glands of another two rats, with the same result, to
verify its reliability and improve technical dexterity in
sample processing.

The Na,K-ATPase technique was again successful on Rat
kidney cortex and footpad sweat gland so it was then
applied to the sweat glands from the required species.

Fig.7.2. Rat kidney cortex: Proximal convoluted tubule
with no reaction product present on the highly folded
basal membrane

Fig.7.3. Rat footpad sweat gland fundus: Low power
electron micrograph illustrating reaction product on the
basal and basolateral membranes of the secretory
epithelium.

Fig.7.4. Rat footpad sweat gland fundus: Reaction product
on the apical lateral membranes between secretory cells.

Fig.7.5. Rat footpad sweat gland fundus: Reaction product
on the basal infoldings of a secretory cell.

7
1

M

L

N

M

0·5μm

7
2

M

M

M

BL

Cap

0·5μm

73

74

75

L

SE

SE

N

MyoE

BI

BI

2µm

L

SE

SE

N

0·5µm

N

SE

BI

0·5µm
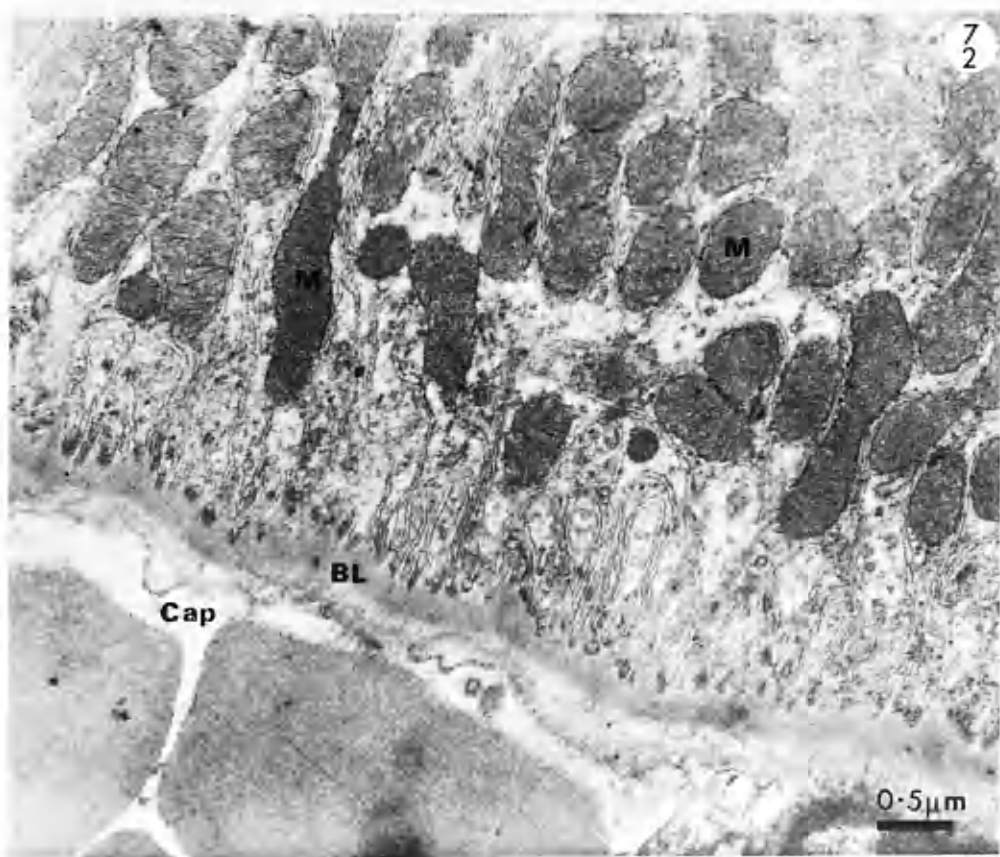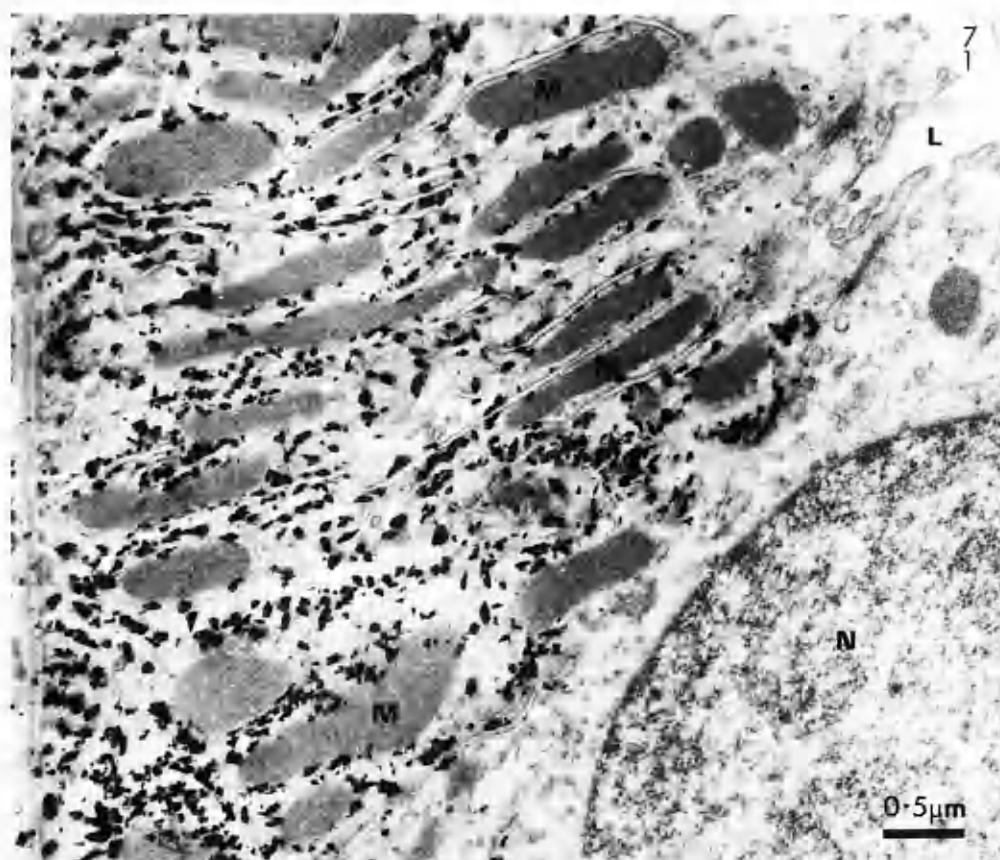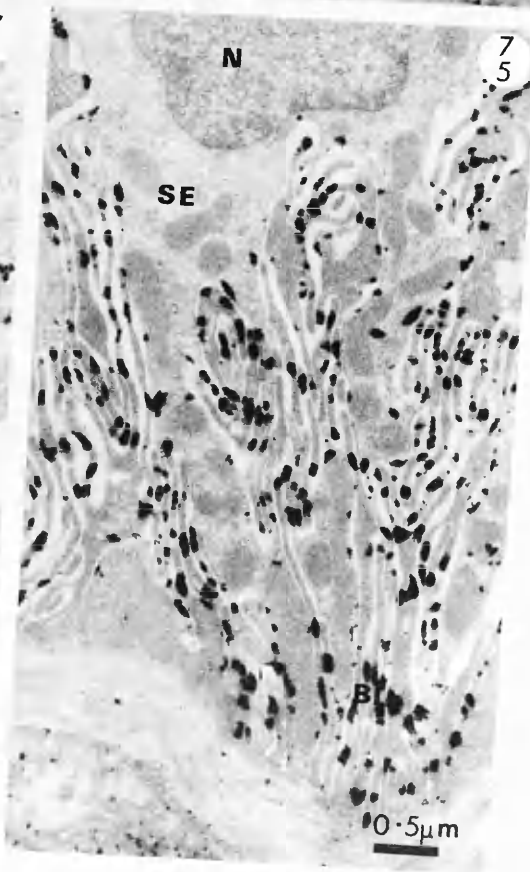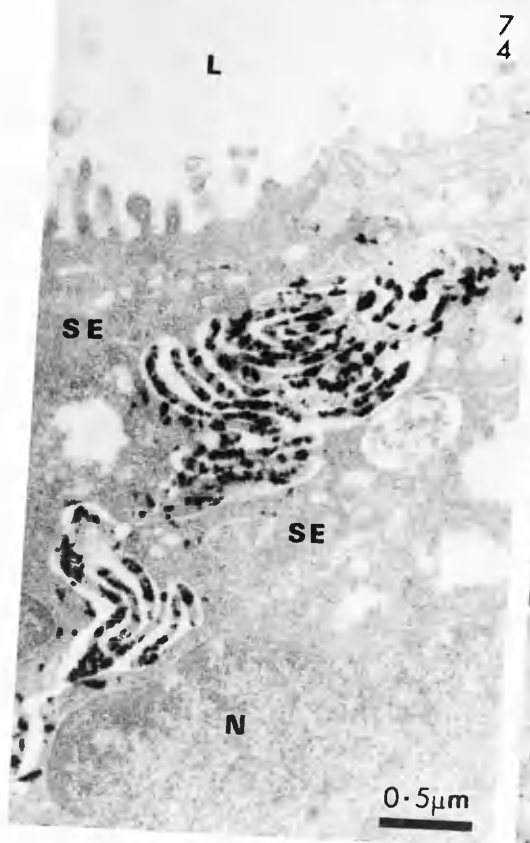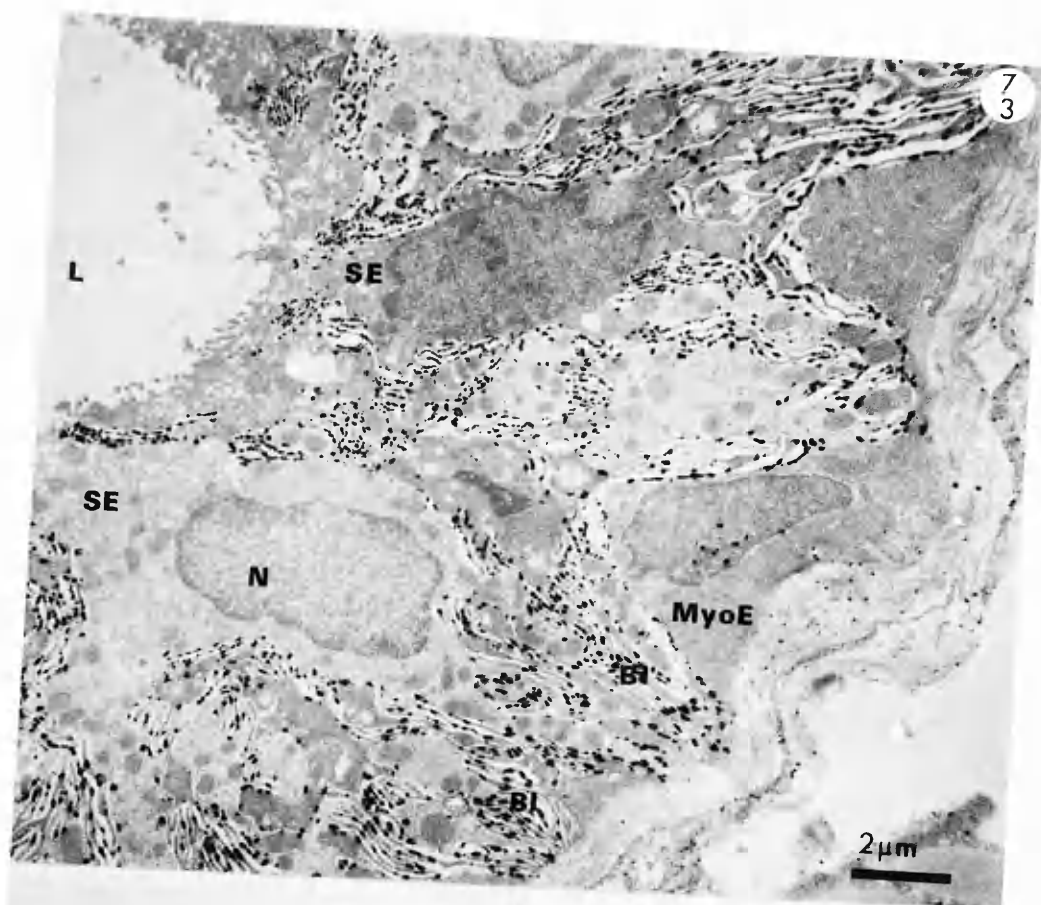
<u>Cattle and Sheep</u>.

<u>Procedure</u>.

One cow and two sheep were placed in an environment of 40°C Dry Bulb (D.B.) /26°C Wet Bulb (W.B.) and the sweating response was monitored throughout the exposure period of 3 hours. A skin sample was taken from each animal on entry to the chamber. The cow was sampled again after three hours exposure and the sheep during the second sweating peak (Ch.4, Fig.4.1) and at fatigue. Samples were fixed and processed as described in appendix 2. Rat footpad sweat gland was used in this and all further experiments as a control tissue for the reaction.

<u>Result</u>.

No reaction product was found on the secretory or myoepithelial cells of the unstimulated fundus of the cow (Fig.7.6). After thermal stimulation, reaction product was detected on the outer leaflet of the luminal membrane of the secretory cells (Fig.7.7).

The enzyme was present, albeit at a very low level, on the basal and basolateral membranes of the unstimulated

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<u>Fig.7.6</u>. Cow sweat gland fundus: The unstimulated sweat gland fundus with no reaction product present on any cell membranes.

<u>Fig.7.7</u>. Cow sweat gland fundus: After thermal stimulation reaction product is present on the luminal membrane of the secretory epithelium (arrows).
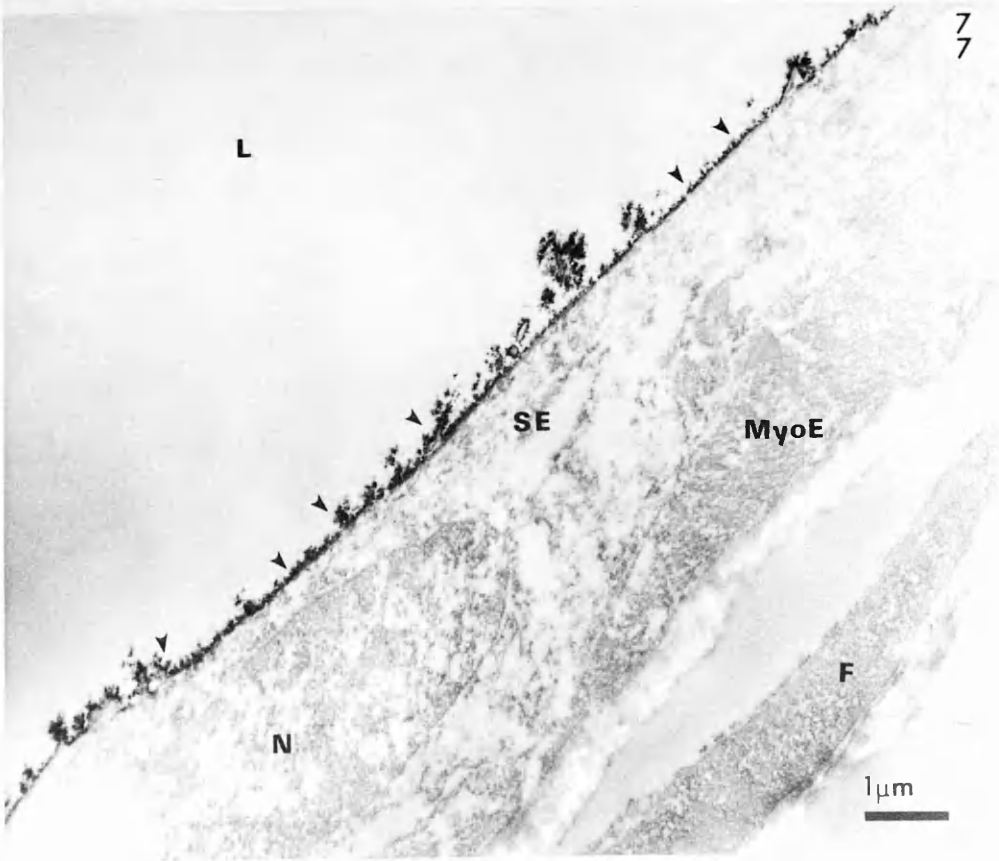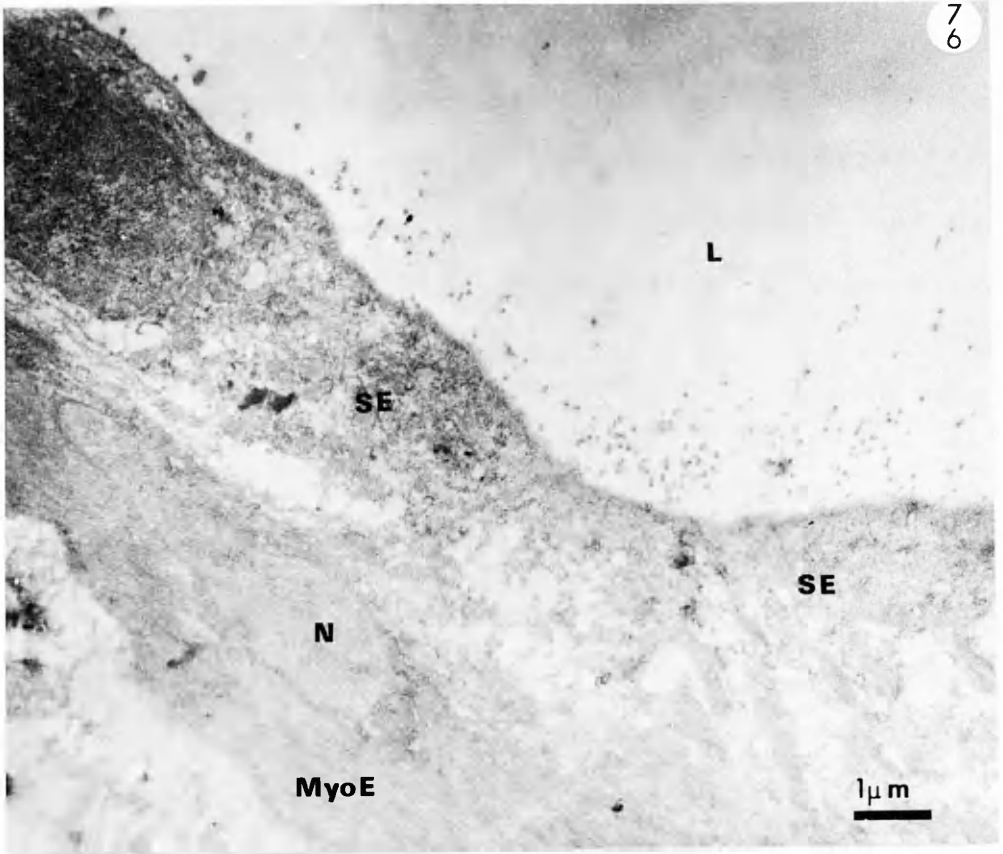
secretory cells of the sheep (Fig.7.8). At the second

sweating peak the amount on the basal and basolateral

membranes increased (Fig.7.9) and reaction product was

also present on the luminal microvilli (Fig.7.9.10). At

fatigue there was no reaction product present on any of

the secretory (Fig.7.11) or myoepithelial cell membranes.

The reaction in all instances was again potassium

dependent and inhibited by ouabain.

Horse.

A comparable trial was carried out on the sweat

glands of two horses. Both were sampled at rest and one

after exercise when it had visibly sweated. The horse

which was only sampled at rest was nervous and excited

before the biopsy was taken. Therefore, it is possible

that this specimen may not be representative of the glands

at rest. Reaction product was present on the luminal

Fig.7.8. Sheep sweat gland fundus: Reaction product

present on the basal and basolateral membranes of the

secretory epithelium.

Fig.7.9. Sheep sweat gland fundus: At the second sweating

peak the secretory epithelium showing reaction product

present on basal, basolateral and luminal membranes of the

secretory epithelium.

Fig.7.10. Sheep sweat gland fundus: Reaction product is

present on the luminal microvilli of a secretory cell

after the second sweating peak.

Fig.7.11. Sheep sweat gland fundus: At sweating fatigue no

reaction product is present on the secretory epithelium.

78

L

MV

SE

N

MyoE

0·5μm

79

L

MV

SE

SE

N

MyoE

1μm

7
10

7
11
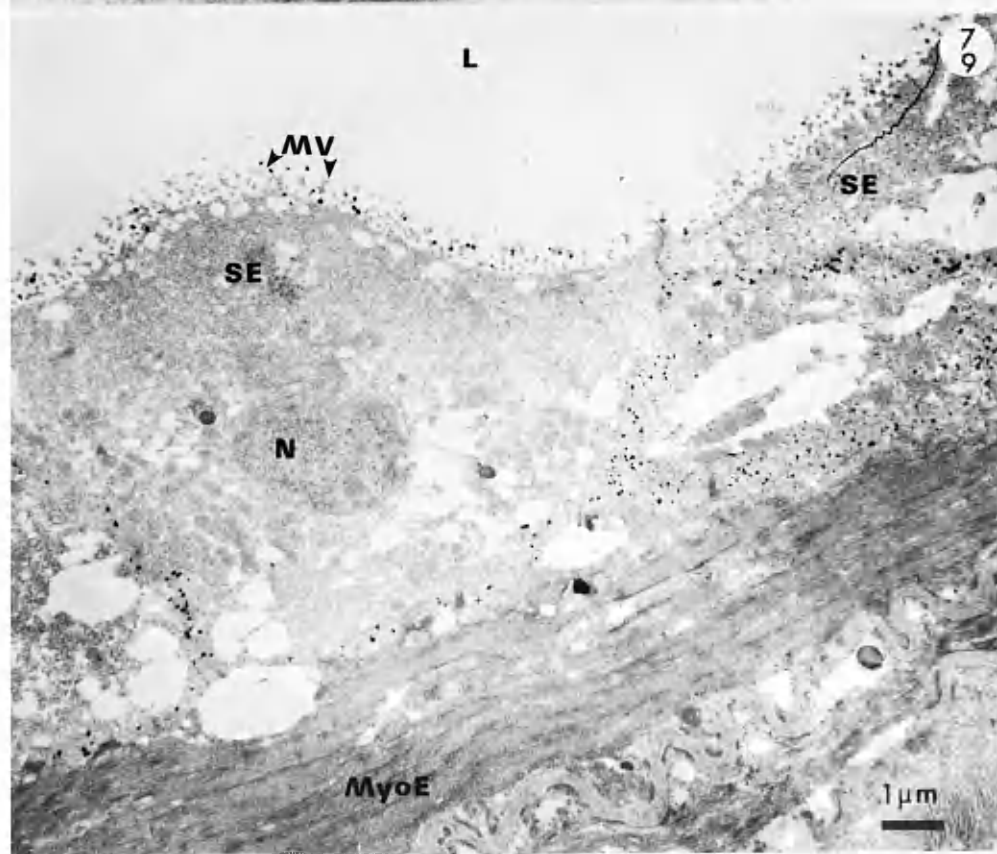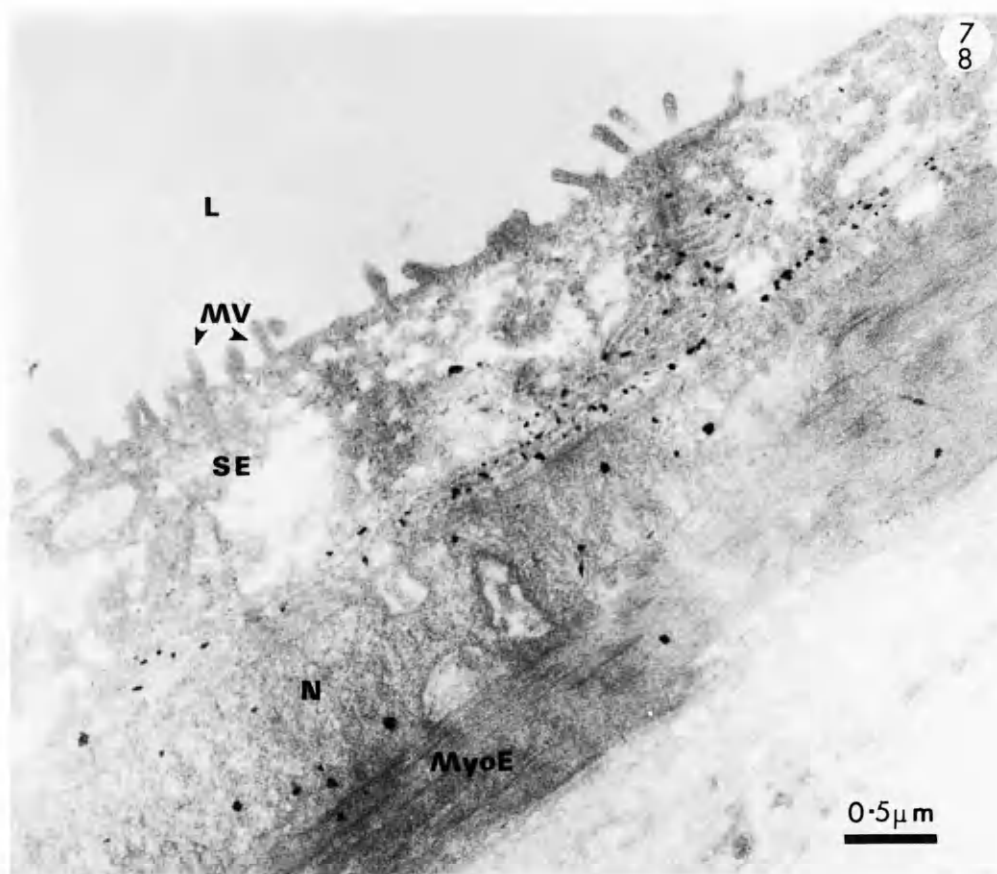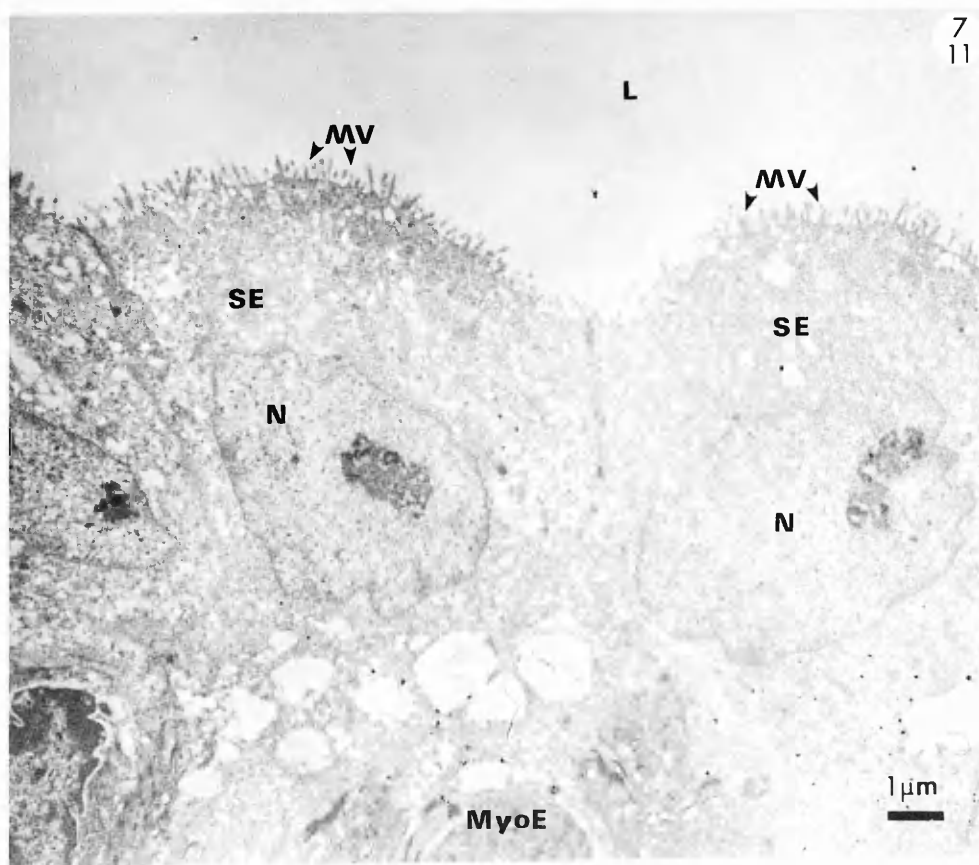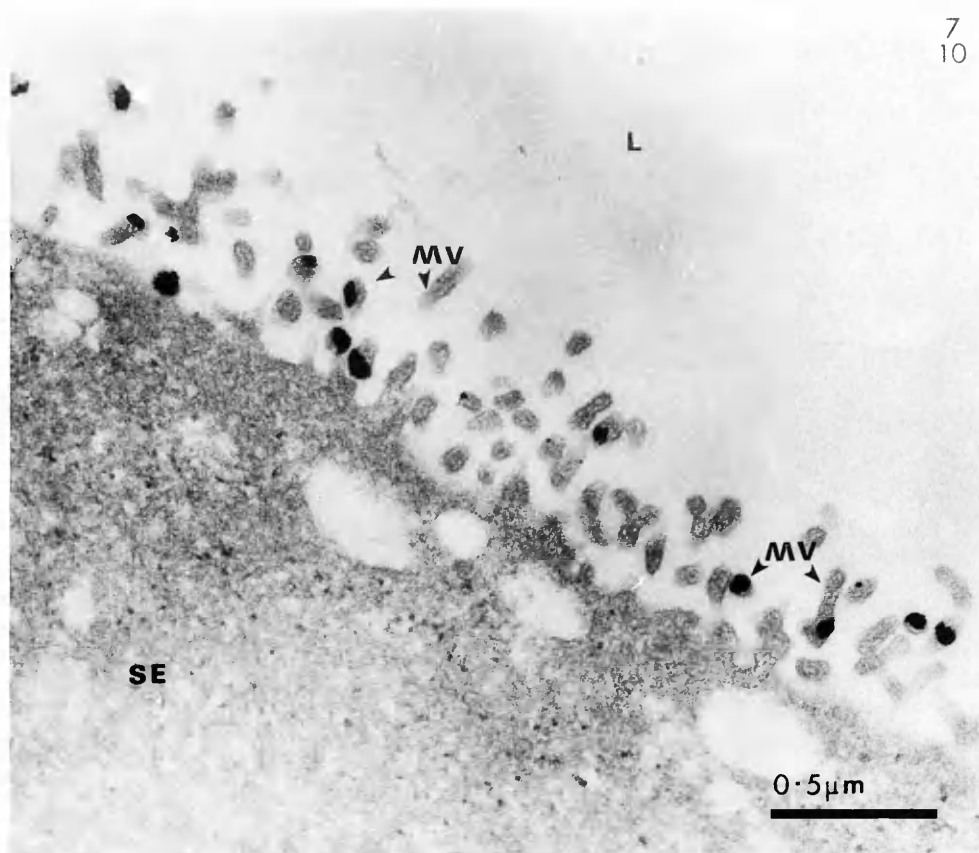
microvilli (Fig.7.12) and basal and basolateral membranes
of the secretory cells (Fig.7.13) of the fundus of the
first (unexercised) horse. At rest the second (exercised)
horse exhibited the reaction only on the basal and
basolateral membranes of the secretory cells (Fig.7.14).
After excercise it was present on the luminal microvilli
(Fig.7.15,16) as well as the basal and basolateral
membranes of the secretory cells (Fig.7.15,17). The
reaction was potassium dependent and inhibited by ouabain.

With the successful completion of the trials more

Fig.7.12. Horse sweat gland fundus: In this horse at rest
reaction product is present on the luminal microvilli of
the secretory cells.

Fig.7.13. Horse sweat gland fundus: Reaction product
present on the basal infoldings of a secretory cell at
rest.

Fig.7.14. Horse sweat gland fundus. In this horse at rest
reaction product is only present on the basal and
basolateral membranes of the secretory epithelium.

Fig.7.15. Horse sweat gland fundus. After exercise
reaction product is also present on the luminal membrane
of the secretory epithelium.

Fig.7.16. Horse sweat gland fundus: Reaction product on
the luminal microvilli of a secretory cell after the horse
was exercised.

Fig.7.17. Horse sweat gland fundus: Reaction product on
the basal infoldings of a secretory cell after the horse
was exercised.

7
12

SE

MV

L

MV

SE

0·5μm

7
13

SE

SE

BI

BI

F

0·5μm
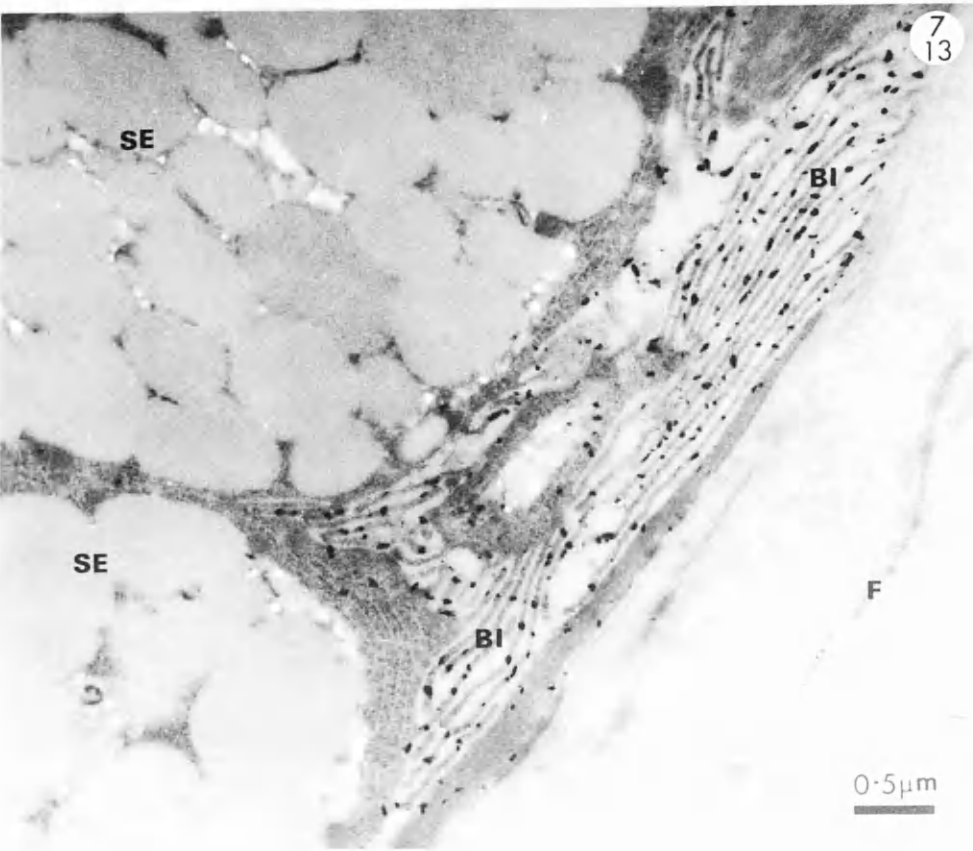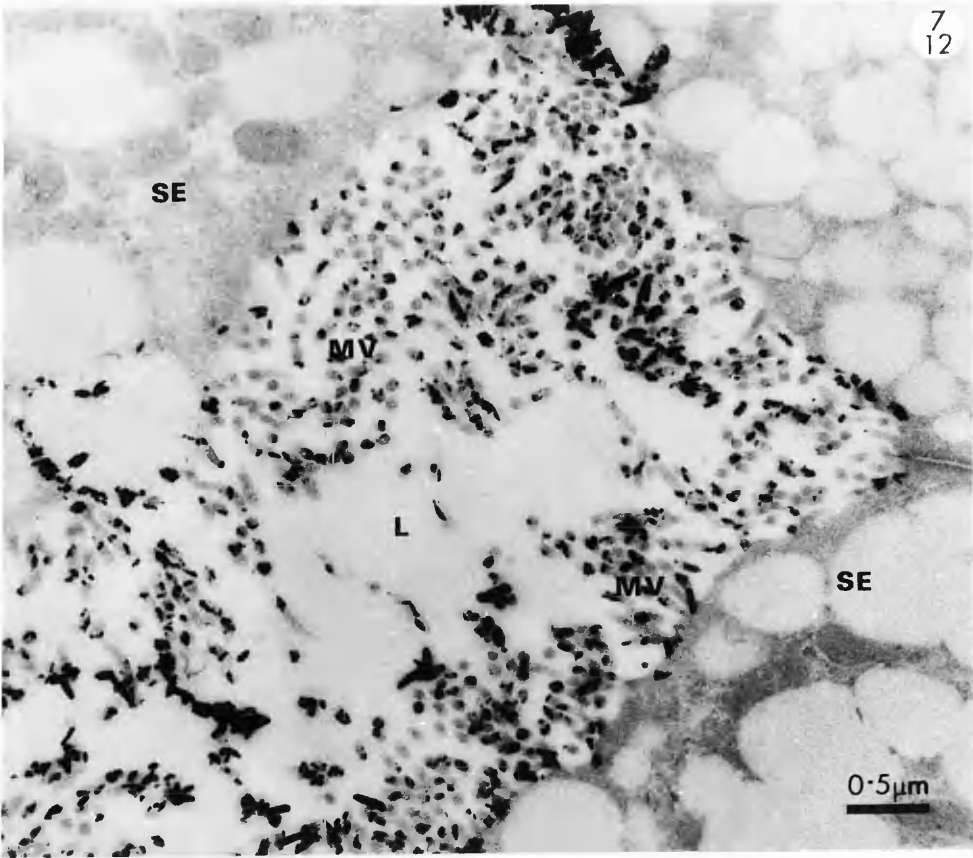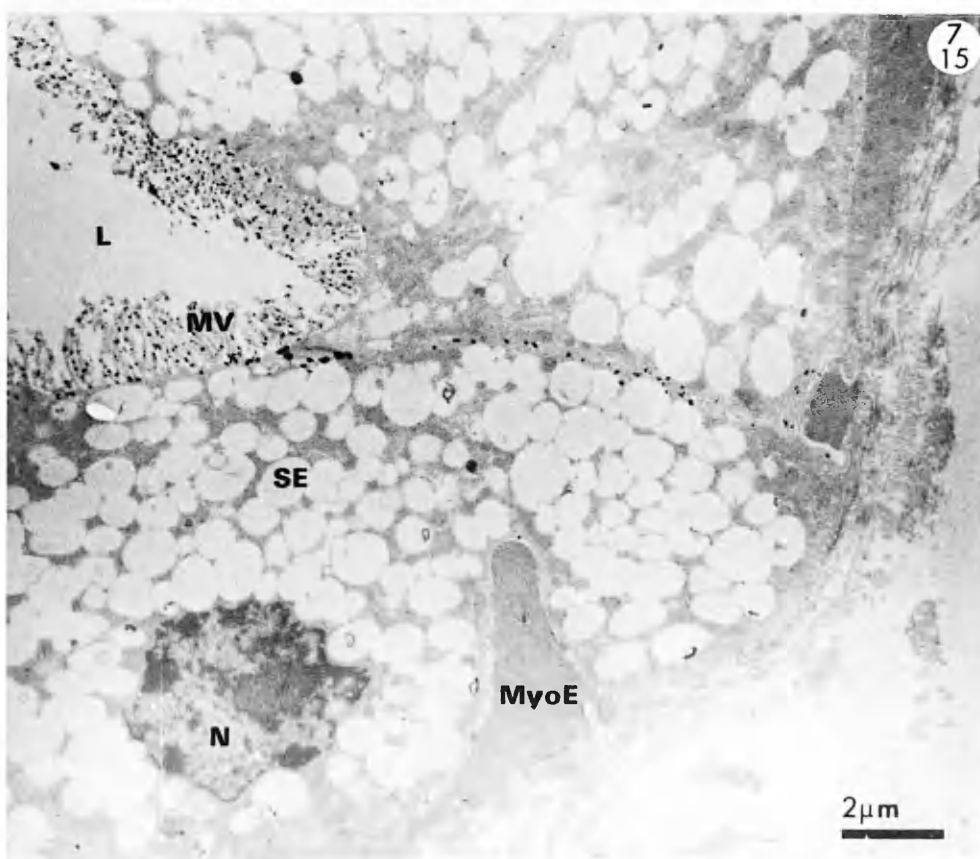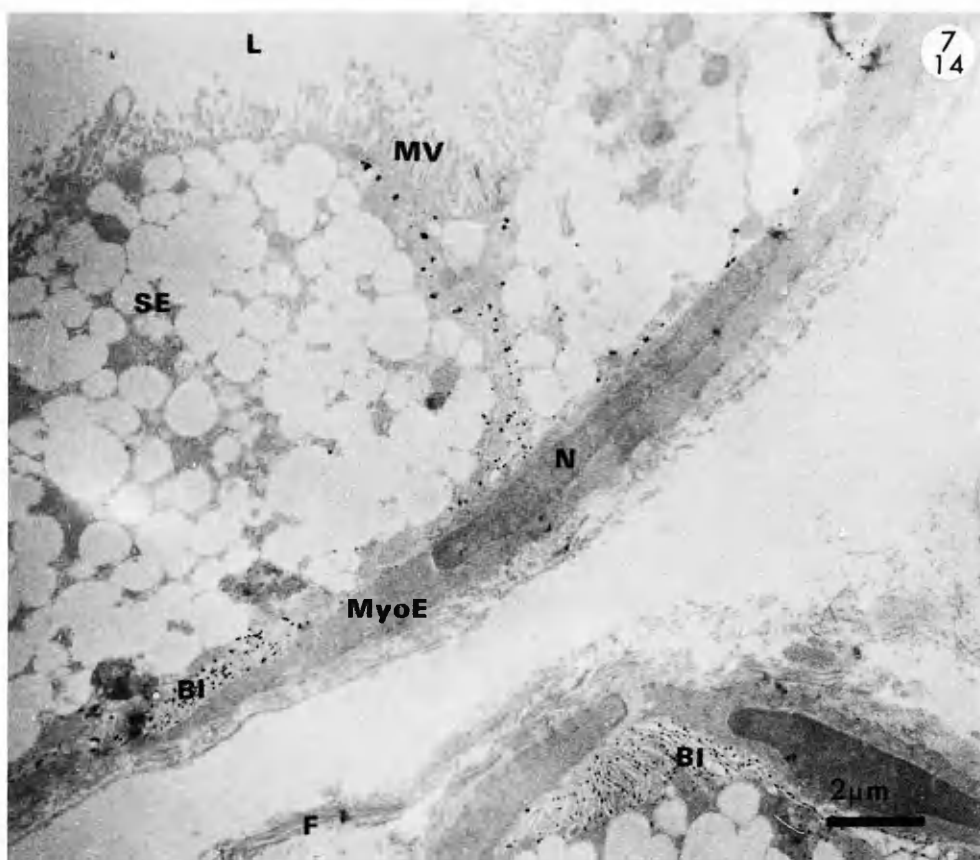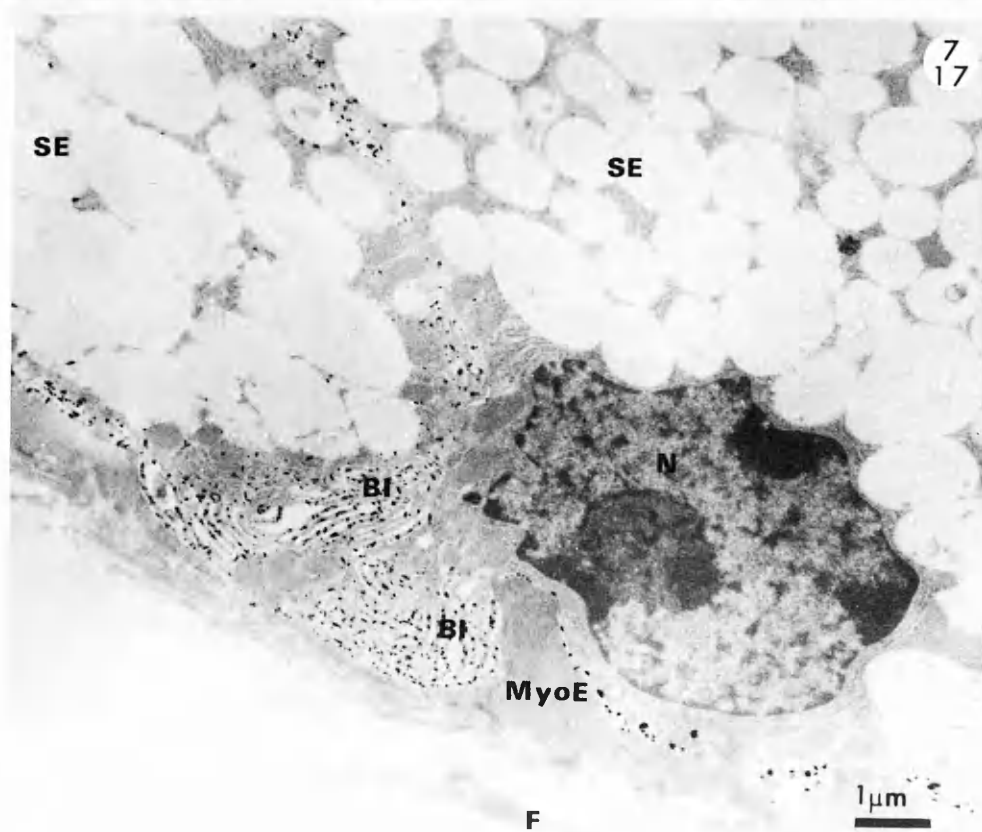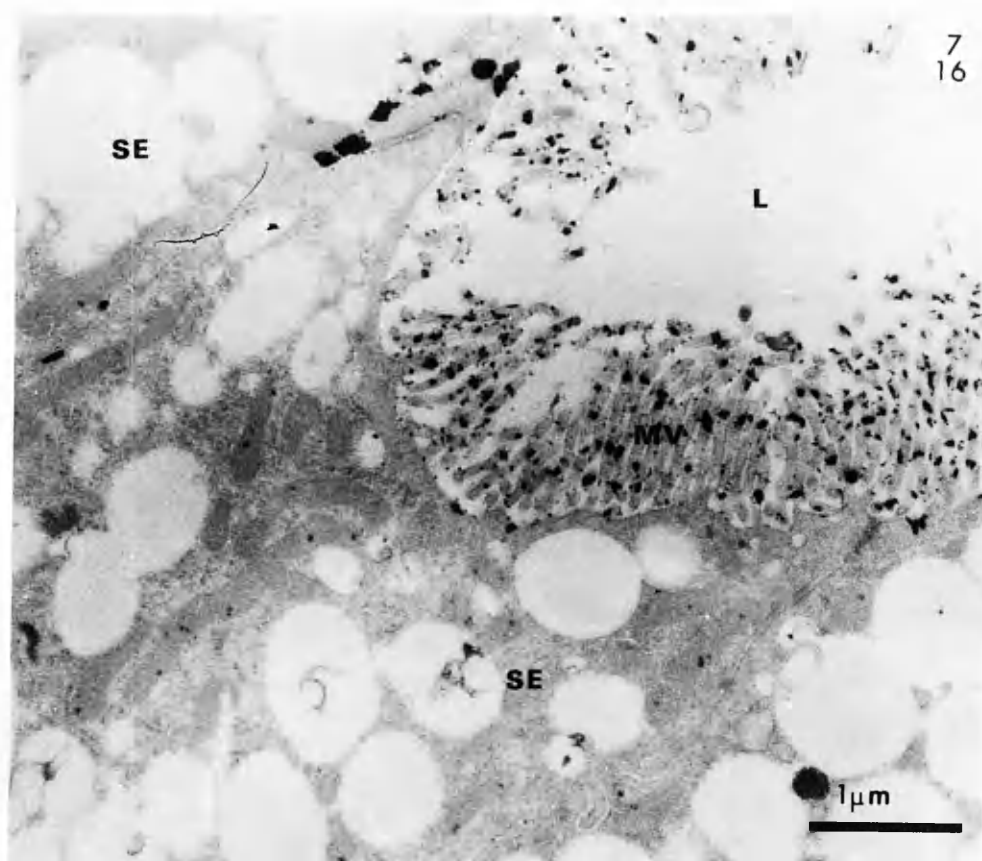
detailed experiments on the effects of thermal stimulation on sweat gland Na, K-ATPase were undertaken.

Effects of thermal stimulation.

Cattle, Sheep and Goat.

Four animals from each species were placed in an environment of 40°C DB/26°C WB. Skin samples were taken from the cattle upon entry into the environmental chamber at the onset of sweating and after 3 hours of continuous sweating. The sheep and goats were sampled at entry, immediately after the second sweating peak and at glandular fatigue. The results from these experiments were negative at all sample times in every instance. Rat footpad sweat glands processed at the same time were positive.

Horse.

The above experiment was repeated at the same environmental conditions using four horses. Samples were taken upon entry at sweating plateau and after 3 hours of sweating. On two of the horses reaction product was not detected either before or after thermally-induced sweating. The reaction was present on the basal and basolateral membranes of the secretory cells on the other two horses (Fig.7.18,19) but was absent from the luminal microvilli. At plateau and after 3 hours of sweating it

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Fig.7.18. Horse sweat gland fundus at rest: Reaction product present on the basal membranes of the secretory cells.

was still present on the basal and basolateral membranes but absent from the luminal microvilli. Rat footpad sweat glands processed at the same time were positive.

The results prompted a series of experiments on rats, cattle, sheep and goats to evaluate critically the reason for the discrepancies between the results of the experiments and those from the trials.

Technique evaluation.

Fixation.

Biochemical studies (Ernst & Philpott, 1970) on the avian salt gland and on teleost gill filaments showed that more than 70% of the Na,K-ATPase remains after fixation with 2-3% paraformaldehyde. Subsequent studies (Ernst, 1975) demonstrated that the inclusion of a low concentration of glutaraldehyde (0.25%) to this fixative resulted in superior morphological preservation while maintaining enzymatic activity. Using this information the following primary fixatives were tested on skin samples from Cattle, Sheep, Goat and Rat footpad.

Fix.1. (Ernst, 1975; Cutler et al., 1980).

1% Paraformaldehyde plus 0.25% Glutaraldehyde in 0.1M sodium cacodylate at pH7.3.

Fig.7.19. Horse sweat gland fundus at rest: Reaction product on the basolateral membranes of the secretory cells.

SE

N   BI

SE

MyoE

BI

1μm

MV

L

SE

SE

0·5μm
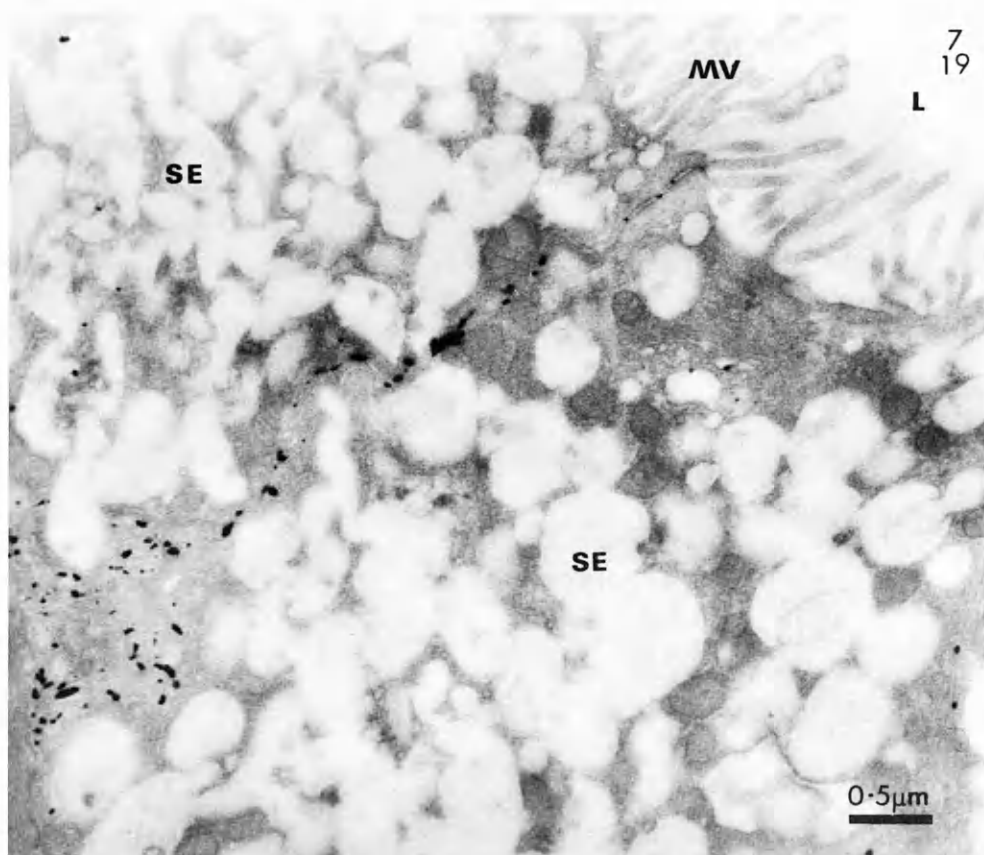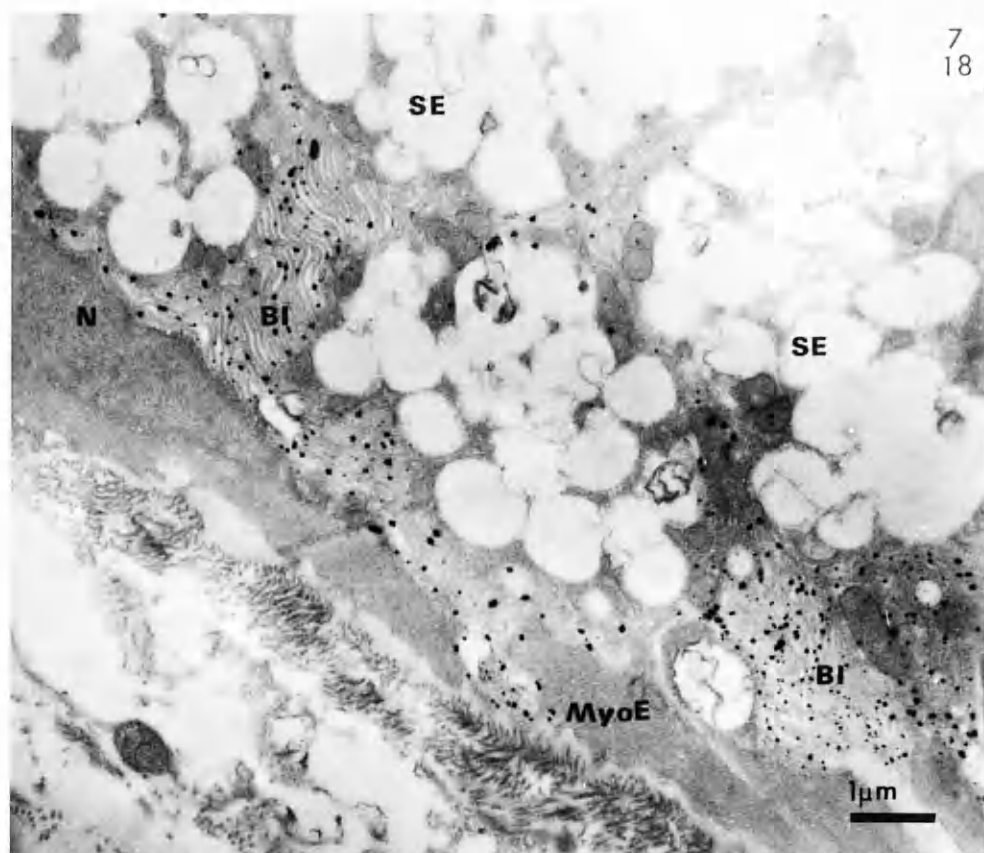
Fix.2 (Broderson, Patton & Stahl, 1978).

3% Paraformaldehyde plus 0.25% Glutaraldehyde in 0.1M sodium cacodylate at pH7.3.

Fix.3. (Ernst, 1972a,b).

3% Paraformaldehyde in 0.1M sodium cacodylate at pH7.3.

The skin samples were simultaneously fixed for 1 hour and processed together as described in appendix 2 for the presence of a K-dependent, ouabain sensitive reaction.

Result.

The distribution of the reaction product on the Rat footpad sweat gland fundus was as consistently found previously. There was no reaction present in the cow, sheep or goat. Fixative 1 was retained for further experiments as this gave maximum enzyme retention combined with reasonable ultrastructural preservation in footpad sweat glands.

Although the technique was successful on footpad sweat glands the quality of the glutaraldehyde used in the primary fixative was examined. Anderson (1967) concluded that most commercial glutaraldehyde had "impurities" which had an inhibitory effect on enzyme activity. Davies and Garrett (1972) showed that distilled glutaraldehyde was required for consistent results with minimal inhibition. Although the glutaraldehyde used in the primary fixative of the initial trials was of E.M. grade (Agar Aids), a sample was examined spectrophotometrically. The spectral UV absorbance curve (Fig.7.20) of this sample showed two maxima at wavelengths of 280nm and 233nm . Anderson (1967) from chromatographic experiments and comparisons with

spectral absorption curves found a peak of absorption at 280nm due to dialdehyde and another at 233nm caused either by contaminating material eg. glutaric acid or polymeric forms of glutaraldehyde. Distillation of the glutaraldehyde solution removed this second peak leaving a pure solution absorbing at 280nm (Fahimi & Drochmans, 1968). Commercially available distilled glutaraldehyde (Sigma.) was tested and as can be seen in Fig.7.20 absorption was only present at 280nm.

The fixation trial was repeated using distilled glutaraldehyde in fixative 1 and 2. Again the rat footpad sweat gland fundus gave a positive result, while with both fixatives cattle, sheep and goat glands were negative. Despite these negative results, in all further experiments distilled glutaraldehyde was used to avoid the inhibitory effects of the contaminants absorbing at 233nm (Anderson, 1967; Fahimi & Drochmans, 1968; Davies & Garrett, 1972).

Fixative Buffers.

The fixative trials did not resolve the problem so the choice of buffer for the primary fixative was studied. Tris and veronal, poor buffers in the physiological range, contain an amino group and cannot be used with aldehydes. This is because of a Canizarro-type reaction which produces a solution with no buffering capacity in the physiological range of pH values. Phosphate buffers have the advantage that they are the most 'physiological' since

Fig.7.20. Spectral absorbance of 2 samples of glutaraldehyde. E.M. Grade has peaks at 280nm and 233nm whereas distilled glutaraldehyde only has a peak at 280nm.
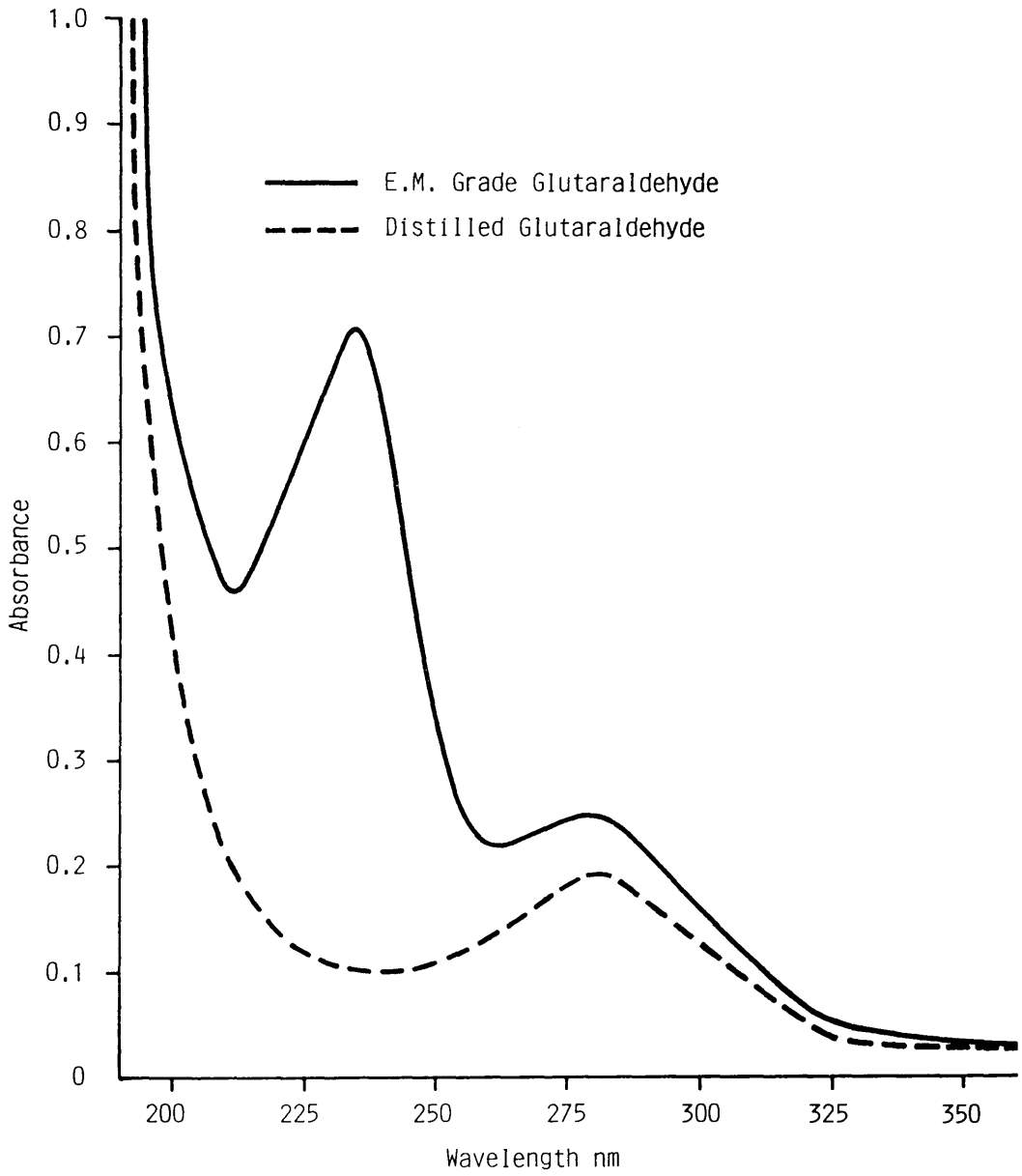
they mimic certain components of extracellular fluids and
are not toxic to cells. Although there is little evidence
of differences amongst phosphate buffers, with respect to
ultrastructural preservation, this may not be true for
cytochemistry. Phosphates cannot be used when calcium ions
are necessary and must be removed before hydrolytic enzyme
techniques are used. In this experiment Sorensen's Na/K
phosphate and Millonig's monosodium phosphate buffers were
tested. Cacodylate, although a scheduled poison due to the
presence of arsenic, has the advantage that calcium can be
included in the buffer and the inhibitory effects of the
arsenic on the Na,K-ATPase are reversible (Ernst and
Philpott, 1970). Collidine when used to buffer
paraformaldehyde leads to lysis of the cytoplasmic matrix
and extensive destruction of membranes (Carson, Lynn &
Martin, 1972). When used with glutaraldehyde Collidine
gives poorer ultrastructure than phosphate or cacodylate
buffers (Busson-Mabillot, 1971). Skin samples from cow,
sheep, goat and the rat footpad were fixed using 1%
paraformaldehyde and 0.25% glutaraldehyde in either
cacodylate, Sorensen's or Millonig's phosphate buffer.
After fixation the samples were processed as described in
appendix 2.

Result.

The rat footpad sweat gland fundus was positive in
the three buffers tested while cow, sheep and goat were
negative. As there was no advantage of phosphates over the
original cacodylate buffer (Ernst, 1972a) cacodylate
remained the fixative buffer during the remainder of the
technique trials.

<u>Direct incubation</u>.

Before commencing trials on substrate composition and optimum conditions, tissues were incubated in the standard substrate without primary fixation. On examination the quality of ultrastructure was so poor that even the rat could be judged a failure.

<u>Incubation Time, Temperature and pH</u>.

In this series of experiments the samples were processed as described in appendix 2. The distribution of the reaction product was as described earlier unless otherwise stated.

<u>Result</u>.

<u>Time</u>.

<u>Sweat gland fundus</u>.

	Rat.	Cow.	Sheep.
30 Minutes.	+ve.	-ve.	-ve.
45 Minutes.	+ve.	-ve.	-ve.
1 Hour.	+ve.	-ve.	-ve.
1.5 Hours.	+ve.	-ve.	-ve.
2 Hours.	+ve.	-ve.	-ve.
3 Hours.	+ve.	-ve.	-ve.

Sections, from all animals, incubated for 2-3 hours showed an increase in non-specific background staining, particularly on the cisternae of the nuclear envelope.

Temperature.

Incubation temperature was raised to 37°C.

Sweat gland fundus.

	Rat.	Cow	Sheep.
30 Minutes.	+ve.	-ve.	-ve.
45 Minutes.	+ve.	-ve.	-ve.
1 Hour.	+ve.	-ve.	-ve.
1.5 Hours.	+ve.	-ve.	-ve.
2 Hours.	+ve.	-ve.	-ve.
2.5 Hours.	+ve.	-ve.	-ve.
3 Hours.	+ve.	-ve.	-ve.

Raising the incubation temperature resulted in an increase of non-specific background staining in all animals otherwise the result was the same as before.

pH.

Sweat gland fundus.

	Rat.	Cow.	Goat.
pH 7.2	-ve.	-ve.	-ve.
pH 8.	+/-ve.	-ve.	-ve.
pH 9.	+ve.	-ve.	-ve.
pH 10.	-ve.	-ve.	-ve.

Cow and goat were negative at all pH values. The rat footpad sweat gland showed changes from the original distribution which agreed with those of Ernst (1972a). The reaction product was markedly reduced at pH 8 and absent at pH 7.2 due to insufficient trapping of the hydrolysed phosphate and solubilization of the strontium phosphate. At pH values above 9 the potassium-dependent activity fell rapidly.

Incubation substrate composition.

In this series of experiments the samples were
processed as described in appendix 2 but with the
incubation substrate altered in the following ways:-

Potassium.

Sweat gland fundus.

	Rat.	Cow.	Sheep.	Goat.
10mM KCl.	+ve.	-ve.	-ve.	-ve.
20mM KCl.	+ve.	-ve.	-ve.	-ve.
50mM KCl.	+ve.	-ve.	-ve.	-ve.
100mM KCl.	+ve.	-ve.	-ve.	-ve.

The distribution of the reaction product on the Rat
footpad sweat gland was as before, cow, sheep and goat
were negative.

p-Nitrophenyl Phosphate. (p-NPP.)

Sweat gland fundus.

	Rat.	Cow.	Sheep.	Goat.
10mM p-NPP	+ve.	-ve.	-ve.	-ve.
20mM p-NPP	+ve.	-ve.	-ve.	-ve.
30mM p-NPP	+ve.	-ve.	-ve.	-ve.

The distribution of the reaction product on the Rat
footpad sweat gland was as before, cow, sheep and goat
were negative.

Strontium chloride.

Strontium, the capture ion for the hydrolysed
phosphate of the reaction, is a strong inhibitor of the
enzyme, although less inhibitory than other heavy metals
(Ernst, 1972a,b). The concentration of the strontium was
lowered, from 20mM, to ascertain if the ion was indeed

causing excess inhibition of the enzyme in the experimental animals.

Sweat gland fundus.

	Rat.	Cow.	Goat.
20mM SrCl.	+ve.	-ve.	-ve.
10mM SrCl.	+ve.	-ve.	-ve.
5mM SrCl.	+ve.	-ve.	-ve.

Decreasing the concentration of strontium in the incubation substrate had no effect on the result. The distribution in the rat footpad sweat gland was as before.

Lead nitrate substitution.

This step of the technique was necessary to convert strontium phosphate to lead phosphate which can be visualized in the electron microscope. The standard time of 2 x 5 minutes was altered to 1 x 5 minutes and 3 x 5 minutes. Again the rat was positive at all times with cattle, sheep and goat negative.

The changes in technique failed to enable repetition of the original result except with the rat which gave a consistently positive result. As the Ernst technique proved unsuccessful on the experimental animals a second technique for the electron microscopic localization of the ouabain sensitive, potassium-dependent p-nitrophenylphosphate activity of the Na,K-ATPase complex was tested. The technique (Mayahara, Fujimoto, Ando & Ogawa, 1981), which uses p-nitrophenylphosphate as substrate, lead citrate as the capture reagent and dimethylsulphoxide as an activator, was tested on Rat footpad. In the sweat gland fundus, reaction product was again localized on the basal and basolateral membranes of

the secretory epithelial cells (Fig.7.21,22,23). The
reaction product was ouabain sensitive and potassium
dependent. Its extremely fine size enabled visualization
on the cytoplasmic side of the plasma membrane. The
technique was tried on cattle, sheep and goat but again
with a negative result. A series of trials was started on
the chemical composition of the substrate but abandoned as
precipitates formed readily making the substrate
unsuitable for use.

A final experiment on the localization of Na,K-ATPase
in sweat glands was undertaken after personal
communication with Dr J. A. Firth. The light microscopic
technique (Guth & Albers, 1974) described in appendix 2
was carried out on unfixed cryostat sections. As
previously Rat kidney and footpad were used as +ve control
tissues. Reaction product was localized in the tubules of
the kidney cortex and at the base of secretory cells of
the sweat gland fundus. However, when carried out on the

Fig.7.21. Rat footpad sweat gland fundus: Reaction product
present on the basal and basolateral membranes of the
secretory epithelium after staining by the Mayahara (1981)
technique.

Fig.7.22. Rat footpad sweat gland fundus: High power
electron micrograph of the basal infoldings after staining
by the Mayahara et al., (1981) technique.

Fig.7.23. Rat footpad sweat gland fundus: High power
electron micrograph of the apical membranes between
secretory cells stained by the Mayahara et al.,(1981)
technique.
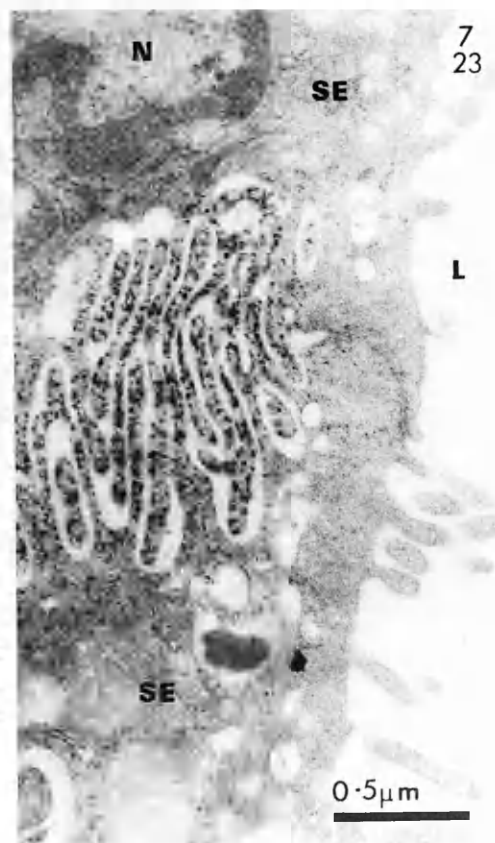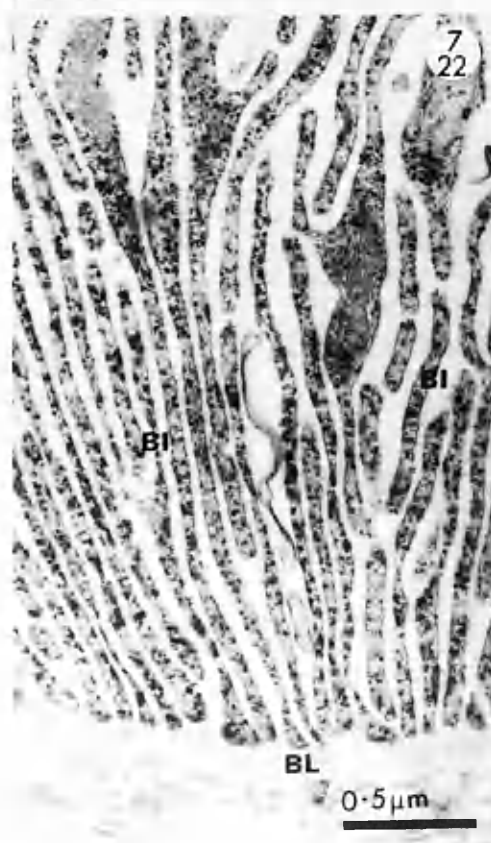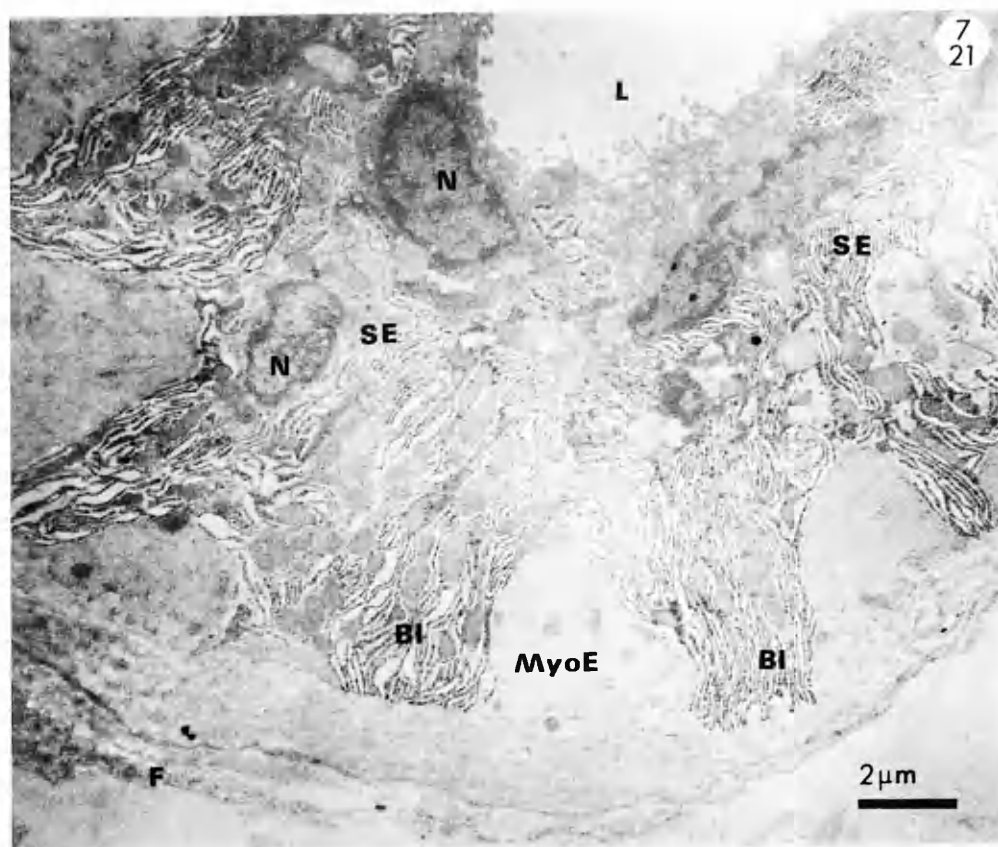
skin of cattle, sheep and goat no reaction product developed in the sweat glands.

The distribution of alkaline phosphatase in sweat glands was also examined. Although alkaline phosphatase was inhibited by levamisole (Borgers, 1973) in the Na,K-ATPase incubation medium, the enzyme is capable of catalysing the hydrolysis of various phosphatase esters at alkaline pH. Therefore the possibility that alkaline phosphatase is being demonstrated by the p-NPPase technique cannot be eliminated.

GLANDULAR ALKALINE PHOSPHATASE.

Trials.

A series of technique trials on the sweat glands from rat footpad, cow, sheep and goat was undertaken before an experiment was attempted in the environmental chamber.

Procedure.

Samples were fixed and processed for alkaline phosphatase (Alk.Pase) as described in Appendix 2.

Rat footpad.

Alkaline phosphatase was located in the fibrocyte sheath surrounding the fundus of the rat footpad sweat gland (Fig.7.24), and on the plasma membranes of the myoepithelial cells (Fig.7.25). It was, however, also

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Fig.7.24. Rat footpad sweat gland fundus: Reaction product present in the fibrocyte sheath and on the plasma membranes of the myoepithelium.

Fig.7.25. Rat footpad sweat gland fundus: High power electron microgragh of reaction product on the plasma membrane of the myoepithelium.

detected in low concentration on the basal infoldings of the secretory cells (Fig.7.24). The reaction product was completely inhibited by levamisole (Fig.7.26).

Cow, Sheep and Goat.

The distribution of alkaline phosphatase in the sweat glands was essentially the same as that found in the rat. The main concentrations of enzyme in the cow were on the basal membranes of the secretory cells and around the myoepithelial cells (Fig.7.27), particularly at the surface adjoining the secretory cells, where caveolae were regularly electron opaque (Fig.7.28). It was also present in the surrounding fibrocyte sheath. The enzyme in sheep and goat was generally seen in very low concentration, laterally between secretory cells, but was never found at the luminal surface (Fig.7.29). The fibrocytes in the surrounding sheath reacted strongly for alkaline phosphatase. The reaction was completely inhibited by levamisole.

Fig.7.26. Rat footpad sweat gland fundus: No reaction product is present after levamisole inhibition.

Fig.7.27. Cow sweat gland fundus: Reaction product present on the basal membranes of secretory cells and around the myoepithelium.

Fig.7.28. Cow sweat gland fundus: High power electron micrograph of the reaction product present at the surface between secretory and myoepithelium.

Fig.7.29. Goat sweat gland fundus: Reaction product between secretory cells and myoepithelium.

Effect of thermal stimulation.

Four cattle, sheep and goats were placed in an environment of 40°C DB/26°C WB. Skin samples were taken from the cattle upon entry into the environmental chamber, at the onset of sweating, and after 3 hours of continuous sweating. Samples were taken from the sheep and goats at entry, immediately after the second sweating peak and at gland fatigue. The samples from each species were fixed and processed for alkaline phosphatase as described in appendix 2. In this series of experiments the effect of the Na,K-ATPase inhibitor, ouabain, was also tested.

Result.

Thermal stimulation had no effect on the distribution of alkaline phosphatase. The reaction product in cattle was localised to the membranes of the myoepithelium, particularly those adjacent to the secretory epithelium (Fig.7.30) and never found on the luminal membrane of the secretory epithelium. The reaction was completely inhibited by levamisole (Fig.7.31) but unaffected by ouabain (Fig.7.32).

Fig.7.30. Cow sweat gland fundus: At sweating plateau reaction product is still present between secretory and myoepithelium.

Fig.7.31. Cow sweat gland fundus: Reaction product is absent after levamisole inhibition.

Fig.7.32. Cow sweat gland fundus: Reaction product is still present after treatment with ouabain.

7
27

7
28

L

N

SE

MyoE

0·5µm

SE

MyoE

0·5µm
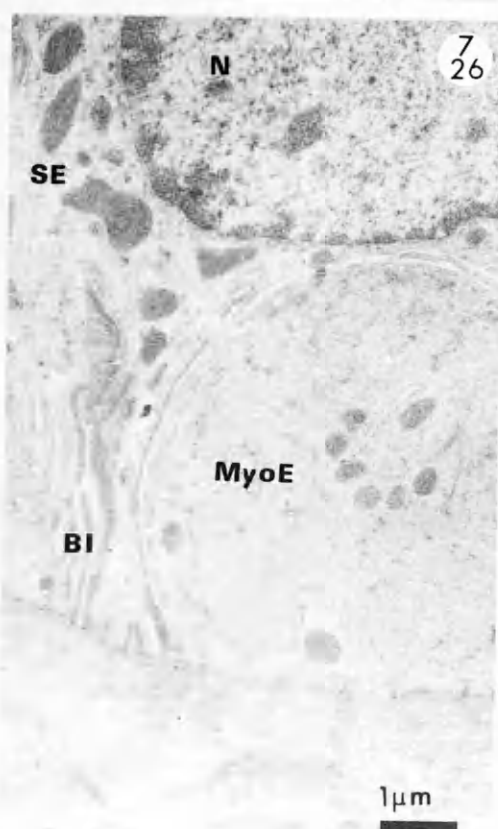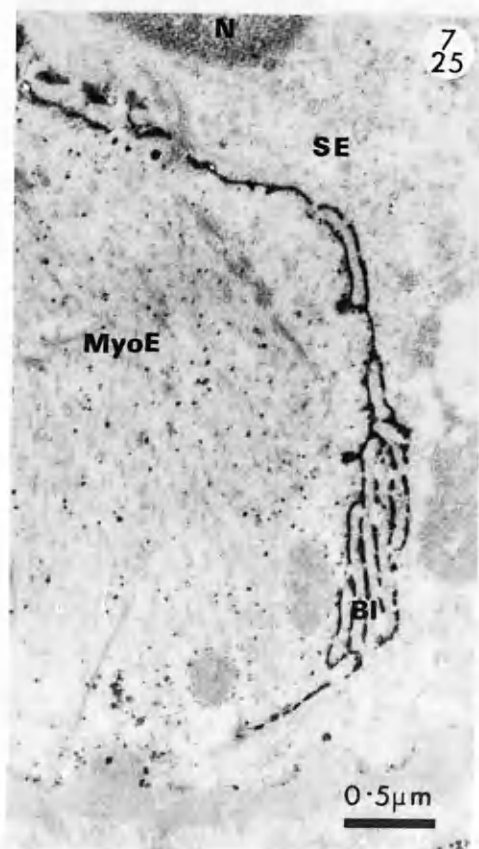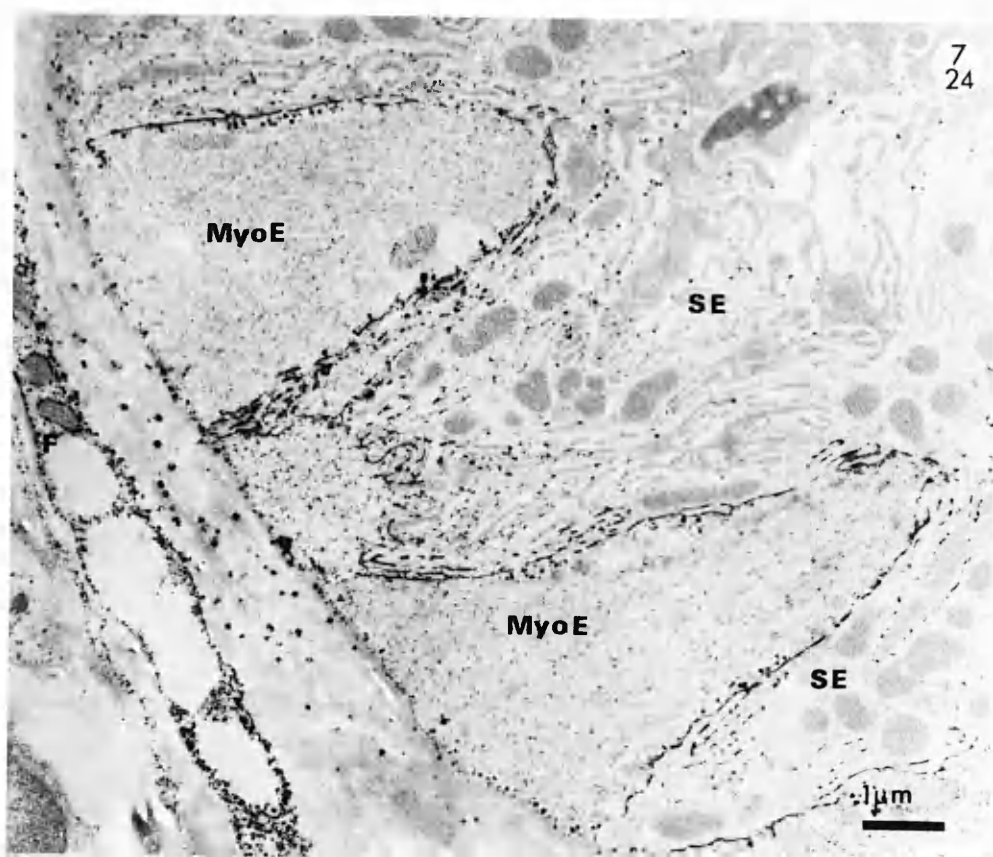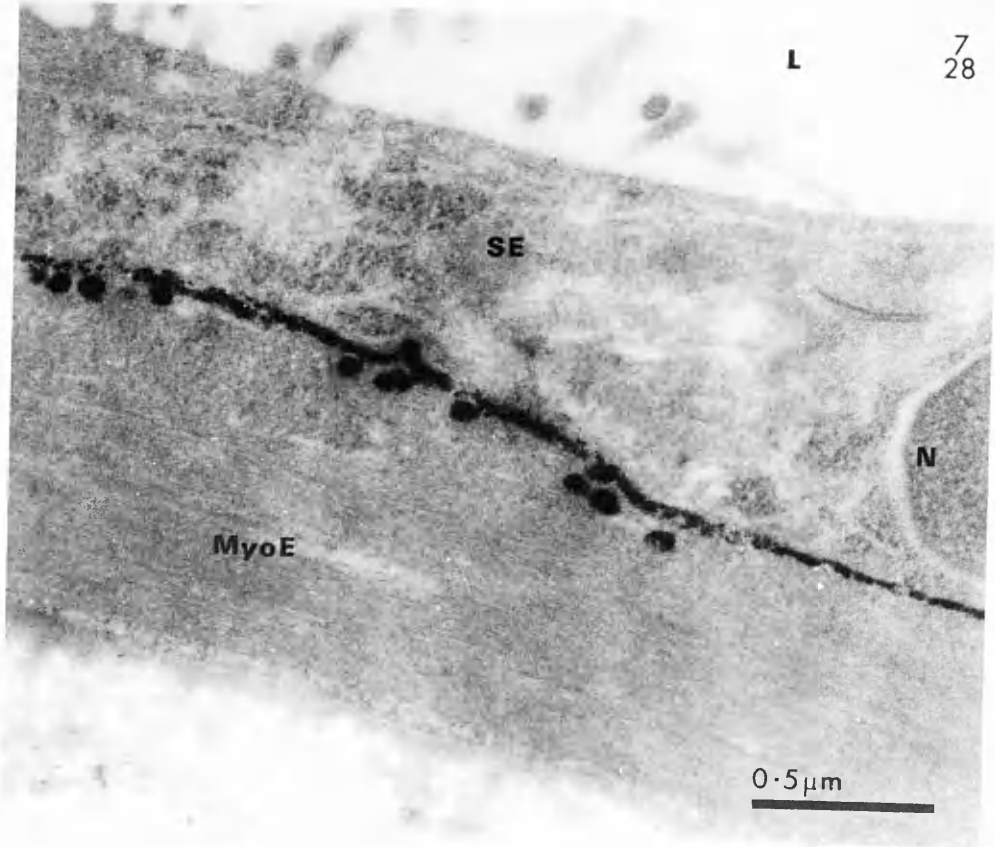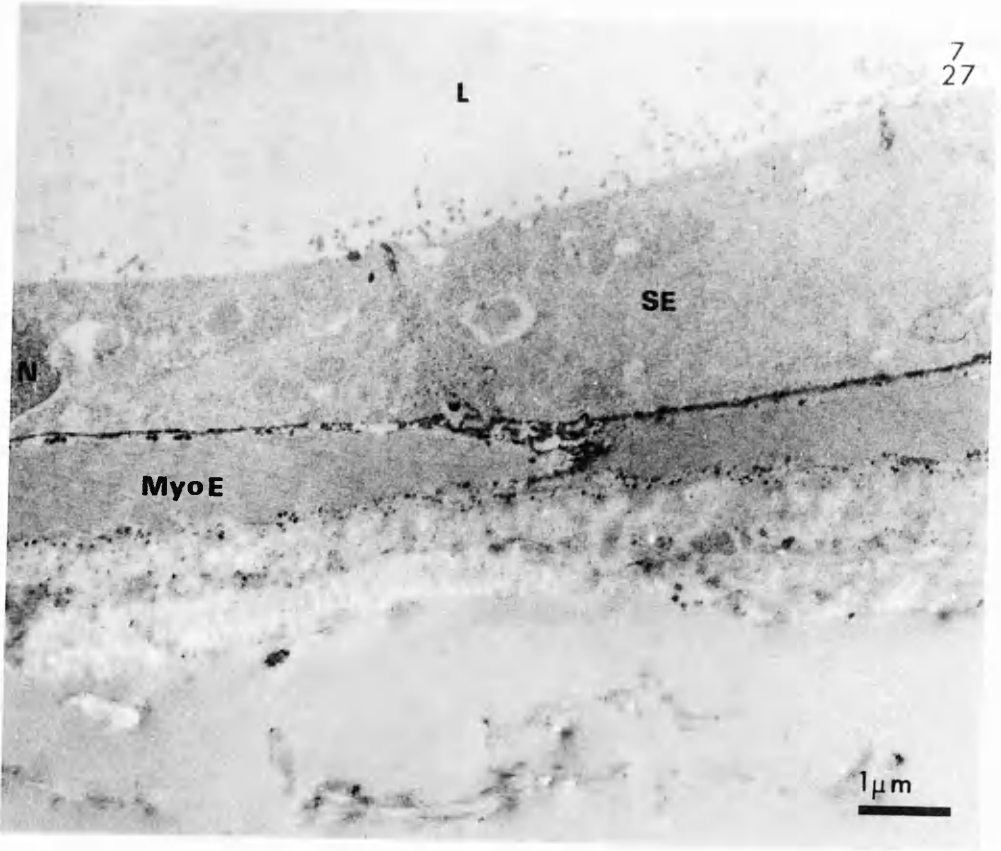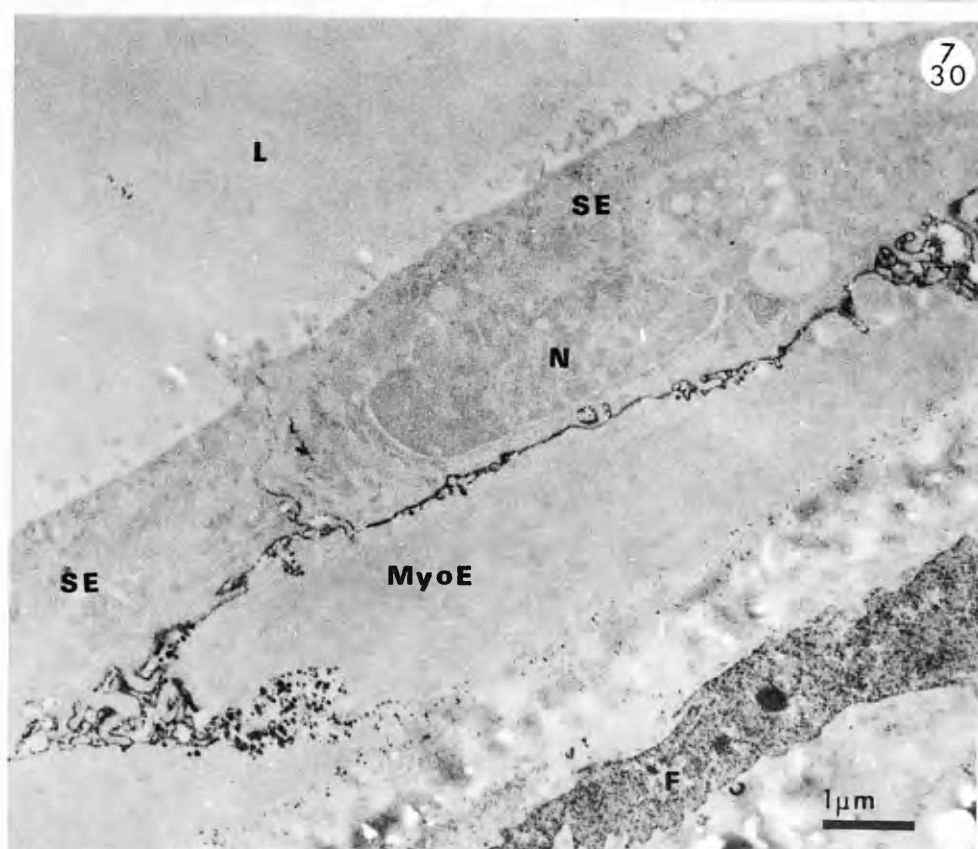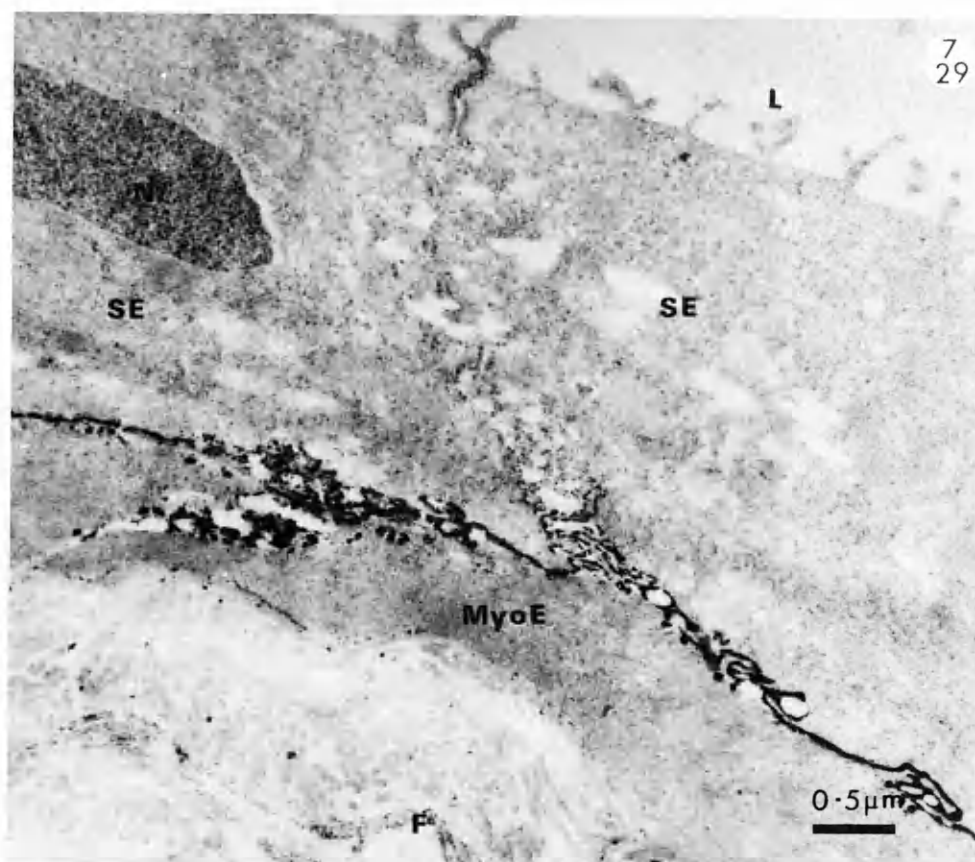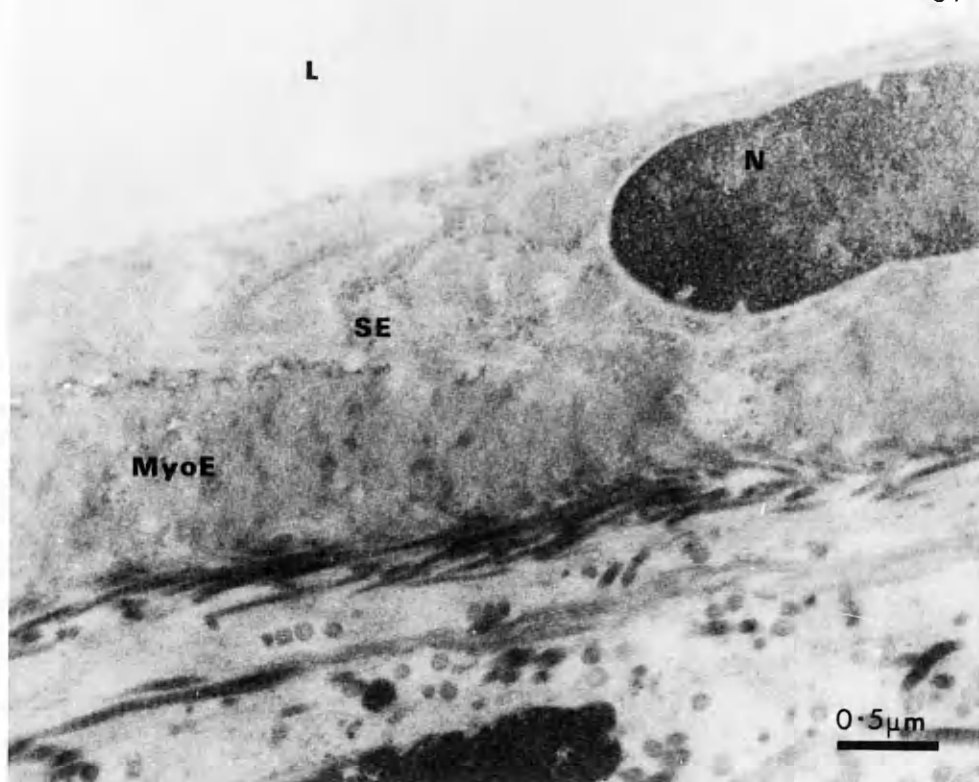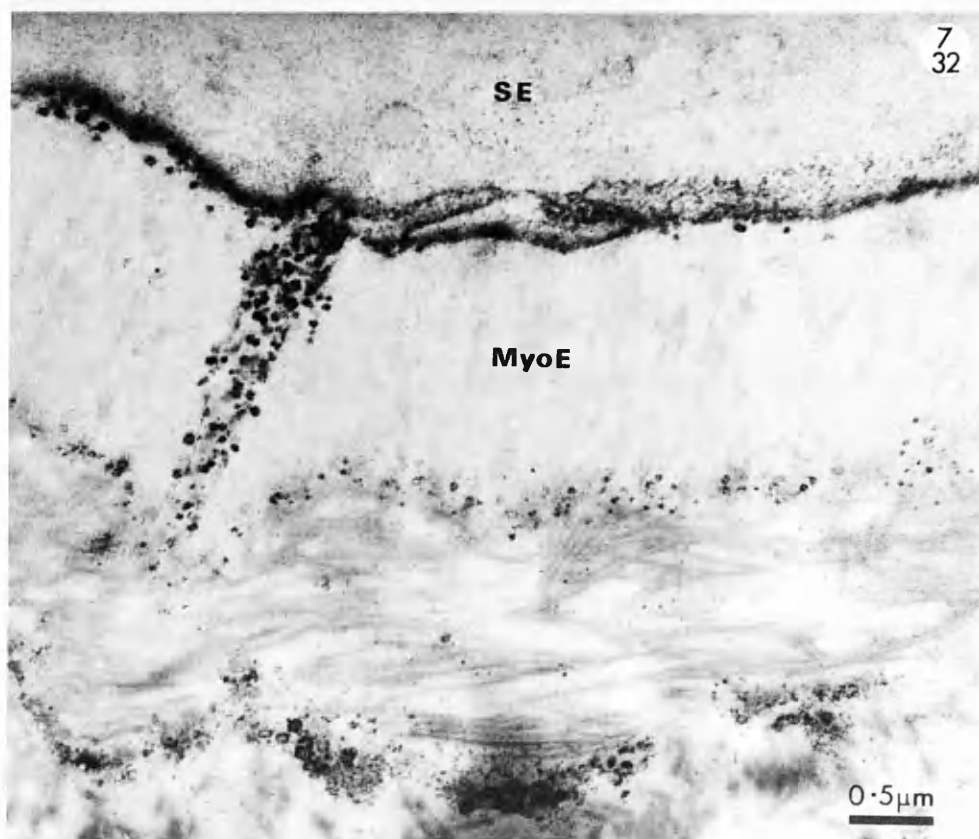
Sheep and goats had the same distribution of enzyme in the active and fatigued gland (Fig.7.33,34) as at rest. The reaction was inhibited by levamisole but unaffected by ouabain.

Horse.

In the unstimulated equine sweat gland fundus the reaction product was localised at the luminal membrane and on the microvilli of the secretory cells, but was absent from their lateral and basal surfaces (Fig.7.35). No enzyme was found on the myoepithelial cells although it was again present in the surrounding fibrocytes.

The distribution of the enzyme was unaffected by glandular stimulation, although the amount of reaction product present after 3 hours of sweating, as assessed by comparison of corresponding histological sections from the same horses processed in the same incubation bath, appeared to have been considerably reduced (Fig.7.36). The reaction was inhibited by levamisole and unaffected by

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Fig.7.33. Sheep sweat gland fundus after the second sweating peak: Reaction product is present between secretory and myoepithelium.

Fig.7.34. Goat sweat gland fundus: Reaction product is present between secretory and myoepithelium.

Fig.7.35. Horse sweat gland fundus: Reaction product present on the luminal microvilli of the secretory epithelium.

Fig.7.36. Horse sweat gland fundus: After 3 hours of thermal stimulation reaction product is still present on luminal microvilli of the secretory cells.

7
35

MyoE

N

SE

SE

MV

L

1µm

7
36

MyoE

SE

L

N

MV

0·5µm
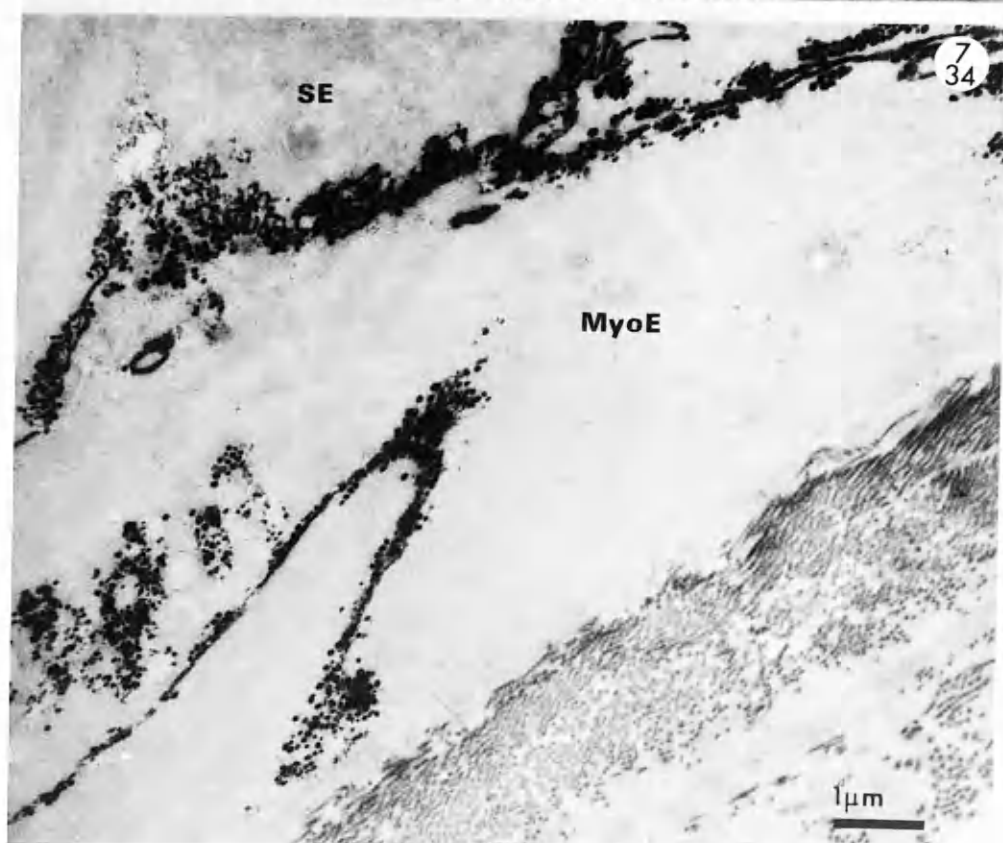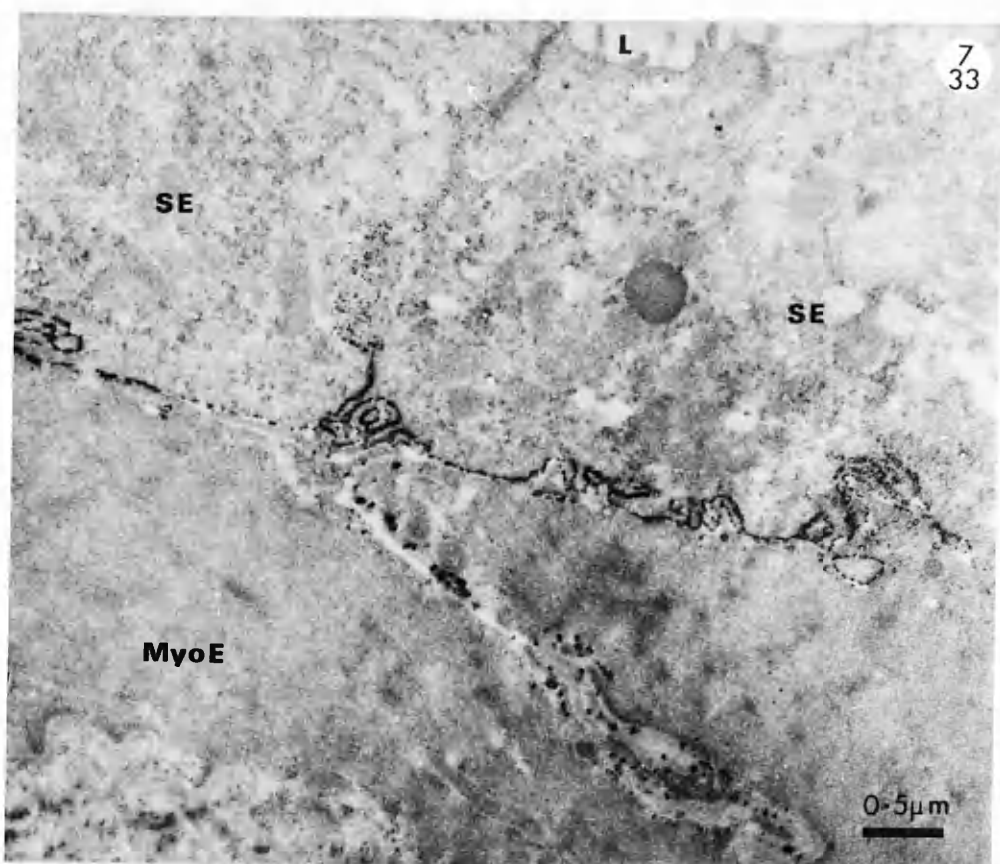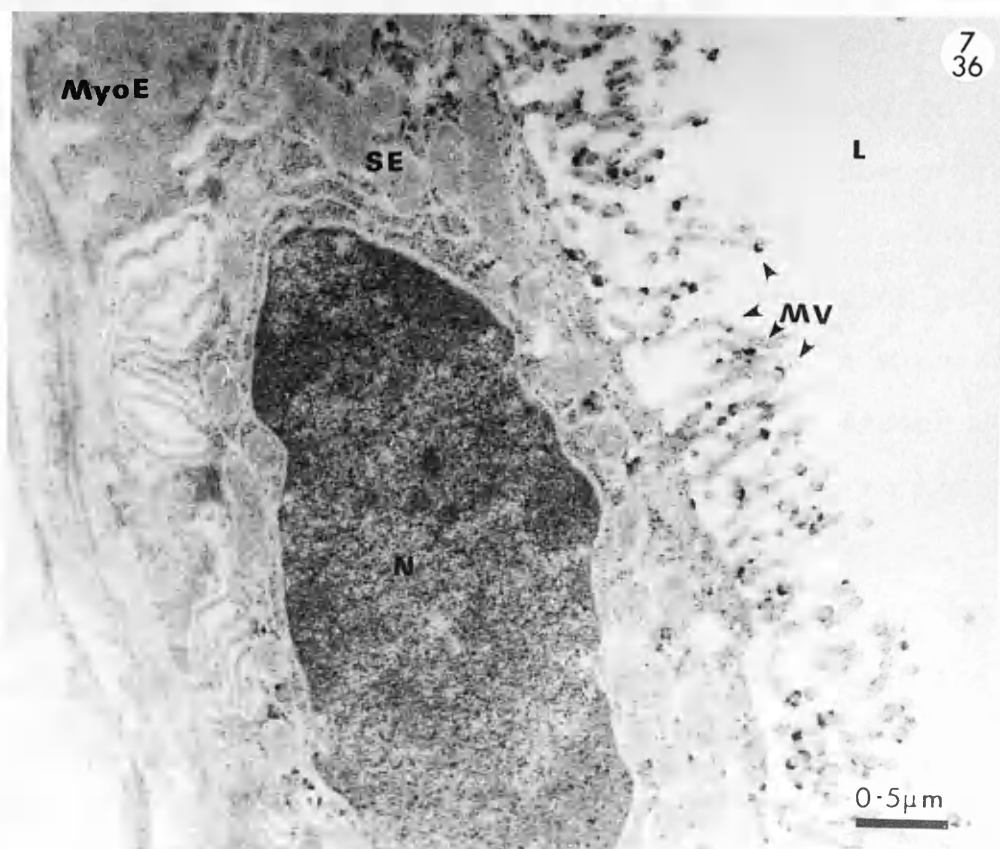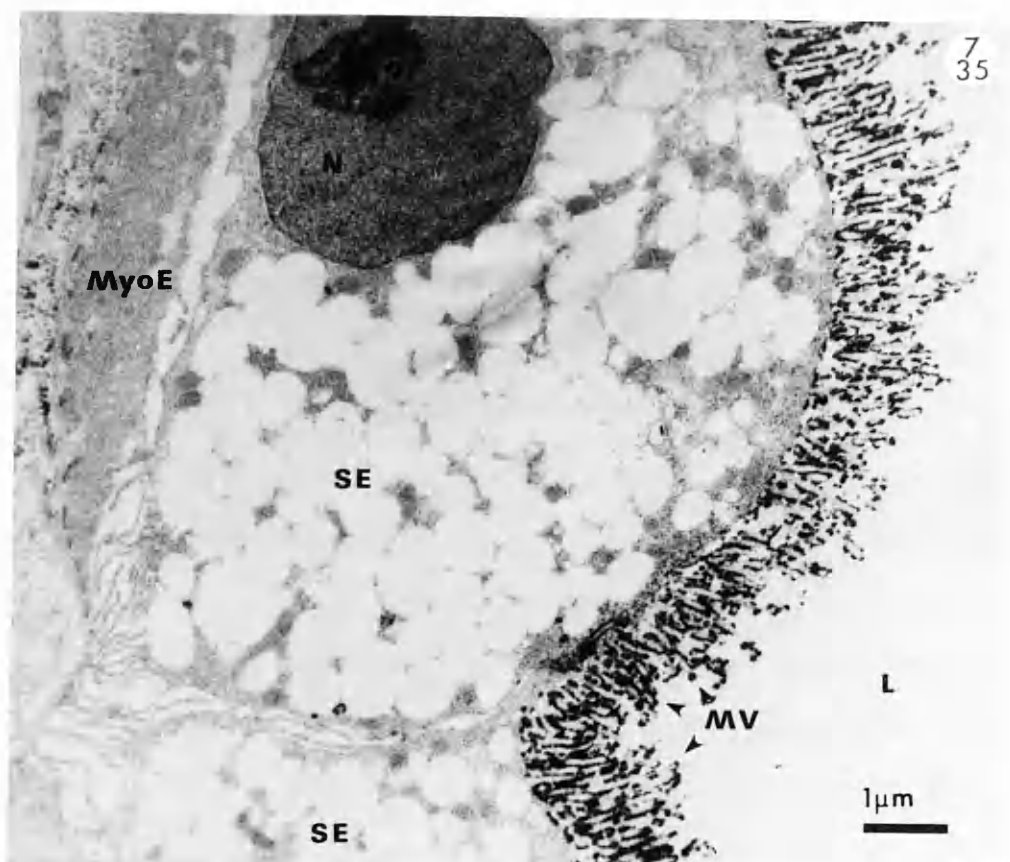
ouabain.

DISCUSSION.

   The results for the glandular localization of
Na,K-ATPase although interesting were disappointing as the
technique was not repeatable. When a positive reaction was
obtained it satisfied the criteria suggested by Ernst and
Hootman (1981). The reaction was potassium dependent,
inhibited by ouabain, present when an alkaline phosphatase
inhibitor was included and, in all but one instance,
localized to the cytoplasmic side of the plasma membrane.
Rat kidney and the basal and basolateral membranes of
secretory cells from rat footpad, sheep and horse sweat
glands satisfied these criteria. When present on the
luminal membrane of secretory cells from the sheep and
horse sweat glands it also satisfied these criteria. The
reaction product in the cow was found on the outer leaflet
of the secretory cell luminal membrane. Leuenberger and
Novikoff (1974) in the rat cornea and Milhorat, Davis and
Hammock, (1975) in the choroid plexus also found reaction
product only on the extracellular side of the plasmalemma.
Quinton, Wright and Tormey, (1973) using tritiated ouabain
on the choroid plexus localized the reaction to the
ventricular surface of the epithelium agreeing with the
physiological data of Wright (1972). Although Milhorat et
al., (1975) and Quinton et al., (1973) localized the
enzyme on different sides of the membrane, the reaction
deposition, like that found in cow and sheep, was
K-dependent and ouabain sensitive.

   The failure of the technique to provide reproducible
results, despite the range of modifications, may not be

due to technical shortcomings but specific requirements of
the enzyme. The enzyme may be extremely labile and
deteriorate rapidly after the biopsy has been taken. This
seems unlikely as unfixed tissue from cattle, sheep and
goat gave a negative result. Studies have shown activation
of Na,K-ATPase by secretagogues such as carbachol, CCK_8,
secretin and VIP in guinea pig pancreatic acinar cells
(Hootman, Ernst & Williams, 1983) and by carbachol and
cGMP in rat submandibular gland (Shi, Stewart & Sen,
1980). The localization of Na,K-ATPase on the luminal
microvilli of the nervous horse at rest indicates an
adrenergic component may be necessary for activation of
the enzyme in sweat glands. Both cholinergic and
adrenergic components are involved in the sweating
response of the horse (Lovatt Evans & Smith, 1956;
Jenkinson, Montgomery & Elder, 1978; Johnston & Creed,
1982). The effects of secretagogues on Na,K-ATPase
activity in the sweat gland requires further study. There
is evidence for the appearance of ATPase sites in response
to aldosterone and antidiuretic hormone (ADH) in cultured
monolayers of amphibian bladder epithelial cells (Lewis,
1983; Kraehenbuhl, Bonnard, Geering, Girardet & Rossier,
1983). This system relies on a "cytoskeleton with
vesicles" containing transport proteins which can be
inserted into the appropriate membrane upon activation
(Lewis, 1983). Such a system in sweat glands is unlikely,
as if vesicles of this type were present they would be
expected to stain for Na,K-ATPase and be present in the
cytoplasm of unstimulated sweat gland secretory cells.

The main distribution of alkaline phosphatase was on the myoepithelium and the adjacent secretory cell membranes. Only the horse showed reaction product on the luminal microvilli of secretory cells and not on the membranes of the myoepithelium. The distribution of alkaline phosphatase was unaffected by ouabain and thermal stimulation, except for the reduction in the horse, but was completely inhibited by levamisole. These findings do not rule out the possibility that the reaction product found using the Ernst (1972a,b) technique for Na,K-ATPase in the cow, sheep and horse was a levamisole-resistant alkaline phosphatase (Firth & Marland, 1975). The question remains however, why did the reaction for Na,K-ATPase only occur in the cow after thermal stimulation and why was it abolished in sheep after gland fatigue.

Capture techniques using other metal ions such as Cerium (Robinson & Karnovsky, 1983) can be used and visualised directly or be detected by Electron Spectroscopic Imaging producing an elemental distribution map of the specimen. The autoradiographic localization of Na,K-ATPase using the specificity of tritiated ouabain binding to the enzyme (Stirling, 1972) and immunocytochemical techniques with labelled antibody (Kyte, 1976a,b; Sztul, Biemesderfer, Caplan, Kashgarian & Boyer, 1987) on lightly fixed or ultrathin frozen sections will have to be tested before more experiments on thermally stimulated sweat glands are undertaken. The isolation of sweat glands by the shearing technique of Lee, Jones and Kealey (1984) and their maintenance in culture (Lee et al., 1984) before examination by

cytochemistry can also be tested. Biochemical assays of
sweat glands using the techniques described by Schuurmans
Stekhoven and Bonting (1981) would also be of importance
in determining the overall activity of Na,K-ATPase.
Using a combination of these techniques, sweat glands can
be examined in an attempt to explain the results obtained
in the initial experiments using the Ernst technique for
Na,K-ATPase.

CHAPTER 8.

GENERAL DISCUSSION.

These, the first comparative dynamic studies of sweat
gland ultrastructure, illustrate that the sweat gland duct
in all species can be divided into two main regions, the
intraepidermal region where it traverses either the
epidermis or the hair follicle and the intradermal region.
The structure of these two regions is basically the same
in all species. The intradermal region can be sub-divided
into an upper and lower ascending duct and a transition
zone. The only difference observed between species was the
presence in man of a coiled ductal zone adjacent to the
transition zone with the fundus. The keratinized cells at
the mouth of the duct in all the species examined are
likely, as are those of the epidermis (Jenkinson, 1980),
to protect against physical damage and the penetration of
foreign bodies. Jenkinson and Lloyd (1979) postulated that
sebum and sweat form an emulsion which permeates between
the squames and exudes from this intercellular reservoir
on to the skin surface at the cell margins, where it may
well change in composition and harden to form a sealant.
Therefore, since the duct pore in this zone is contiguous
with the epidermis it would seem to have a role in
spreading sweat over the surface of the skin.

The underlying portion of the intraepidermal zone and
upper portion of the intradermal duct in all species
exhibit small membrane bound vesicles associated with
luminal microvilli (Figs. 3.11 inset, 4.13 inset, 5.8
inset & 6.16,17). Their function in this region is still

unknown, as is their source. Vesicles of this type have
also been illustrated and described in the unstimulated
human eccrine and apocrine gland (Kurosumi, 1977;
Hashimoto, 1978a,b; Tani, Yamamoto & Mishima, 1980). They
were considered by Shibasaki and Ito (1967), Kurosumi
(1977) and Hashimoto (1978a) to originate from the
microvilli by budding and were ascribed, without
supporting evidence, an 'excretory' role. There was no
ultrastructural evidence from the species examined in this
study to indicate that these vesicles were formed and
released by the luminal cells in the duct in this zone
although this would seem to be the most likely
possibility. However, they could be derived from the
secretory products of the fundus or remnants of dead
secretory cells trapped by the microvilli since after
thermal stimulation and increased fluid output, there is a
marked reduction in their quantity in the duct. There is
also no evidence from this study on whether or not
absorption of these vesicles by luminal duct cells occurs.

Within the luminal cells of the lower intraepidermal
and upper dermal zones, vesicles of different morphology
were found closely associated with the luminal plasma
membrane. These vesicles had bristle-like structures on
their cytoplasmic surface and formed coated pits
(Fig.6.17). Similar coated vesicles isolated from brain,
have been found to contain several major proteins, the
best characterised of which is clathrin (Ungewickell &
Branton, 1981). In the sweat glands these vesicles
probably provide a specialised pathway for macromolecules
in the lumen to be taken into the luminal cells by

absorptive endocytosis (Goldstein, Anderson & Brown, 1979; Pastan & Willingham, 1981). These components of sweat secretion, probably proteins, may later be hydrolysed in the numerous multivesicular bodies at the base of the terminal web within the luminal cells (Fig.6.15). This view was supported by Kurosumi (1977) who considered that the small, empty vesicles of this type (Fig.6.17) appearing near the luminal surface are pinocytotic and involved in the reabsorption of substances from the sweat. Dynamic studies are now required for the elucidation of the roles in sweat formation of both types of vesicle found in the duct.

In the lower ascending duct in all species and in the additional coiled zone of man, uncoated cytoplasmic vesicles, which increased markedly in number during sweating (Fig.6.39,40), were present in the luminal cells. Kurosumi et al., (1982) considered that these vesicles, which stain with methenamine-silver, arise from the Golgi apparatus and are likely to be involved in transport of secretory products to the luminal membrane. The coated vesicles common in the intraepidermal and upper intradermal duct were not present in the cells of this zone.

The most marked changes in ductal morphology occurred in the coiled zone of the human duct during thermal stimulation. The luminal cells exhibited varying degrees of apical distention which were never seen in the unstimulated duct. Although this appearance may have been influenced by glutaraldehyde fixation (Shelton & Mowczko, 1978; Sletyr & Robards, 1982) there is little doubt that a

change in the morphology of the luminal membrane and apical cytoplasm occurs during sweating. The distention of the plasma membrane and distortion of the microvilli could be the result of fluid filtering through the apical terminal web of microfilaments. The microfilaments of the terminal web are probably maintained by the large electron-opaque bodies which are more prominent within the terminal web of luminal cells in the active duct (Fig.6.38). These structures strongly resemble the 'junctional-complex associated bodies' previously reported for the clear (non-granular) cells of the human eccrine sweat gland fundus by Spicer, Martin and Simon (1972). The apical blebbing of the luminal membrane in the coiled duct of man during stimulation is similar to the configuration found in the secretory epithelium of rat footpad sweat glands after pilocarpine stimulation (McWilliams, 1986). This suggests that the coiled duct of man has a secretory role during prolonged stimulation. However, the coiled duct of man is also an area where ductal sodium reabsorption occurs (Sato, 1977). Sodium crosses the luminal membrane either by Na channels or a sodium proton exchange system and is pumped out at the basolateral membrane by Na,K-ATPase (Quinton, 1981,86). Quinton (1986), suggested that the epithelium has a high chloride permeability with chlorine following a paracellular route to maintain electrical neutrality. Whether a similar or different system occurs in the lower ascending duct of cow, sheep, goat and horse remains to be determined.

A junctional complex is present at the luminal surface throughout the duct in all species, except for the

keratinized intrafollicular/intraepidermal regions. Studies in man (Hashimoto, 1971,1978a), cattle, sheep, goat and horse using lanthanum as a tracer (Jenkinson et al., 1983) have demonstrated that these are tight junctions and suggest that they constitute a barrier to fluid flow even during glandular activity. The presence of discrete gap junctions between neighbouring basal duct cells in the lower body region in all species may indicate electrical coupling between the cells in this zone. In the cow these gap junctions were sometimes seen to have interconnections across the gap similar to those of septate junctions. Since gap junctions are areas of ionic and metabolic coupling and regions of low electrical resistance for cell to cell propagation of excitation impulses, these cells are likely to act in concert. The dye coupling experiments of Jones and Kealey (1985, 1987) on isolated human sweat glands illustrate that cells of the inner and outer layers of the duct act as a syncytium. Therefore, apical and basal cell compartments will be able to comunicate and react rapidly to changes in the composition of sweat in this part of the duct during secretion.

Sweat in all the species studied was formed by secretion which consisted of two components, exocytosis and fluid transport. In the horse and man a minor microapocrine mechanism was also present. Cell death which was found in all species must also be considered as a component of sweat formation. The secretory processes of exocytosis and fluid transport are undertaken by one type of secretory cell in the cow, sheep, goat and horse.

However, the secretory epithelium of man appears to have specialized and devolved the function of exocytosis of proteins to the granular cells while the non granular cells have become pumps for fluid transport. Exocytosis in equidae and man results in total depletion of the stored vesicles in the secretory cells and although cattle have fewer stored vesicles, loss during activity is still prominent. Sheep and particularly the goat exhibit vesicle release with marked Golgi and RER dilatation and activity for a short period following stimulation. Secretory vesicles in all the species were derived from the Golgi apparatus although there was evidence that in cattle they are also formed by mitochondrial transformation.

The ultrastructure of the myoepithelium in all the species examined was similar. The only differences were in the horse and man where the myoepithelium did not overlap at the poles thus allowing the secretory cells to reach the basement membrane. The orientation of the myoepithelium was with the longitudinal axis of the cells approximately parallel to the length of the glands. This result agrees with the comparative results of Jenkinson (1971). During sweating the myoepithelium, of all species, appeared contracted, but otherwise was morphologically the same as in the unstimulated gland. This finding lends support to the thesis of Sato (1980) that the myoepithelium is probably not directly involved in sweat expulsion. The principal role of the well-developed myoepithelial cells may therefore be the provision of structural support for the secretory epithelium, as suggested by Montagna and Parakkal (1974) and Sato,

Nishiyama and Kobayashi, (1979). However, observations of the sweat glands of the living bat wing (Murphy, 1960; Cortese & Nicoll, 1970) and human sweat gland (Nicolaidis & Sivadjian, 1972) demonstrate that pulsatile contractions of the myoepithelium can occur in mammalian sweat glands. Therefore, the possibility of phasic myoepithelial contraction cannot be eliminated from an appearance of continued contraction obtained from purely morphological evidence.

The process of fluid transport is associated with extensive folding of the cell surfaces of the secretory epithelium, a structural characteristic of transporting epithelium. Equidae have long closely packed apical microvilli with lateral and basolateral interdigitations which reach the basement membrane. The non granular secretory cells in man have short, sparse, apical microvilli and complex basal infoldings on the serosal membrane with canaliculi between neighbouring non granular cells. Sheep and goat follow this pattern with numerous short and frequently branched luminal microvilli but with fewer basal infoldings. Cattle have short widely spaced apical microvilli and microvillous projections at the serosal membrane. Although the mechanism of fluid transport could be explained using the standing gradient model of Diamond and Bossert, (1967) it cannot be explained on the basis of microvilli or interdigitations. The discrete, stepwise and smooth onset patterns of sweating proposed by Bligh, (1967) are not explained by differences in the ultrastructure of the glands during thermal stimulation. The smooth onset pattern obtained

from horse and man is believed to be the result of secretion with little or no myoepithelial action. The intermittent and decremental pattern to fatigue exhibited by the sheep and goat is thought to be due to the rate of sweat expulsion exceeding that of production (Jenkinson & Robertshaw, 1971; Johnson, 1973). On the basis of the present results it seems more likely that the patterns of sheep and goat are due to gradual secretory cell exhaustion and failure and not as suggested by Allen and Bligh, (1969) and Johnson, (1973) to an imbalance between the secretory and discharge mechanisms. The stepwise pattern obtained from the cow is interpreted as secretion superimposed on myoepithelial contractions. Although cattle can maintain sweating for more than five hours the secretory cells have less extensive folding of the cell surfaces when compared to equidae and man which also display a sustained pattern of sweating.

Since the available ultrastructural evidence does not fully explain the patterns of sweat output and since fluid output is one of the most important features of sweating, the mechanism of fluid transport in sweat glands during thermal stimulation was considered. Fluid transport is now known to be driven by Na,K-ATPase in most instances and hence the localization of this enzyme which would be of importance in establishing the cellular route of electrolyte flow across the secretory epithelium was studied. Trial experiments for the cytochemical localization of Na,K-ATPase in cow, sheep and horse demonstrated the enzyme on basolateral membranes of the secretory cells at rest and activity. The enzyme was also

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present on the luminal membrane of one horse at rest and in the cow, sheep and horse after activity. Unfortunately when the localization of Na,K-ATPase was repeated on a larger sample during thermal stimulation this initial result was not reproducible. Na,K-ATPase has been localized on the luminal membrane of the dog submandibular gland acini by Nakagaki, Goto, Sasaki and Imai (1978), using p-nitrophenylphosphatase cytochemistry. Tanaka, Akayama, Yamamoto, Omori and Tashiro, (1987), using immunoelectron microscopy, also localized Na,K-ATPase on the luminal membrane of secretory cells in the rat exocrine pancreas. Therefore, it is possible that the cow, sheep, goat and horse may have a Na,K-ATPase, which unlike that of the rat, does not respond to the Ernst technique (1972a,b) on every occasion despite efforts to standardise the proceedure. The physiological state of sweat glands in these species after sampling may be such that the localization of Na,K-ATPase using the K-dependent p-nitrophenylphosphatase activity of the complex cannot be repeated. Immunoelectron microscopy and the use of tritiated ouabain may therefore be methods which may be valuable for the localization of Na,K-ATPase in sweat glands.

Langerhans cells were found throughout the entire length of the duct and fundus in all five species. These cells are almost certainly, as postulated for those in the epidermis of man (Stingl, Katz, Green & Shevach, 1980) involved in immuno-defence. They were most commonly found in the glands of horse and man where they were readily identified by their cytoplasmic Birbeck granules. Cattle

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had the fewest number present and identification was more difficult because of the paucity of Birbeck granules in the cytoplasm. The study of Tanaki, Stingl and Katz (1980) indicates that epidermal Langerhans cells are derived from and continuously replenished by a mobile pool of precursor cells which originate in the bone marrow. Their presence between cells of the hair follicle and duct of the human sebaceous gland (Jimbow, Sato & Kukita, 1969) suggests migration from the hair follicle down the duct between cell layers. There was no evidence in the present study to support the alternative hypothesis of cell penetration through the basal membrane.

The failure to detect nerve terminals in the vicinity of the duct in cow, sheep, goat and horse strengthens the conclusion of Jenkinson, Montgomery and Elder (1978) that the sweat glands of these species are not innervated. Although nerves were never found in the vicinity of the duct in man, varicosities occured within the fibrocyte sheath of the fundus (Jenkinson et al., 1978) close enough to have a direct influence on the glands. The evidence is thus consistent with the view of Jenkinson et al., (1978) that the sudomotor mechanism is basically the same in all species. They suggested that adrenergic and cholinergic nerves act on the cutaneous blood vessels and that local catecholamine transfer to the gland ensues. According to Jenkinson et al., (1978) there is an additional mechanism in man where transmitters released at parasympathetic nerve endings, in close proximity to the glands, in particular acetylcholine, also have a direct action on the glands.

The presence of cell debris in the duct and gaps in the secretory epithelium in man caused by cell death, raises the question as to the mode of cell replacement in both the fundus and duct. The embryological development of mammalian sweat glands is from a down-growing column of cells emanating from the hair follicle bud (Hardy & Lyne, 1956; Lyne & Heideman, 1959) or as in man the epidermis (Hashimoto, Gross & Lever, 1965). Three possible sources of postnatal cell replacement have been suggested. Christophers and Plewig (1973), on the basis of studies with tritiated thymidine, suggested that the cells arise at a germinal zone in the lower epidermis and migrate to form the duct cells of the acrosyringium. Hashimoto (1978a) on the other hand considered that the epithelium of the duct is produced by germinative cells deep within the duct at the transition portion. The evidence to date in the human indicates that basal cells of the coiled zone of the duct are the source of new cells (Lobitz, Holyoke & Montagna, 1954; Dobson, Abele & Hale, 1961). In the footpad sweat glands of the mouse, Bullough and Doel (1972) found mitosis in the intradermal duct where it leaves the dermis and in the fundus duct transition zone. A feature common at the fundus duct transition zone was the differentiation of basal duct cells into myoepithelium (Chapter 3, 4). It is possible that Bullough and Doel (1972) interpreted these transforming cells as mitotic cells in a zone where mitosis was thought to occur. A third possible source of replacement is indicated from studies of the fundus using light microscopy (Dobson & Lobitz 1958). Configurations resembling post mitotic

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daughter cells were found in the secretory epithelium of the human gland during recovery from sweating. After injury all the cells of the secretory coil, including myoepithelium, show increased mitotic activity (Lobitz & Dobson, 1957). The mitotic activity observed in the human sweat gland fundus after injury (Lobitz & Dobson, 1957) is however a pathological response to trauma and may not represent the normal response after stimulation. The gaps in the active secretory epithelium of the human do not suggest the rapid replacement of the fundus epithelium by re-attachment of adjacent cells. This is in contrast to the domestic animals, where the luminal intercellular connections between epithelial cells remain tight to lanthanum even after prolonged sweating (Jenkinson et al., 1983). Although Dobson and Lobitz (1958) described configurations resembling mitotic daughter cells in the fundus of the human during recovery after sweating, configurations such as this were never found during recovery in sheep and goat.

Of the three possible sources of cell replacement the conclusion of Christophers and Plewig (1973) which is based on studies of mitosis and cell migration using tritiated thymidine autoradiography, planimetry and three-dimensional reconstruction is the most plausible. These have been the only dynamic studies of cellular proliferation in the human eccrine sweat gland and the exact location of mitosis in sweat glands of cow, sheep, goat and horse would require a further series of comparable experiments. Such experiments would be particularly valuable after activity of the glands of man, horse, sheep

and goat where cell death and the production of replacement cells has been induced.

The results of the comparative study illustrate that sweat glands can no longer be defined using the classification proposed by Schiefferdecker (1917). Those of the cow, sheep, goat and horse satisfy two of the criteria of the apocrine gland as they develop from epidermal hair follicle buds and are associated with hair follicles. They do not however, satisfy the principle criterion as they do not display classical apical degeneration. The present results illustrate that secretion in these species is by vesicle exocytosis through the luminal membrane and fluid transport, although the horse exhibits a secondary microapocrine process. The human sweat gland, eccrine according to Schiefferdecker (1917), develops from the epidermis and is not associated with hair follicles. However the evidence of cell death and microapocrine secretion after stimulation illustrates that secretion is not basically different from that of the other species and is more than the release through the luminal membrane without rupture and loss of cytoplasm required of eccrine secretion

The occurence of cell death in all the species studied suggests a classification of holocrine. However, since degenerate cells only contribute a proportion of the sweat constituents the process cannot be holocrine (G. Holos, all + crinein, separate) as defined. Also, in the species where increased cellular disruption follows stimulation, some of the cells almost certainly secrete vesicles before degeneration and hence contribute to the

sweat more than their cell contents after disruption as
with holocrine secretion. The term, partly holocrine, is
also unacceptable since it is not yet known if in all
species the cell degeneration is a true secretory process
induced or accelerated by stimulation or simply a
manifestation of an associated mechanism of cell
replacement.

The classification of sweat glands therefore requires
reassessment and until more detailed information on the
processes involved in sweat formation is forthcoming, it
would seem prudent to dispense with the current misleading
categories 'apocrine' and 'eccrine' and revert to
anatomical definitions. The anatomical definitions,
epitrichial and atrichial (Bligh, 1967), adequately
distinguish glands associated with hair follicles from
those which open directly to the epidermis and, if applied
provide the basis for fresh assessment of sweat gland
physiology.

TRANSMISSION ELECTRON MICROSCOPY.

The skin samples were fixed and processed using the following solutions and method.

FIXATIVES.

The fixative solutions are from those of Sabatini, Bensch and Barrnett (1963).

PRIMARY FIXATIVE.

1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	70ml.
25% GLUTARALDEHYDE.	=	8ml. (E.M. GRADE.)
SUCROSE.	=	1.7g.
1M. CALCIUM CHLORIDE.	=	0.1ml.

Adjust to pH 7.2, bring to 100ml with distilled water.

BUFFER WASH.

1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	80ml.
SUCROSE.	=	5.9g.

Adjust to pH 7.2, bring to 100ml with distilled water.

POST FIXATIVE.

1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	50ml.
SUCROSE.	=	5.1g.

Adjust to pH 7.2, bring to 100ml with distilled water.

BUFFER.	=	3 PARTS.
4% OSMIUM TETROXIDE.	=	1 PART.

METHOD.

The skin samples were divided into E.M. blocks as shown in Fig.A.1 and processed using the following schedule.

1.)   Primary fixative. = 3 hours.

2.)   Buffer wash.   = 3 x 20 minutes. Specimens can be left overnight in buffer if necessary.

3.)   Post fixative.   = 2 hours.

4.)   Buffer wash.   = Rinse.

5.)   Dehydrate slowly using graded alcohol or acetone.

6.)   Propylene oxide as intermediate solvent.

7.)   Propylene oxide/Araldite. = Overnight. (Glauert, Rogers & Glauert, 1956; Glauert & Glauert, 1958)

8.)   Fresh unpolymerised resin for 3-4 days.

9.)   Embed in flat embedding trays and polymerize at 60°C.

STAINING.

1µm SECTIONS.

1µm sections were mounted on gelatine/chrome alum subbed slides then stained using the following technique.

TOLUIDINE BLUE/PYRONIN Y. (Ito & Winchester, 1963)

SODIUM BORATE. (BORAX)   = 0.8g.

DISTILLED WATER.   = 100ml.

Dissolve, then add in order

TOLUIDINE BLUE.   = 0.8g.

PYRONIN Y.   = 0.2g.

Dissolve, then filter into a stock bottle.

Stain sections at room temperature until the desired depth of staining is obtained. Wash in running tap water, air dry and mount in immersion oil or synthetic mounting

A.) WHOLE BIOPSY.

DURING DIVISION THE BIOPSY IS FLOODED WITH THE PRIMARY
FIXATIVE.

B.) THE BIOPSY IS HALVED AND LAID ONTO THE CUT SURFACE.
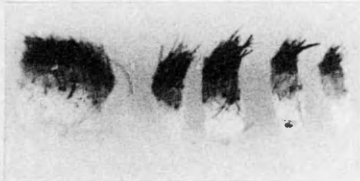
C.) ONE HALF IS DIVIDED INTO FOUR. THE OTHER HALF CAN BE
USED FOR CYTOCHEMISTRY OR DIVIDED.

D.) THE QUARTERS ARE LAID ONTO THEIR BROAD SURFACE.
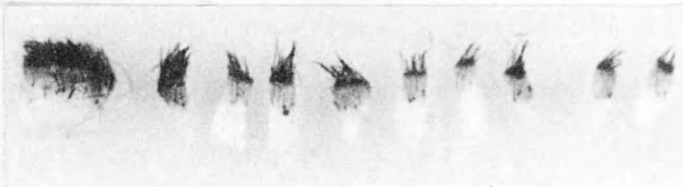
E.) EACH QUARTER IS FURTHER DIVIDED TO PROVIDE BLOCKS OF A
SUITABLE SIZE AND SHAPE FOR PROCESSING AND SECTIONING.

medium. The stain gives a very pleasing result of varying shades of blue.

ULTRATHIN SECTIONS.

Ultrathin sections, cut using a diamond knife, were mounted on copper grids with a formvar foil then stained with 2% Uranyl Acetate in 50% Ethanol or Methanol (Stempak & Ward, 1964) followed by Reynolds Lead Citrate (Reynolds, 1963).

SECTIONING REGIME.

FUNDUS.

Using glass knives and examined by light microscopy blocks were semithin sectioned perpendicular to the skin surface until the sweat gland fundus was found. The blocks were then trimmed and ultrathin sections cut using a diamond knife. Semithin sections were then cut at 10μm intervals through the block until the fundus/duct transition zone was found. The block was trimmed if necessary and ultrathin sections cut using a diamond knife.

DUCT.

The blocks used for studies of the duct were sectioned parallel to the skin surface. Starting at the epidermis blocks were sectioned at 10μm intervals until a duct was found. The duct chosen was from a central position in the block as experience illustrated that ducts from the periphery were frequently lost. A selected duct from each sample was sectioned at 1μm and ultrathin sections were taken every 20μm throughout its entire length. The remaining blocks were again sectioned at 1μm and ultrathin sections cut at 20μm intervals in the

intrafollicular region and perifollicular zone of the intradermal region. The duct body was ultrathin-sectioned at 50μm intervals to the fundus.

LIGHT MICROSCOPY.

The stained 1μm sections were examined using a Zeiss GFL Standard microscope.

ELECTRON MICROSCOPY.

The stained ultrathin sections were examined with an A.E.I.EM6B electron microscope at 60kV or a J.E.O.L.100C electron microscope at 80kV. Electron micrographs were taken on Ilford E.M. plates and developed with Ilford PQ Universal developer.

APPENDIX 2.

CYTOCHEMISTRY.

SODIUM, POTASSIUM – ADENOSINE TRIPHOSPHATASE.

(Ernst, 1972a,b; Firth et al., 1979)

The following technique was used to localize the ouabain sensitive K-dependent p-nitrophenylphosphate (K-NPPase) activity of the Na,K-ATPase complex at the ultrastructural level.

SOLUTIONS.

PRIMARY FIXATIVE.

25% GLUTARALDEHYDE.	=	1ml. (DISTILLED.)
10% PARAFORMALDEHYDE.	=	10ml.
1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	70ml.
SUCROSE.	=	1.7g.

Adjust to pH 7.2, bring to 100ml with distilled water.

CACODYLATE BUFFER WASH.

1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	80ml.
SUCROSE.	=	5.9g.

Adjust to pH 7.2, bring to 100ml with distilled water.

POST FIXATIVE.

1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	60ml.
SUCROSE.	=	5.1g.

Adjust to pH 7.2, bring to 75ml with distilled water.

```
BUFFER.                        =   3 PARTS.

4% OSMIUM TETROXIDE.           =   1 PART.
```

PRE-POST INCUBATION RINSE. (100mM TRIS.)

```
TRIZMA BASE.                   =   6.05g.

DISTILLED WATER.               =   450ml.

SUCROSE.                       =   25g.
```

Adjust to pH 9, bring to 500ml with distilled water.

7% AGAR.

2% LEAD NITRATE.

8.6% SUCROSE. (250mM.)

INCUBATION SUBSTRATE.

```
TRIZMA BASE.                   =   1.21g. (100mM.)

DISTILLED WATER.               =   90ml.
```

Adjust to pH 9, bring to 100ml with distilled water.

```
100mM TRIS BUFFER.             =   90ml.

p-NITROPHENYL PHOSPHATE.       =   0.26g. (10mM.)

MAGNESIUM CHLORIDE.            =   0.2g. (10mM.)

POTASSIUM CHLORIDE.            =   0.075g. (10mM.)

STRONTIUM CHLORIDE.            =   0.53g. (20mM.)

LEVAMISOLE.                    =   0.12g. (5mM.)
```

Check pH (9), bring to 100ml with TRIS BUFFER.

CONTROL SUBSTRATE.

```
A.)  - POTASSIUM CHLORIDE.

B.)  + 5mM OUABAIN (0.36g/100ml.)

C.)  - LEVAMISOLE.
```

METHOD.

1.) Samples were flooded with the Primary fixative and divided as shown in plate A1.

2.) Cacodylate buffer wash. = 3 x 20 minutes.

3.) Pre-incubation rinse. = 3 x 20 minutes then overnight if necessary.

4.) Mount on a sectioning disc and surround with molten agar (60°C). Allow the agar to set and cool to room temperature.

5.) Cut 50μm sections with Smith/Farquhar· tissue chopper (Sorvall T.C.1.).

6.) Float out sections in pre-incubation rinse.

7.) With glass rods transfer the sections to fresh pre-incubation rinse. During this transfer the better sections can be selected and separated from the agar.

8.) Incubation substrate. = 45 minutes at room temperature on a tissue rotator.

9.) Post-incubation rinse. = 3 rinses.

10.) 2% Lead nitrate. = 2 x 5 minutes.

11.) 8.6% Sucrose. = Rinse.

12.) Post-incubation rinse. = Rinse.

13.) Post fixative. = 1 hour.

14.) Dehydrate in graded alcohol.

15.) Intermediate solvent.

16.) Infiltrate with epoxy resin.

17.) Embed in fresh resin.

18.) Cut ultrathin sections and mount on copper grids.

19.) Lightly stain with uranyl acetate. Sections can be stained with lead citrate to enhance contrast after initial localization of the enzyme deposits.

SODIUM/POTASSIUM-DEPENDENT ADENOSINE TRIPHOSPHATASE.

(Mayahara et al., 1981)

SOLUTIONS.

PRIMARY FIXATIVE.

10% PARAFORMALDEHYDE.	=	20ml.
25% GLUTARALDEHYDE.	=	2ml. (DISTILLED.)
1M. SODIUM CACODYLATE.	=	10ml.
DISTILLED WATER.	=	70ml.

Adjust to pH 7.2, bring to 100ml with distilled water.

BUFFER WASH.

1M. SODIUM CACODYLATE.	=	1ml.
SUCROSE.	=	8.55g.
DIMETHYLSULPHOXIDE.	=	10ml.
DISTILLED WATER.	=	80ml.

Adjust to pH 7.2, bring to 100ml with distilled water.

POST FIXATIVE.

1M. SODIUM CACODYLATE.	=	10ml.
SUCROSE.	=	5.1g.
DISTILLED WATER.	=	60ml.

Adjust to pH 7.2, bring to 75ml with distilled water.

BUFFER.	=	3 PARTS.
4% OSMIUM TETROXIDE.	=	1 PART.

POST INCUBATION RINSE.

1M. SODIUM CACODYLATE.	=	10ml.
SUCROSE.	=	8g.
DISTILLED WATER.	=	80ml.

Adjust to pH 7.2, bring to 100ml with distilled water.

1M. GLYCINE-KOH BUFFER.

POTASSIUM HYDROXIDE.	=	5.61g.

Dissolve and bring to 100ml with distilled water.

GLYCINE.	=	7.5g.
DISTILLED WATER.	=	90ml.

Adjust to pH 9 with KOH, bring to 100ml with distilled water.

1% LEAD CITRATE.

POTASSIUM HYDROXIDE.	=	0.28g.
DISTILLED WATER.	=	95ml.
DISSOLVE.		
LEAD CITRATE.	=	1g.

Dissolve and bring to 100ml with distilled water.

PRE-INCUBATION RINSE.

GLYCINE-KOH.	=	25ml.
DISTILLED WATER.	=	75ml.

INCUBATION SUBSTRATE. (ADD IN THE ORDER GIVEN.)

p-NITROPHENYLPHOSPHATE(Mg).	=	0.24g. (100mM.)
DISTILLED WATER.	=	10ml.
GLYCINE-KOH.	=	25ml.
(GLYCINE-250mM. K-20mM.)		
DIMETHYLSULPHOXIDE.	=	25ml. (25%.)
DEXTRAN.	=	3g. (3%.)
LEAD CITRATE.	=	40ml.
(Pb/Cit.-4mM. K-20mM.)		
LEVAMISOLE.	=	0.06g. (2.5mM.)

CONTROLS.

A.) Substrate free medium.

B.) OUABAIN. Pre-incubate for 30 minutes with pre-incubation rinse plus 10mM ouabain (0.72g/100ml).

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Incubation substrate plus 10mM ouabain (0.72g/100ml).

C.) Exclude Potassium ions from the substrate and replace with Sodium.

GLYCINE-NaOH BUFFER. Adjust pH with 1M (N) NaOH.

1% LEAD CITRATE. Replace KOH with 0.2g NaOH.

D.) Exclude LEVAMISOLE.

METHOD.

1.) PRIMARY FIXATIVE.   = 1 hour. (0-4°C.)

2.) BUFFER WASH.   = 3 x 20 mins.

(Overnight if necessary at 0-4°C.)

3.) 50µm SMITH/FARQUHAR SECTIONS.

4.) PRE-INCUBATION RINSE.   = RINSE.

5.) INCUBATION SUBSTRATE.   = 10-20 mins. (R.T.)

6.) POST INCUBATION RINSE. = 2 x 5 mins

Rinse the incubation vessel well to remove traces of the DMSO. DMSO oxidises the osmium tetroxide of the post fixative. Incomplete rinsing causes the vessel and post fixative to blacken very quickly.

7.) POST FIXATIVE.   = 1 hour.

8.) DEHYDRATE WITH GRADED ALCOHOL.

9.) EMBED IN ARALDITE.

10.) SECTION AND STAIN WITH URANYL ACETATE.

SODIUM/POTASSIUM-DEPENDENT ADENOSINE TRIPHOSPHATASE.

(Firth,J.A. - Personal Communication.)

(Guth & Albers. 1974.)

SOLUTIONS.

2% COBALT CHLORIDE.

1% AMMONIUM SULPHIDE.

BUFFER WASH.

2-AMINO-2-METHYL-1-PROPANOL.	=	1.6ml. (70mM.)
DISTILLED WATER.	=	230ml.

Adjust to pH 9-9.1, bring to 250ml with distilled water.

INCUBATION SUBSTRATE.

2-AMINO-2-METHYL-1-PROPANOL.	=	0.67ml. (70mM.)
DISTILLED WATER.	=	60ml.
DIMETHYLSULPHOXIDE.	=	25ml. (25%)

Adjust to pH 9-9.1, bring to 100ml with distilled water.

POTASSIUM CHLORIDE.	=	0.22g. (30mM.)
MAGNESIUM CHLORIDE.	=	0.4g. (20mM.)
p-NPP. (SIGMA 104)	=	0.13g. (5mM.)

CONTROLS.

A.) 5mM LEVAMISOLE.	=	0.12g/100ml.
B.) 5mM OUABAIN.	=	0.36g/100ml.
C.) -ve POTASSIUM.		

METHOD.

1.) Unfixed cryostat sections.		
2.) Incubation substrate.	=	20 mins.- 2 hours. (RT)
3.) Buffer wash.	=	2 rinses.
4.) 2% Cobalt chloride.	=	5 mins.
5.) Buffer wash.	=	rinse.

6.) Distilled water.   = 3 rinses.

7.) 1% Ammonium sulphide. = 3 mins.

8.) Running tap water.   = wash.

9.) Counterstain as desired.

10.) Mount in glycerin jelly.

11.) or D.C.M. in D.P.X.

RESULT.

    Na,/K-ATPase activity: BLACK.

    Nuclei and cytoplasm as counterstained.

ALKALINE PHOSPHATASE.　(Millonig & Millonig, 1973.)

SOLUTIONS.

FIXATIVES.

Primary fixative, buffer wash and post fixative were the same as those used for the ultrastructural demonstration of Na,K-ATPase by the method of Ernst (1972a,b) described earlier.

7% AGAR.

PRE & POST INCUBATION RINSE. (100mM TRIS.)

TRIZMA BASE.	= 6.05g.
DISTILLED WATER.	= 450ml.

Adjust to pH 9.2, bring to 500ml with distilled water.

0.2M TRIS.

TRIZMA BASE.	= 2.4g.
DISTILLED WATER.	= 90ml.

Adjust to pH 9.2, bring to 100ml with distilled water.

1% SODIUM GLYCEROPHOSPHATE.

3% SODIUM CITRATE.

3% LEAD CITRATE.

INCUBATION SUBSTRATE.　(ADD IN THE ORDER GIVEN.)

0.2M TRIS.	= 76ml.
DEXTRAN.	= 2.28g (OPTIONAL)
1% Na GLYCEROPHOSPHATE.	= 4ml.
3% SODIUM CITRATE.	= 6ml.
3% LEAD NITRATE.	= 6ml.

Add the lead nitrate slowly while stirring, if a weak opalescence is formed several drops of citrate will

dissolve it. The solution is completely clear and stable
for several hours.

CONTROL SUBSTRATE.

A.) - SODIUM GLYCEROPHOSPHATE.

B.) + 5mM LEVAMISOLE. (0.12g/100ml.)

METHOD.

1.) Flood sample with the primary fixative and divide as
shown in plate A1.

2.) Cacodylate buffer wash. = 3 x 20 minutes.

3.) Pre-incubation rinse. = 3 x 20 minutes then overnight
if necessary.

4.) Mount onto a sectioning disc and surround with molten
agar (60°C.). Allow the agar to set and cool to room
temperature.

5.) Cut 50μm sections with Smith/Farquahar tissue chopper
(Sorvall T.C.1.)

6.) Float sections out in the pre-incubation rinse.

7.) With glass rods transfer the sections to fresh
pre-incubation rinse. During this transfer the best
sections can be selected from the agar.

8.) Incubation substrate. = 1-2 hours (38°C.)

9.) Post incubation rinse. = 3 rinses.

10.) Post fixative. = 1 hour.

11.) Dehydrate in graded alcohol.

12.) Intermediate solvent.

13.) Infiltrate with epoxy resin.

14.) Embed in fresh resin.

15.) Cut ultrathin sections and mount on copper grids.

16.) Lightly stain with uranyl acetate. Sections can be stained with lead citrate to enhance contrast after initial localization of the enzyme deposits.

APPENDIX 3.

   KEY TO FIGURES.

CHAPTERS 1 - 5.

A   Atretic luminal cell.

BC   Basal duct cell.

BD   Basal dovetailing.

BMV   Basal microvilli.

C   Centriole.

CT   Connective tissue.

D   Desmosome.

EF   Epithelial fold.

ES   Eccrine secretion.

F   Fibrocyte sheath.

G   Golgi apparatus.

GC   Granular cell.

GJ   Gap junction.

Gly   Glycogen.

H   Hair.

HD   Hemi-desmosome.

HF   Hair follicle.

JC   Junctional complex.

K   Keratohyalin granule.

L   Lumen.

La   Langerhans cell.

LaG   Langerhans cell granule.

LC   Luminal duct cell.

LMV   Lateral microvilli.

LV   Small vesicles in duct lumen.

Ly   Lysosome.

M   Mitochondrion.

MAS   Potential micro-apocrine secretion.

MyoE   Myoepithelium.

MyoF   Myofilaments.

MV   Microvilli.

N   Nucleus.

ND   Necrobiotic discharge.

RER   Rough endoplasmic reticulum.

S   Single inner cell.

SC   _Stratum corneum_.

SE   Secretory epithelium.

T   Tonofilaments.

V   Secretory vesicle.

VC   Ventilated capsule.

WB   Wet bulb.

ZO   _Zonula occludens_.

CHAPTERS 6-7.

AB   Apical blebbing.

BI   Basal infoldings.

BL   Basal lamina.

Ca   Canaliculus.

Cap   Capillary.

CB   Cytopasmic bridge.

CD   Cellular debris.

CP   Cytoplasmic protrusions.

CR   Cell remnants.

CT   Connective tissue.

D   Desmosome.

DG   Dark (electron opaque) granules.

DM   Disrupted membrane.

E   Electron-opaque body.

EOG   Electron-opaque granule.

F   Fibrocyte sheath.

G   Golgi.

GC   Granular cell.

GJ   Gap junction.

Gly   Glycogen.

GO   Granule-containing organelle.

I   Intercellular space.

Id   Interdigitations.

If   Basal cell infoldings.

JC   Junctional complex.

K   Keratinizing cell.

L   Lumem.

La   Langerhans cell.

LaG   Langerhans cell granule.

LV   Luminal vesicles.

Ly   Lysosome.

M   Mitochondria.

Map   Possible microapocrine configuration.

MV   Microvilli.

MVB   Multivesicular body.

MyoE   Myoepithelium.

N   Nucleus.

NGC   Non-granular cell.

PG   Paler granules.

SE   Secretory epithelium.

T   Terminal web.

V   Vesicles.

ZO   Zonula occludens.

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