Intercellular Junction of Urodeal and Phallic Epithelial Cells in the Guinea Fowl, *Numida meleagris*

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**ABSTRACT.** The intercellular junction was observed in urodeal and phallic epithelial cells of the guinea fowl, *Numida meleagris*, by the ultra-thin section and the freeze-fracture methods to clarify the pathway of a lymph-like fluid produced by capillaries. Only desmosomes were found between cells of the stratified squamous epithelium covering the tips of paired lateral phallic bodies. The proximal portion of the lateral phallic body and the urodeal were lined with stratified and pseudostratified columnar epithelia containing numerous mucin-secreting cells. The columnar epithelial cells were linked with junctional complexes and cytoplasmic interdigitations. Half-desmosomes connected cells of the epithelial basal stratum with the basement membrane. The zonula occludens was well developed among mucin-secreting cells, but underdeveloped among columnar epithelial cells. When these junctions were examined in freeze-fracture replicas, the zonula occludens of the mucin-secreting cell exhibited a very distinct network of branching and joining strands. This type of tight junctions appeared to serve as a barrier to prevent lymph from diffusing by the intercellular route. On the other hand, the underdeveloped zonula occludens of the columnar epithelial cell was of incomplete reticular pattern in membrane fusion. It was suggested that the lymph produced by blood and lymph capillaries might be able to pass through the epithelium to play a part in dilution of the ejaculated semen.

The avian phallus is morphologically divided into two major types: the intermittent type observed in the anseriform and the non-intermittent type observed in the galliform species [7]. The guinea fowl, *Numida meleagris*, belongs to the order Galliformes, but its phallus is classified into a type between the two [6].

Nishida *et al.* [10] have recently examined the histological characteristics of the phallus and lymphatic folds of the guinea fowl by a scanning and transmission electron microscope. They failed, however, to observe the detailed structure of an intercellular junction which will be a key for clarification of the pathway of lymph and other high-molecular substances in the epithelia of the phallus and these folds. The authors therefore examined the intercellular junction of these epithelia by using the ultra-thin section and the freeze-fracture methods. The structure of this junction is associated with lymphatic secretion. It is also known as the dilution mechanism of the urodeal mucosa [12].

**MATERIALS AND METHODS**

Seven adult guinea fowls, consisting of five males and two females, were used for this study. Before dissection, they were injected intravenously with 300 units of heparin. The phallus and lymphatic folds were excised from them, fixed in 2% glutaraldehyde buffered with 0.1 M phosphate for 3 hr, and rinsed with the same buffer. For the ultra-thin section method, they were postfixied in 1% osmium tetroxide buffered with 0.1 M phosphate for 3 hr and then dehydrated...
with a graded series of ethanol. They were continually transferred to propylene oxide and embedded in Epoxy resin. For the freeze-fracture method, some specimens were submerged in 30% glycerol buffered with 0.1 M phosphate for 1 hr. Then they were frozen with liquid nitrogen slush, and fractured and replicated in a freeze-fracture device at \(-120^\circ C\) and \(10^{-7}\) torr. The resulting replicas were cleaned by removing the adhering tissues in sodium hypochlorite and washing with water. Electron micrographs were taken with a JEM-1200EX operated at 100 kV.

RESULTS

In both male and female adult guinea fowls, the phallus consisted of only paired lateral phallic bodies and lacked a median phallic body. The paired lateral phallic bodies were smaller in the adult female than in the adult male. Their tips were covered with a stratified squamous epithelium, in which cells were arranged more compactly in the male than in the female. Many cytoplasmic processes and desmosomes were found in spaces among cells of the stratified squamous epithelium (Fig. 1).

At the tip of the lateral phallic body, abundant desmosomes were observed distinctly by the ultra-thin section method. In the region of the desmosomes, the cell membranes were straight and exactly parallel with each other, and separated by an intercellular space about 30 nm in width, which was bisected by a slender intermediate line. The inner leaflet of the cell membrane was closely adhered to a dense homogeneous attachment plaque, about 10 to 15 nm in thickness and less than 500 nm in diameter. Adjacent to the attachment plaque a broad band of rather low density was observed exhibiting a feltwork of 10 nm tonofilaments interwoven. These tonofilaments extended from the filament tracts into the cytoplasm to form a cytoskeleton of cells in the phallic stratified squamous epithelium. They converged upon the attachment plaque, and finally returned to the filament tracts in the cytoplasm (Fig. 2).

The cells of the basal stratum in the phallic stratified squamous epithelium were attached to the basement membrane with half-desmosomes arranged at almost regular intervals along the basal plasma membranes. The cytoplasmic 10 nm tonofilaments converged upon the attachment plaque in the half-desmosome. The basement membrane increased remarkably in density in the region of the half-desmosomes (Fig. 3). No junctions other than those of the type described above were found in the phallic epithelium. There were no morphological differences in the intercellular junction between both sexes.

By the freeze-fracture method, clustering elevations of broken tonofilaments were observed on the fracture face of cytoplasm which appeared to be a desmosomal portion in the epithelial cells of the phallic stratified squamous epithelium. Irregular-shaped intramembranous particles were also seen on the P-face (Fig. 4).

In the stratified squamous epithelial cells of the lateral phallic body, there were large oval nuclei containing nucleoli and heterochromatin, and cell organelles such as mitochondria and endoplasmic reticulum developing moderately. The cells in the outermost layer of the stratified squamous epithelium contained few organelles in their cytoplasm lightly stained and showed a decrease in the number of cytoplasmic processes and desmosomes (Fig. 1).

The proximal portion of the lateral
phallic body was covered with a stratified columnar epithelium. The lymphatic folds were composed of stratified and pseudostratified columnar epithelia. In these epithelia, intercellular spaces were narrow in the apical region and rather wide near the basal region. Desmosomes and cytoplasmic interdigitations were observed in the adjoining portion of cells in the stratified and pseudostratified columnar epithelia of the lateral phallic body and lymphatic folds. The desmosomes in these epithelia revealed the same structure as those in the stratified squamous epithelium at the tip of the lateral phallic body. They were, however, smaller in size and contained fewer 10 nm tonofilaments than those in the stratified squamous epithelium (Fig. 5). There were underdeveloped half-desmosomes in the basal cells of the stratified columnar epithelium, but not in those of the pseudostratified columnar epithelium.

The outermost layer of stratified and pseudostratified columnar epithelia had numerous mucin-secreting cells interspersed among columnar epithelial cells (Figs. 5 and 6). Those cells were linked with one another by the junctional complex and interdigitation. A zonula occludens was recognized in the apical portion of the intercellular space obliterated by a fusion of opposed cell membranes. Those membranes converged within the zonula occludens and their outer leaflets fused with an adjoining cell membrane (Fig. 7). The zonula occludens of mucin-secreting cells was developed well as the first component of the junctional complex. It was longer than that of the columnar epithelial cells. In the female, however, the zonula occludens was well developed not only in the intercellular space between the mucin-secreting cells but in that between the columnar epithelial cells.

In the cell membrane below the zonula occludens, there was a zonula adherens as the second component of the junctional complex. In the zonula adherens, a moderately dense band was present in association with filaments of the terminal web. It covered a distance of 0.2 to 0.5 \( \mu \)m in the cytoplasm close to the cytoplasmic surface of the cell membrane (Fig. 7).

A desmosome was the third component of the junctional complex in the stratified and pseudostratified columnar epithelia. Desmosomes were smaller in size than those in the stratified squamous epithelium and distributed at random all over the adjoining cell surface (Figs. 5 and 7).

When the junctional regions of cells in the stratified and pseudostratified columnar epithelia were examined by the freeze-fracture method, the zonula occludens was recognized as ridges on the P-face and as grooves on the E-face of the cell membrane. The ridges and grooves on the fracture face of the zonula occludens formed complex and highly characteristic polygonal network in the mucin-secreting cells to intersect a wide area in a region very close to the cloacal lumen (Fig. 8). In the columnar epithelial cells, they were composed of particles, rods and short strands to form only one or three discontinuous layers of network (Figs. 9, 10 and 11). In the female, the zonula occludens of columnar epithelial cells was composed of ridges and grooves forming the same well-developed network as that of mucin-secreting cells (Fig. 12).

The mucin-secreting cells had short microvilli on the cloacal surface. They had well-developed cytoplasmic organelles and a large amount of secretory granules. In these cells, nuclei were located in the basal portion and contained well-
developed nucleoli and abundant heterochromatin (Figs. 5 and 6).

Large oval nuclei containing prominent nucleoli and abundant heterochromatin were observed in the stratified and pseudostratified columnar epithelial cells situated in the proximal portion of the lateral phallic body. These cells lined in the outermost layer also possessed short microvilli on their cloacal surface.

**Discussion**

When the cock is sexually excited, the lymph produced in the vascular body flows into the copulatory organs through the lymphatic sinus to cause the erection of the phallus and the swelling of lymphatic folds. On the other hand, the lymph is expelled in part from the lymphatic folds into the urodeal lumen (the ejaculatory fossa) to be added to the semen as its liquid component [8, 11, 12]. No obvious lymphatic route has been observed in the epithelia of urodeum and phallus [12].

The intercellular junctions of epithelial cells of the urodeal and phallic mucosa were examined by the ultra-thin section and the freeze-fracture methods to clarify the lymphatic pathway. The results obtained indicate that the proximal portion of the lateral phallic body and the urodeum, including the lymphatic folds, was lined with stratified and pseudostratified columnar epithelia containing numerous mucin-secreting cells.

Marked differences were found in the junctional complex between the columnar epithelial cells and the mucin-secreting cells. The intercellular junctional complex consisted of three components, a zonula occludens, a zonula adherens and a desmosome. No obvious differences were detected in the fine structure of the desmosome and zonula adherens between the columnar epithelial cells and the mucin-secreting cells. The zonula occludens observed as ridges in the P-face of the epithelial cell membrane (Figs. 8, 10, 11 and 12) was composed of rods or strands varying in length and assumed to be protein particles interspersed in the cell membrane. The grooves present in the E-face (Fig. 9) appeared to be complementary structures of the ridges on the P-face [1, 4, 5, 13].

The freeze-fracture method revealed distinct differences in zonula occludens between the mucin-secreting cells and the columnar epithelial cells. (1) The zonula occludens in the mucin-secreting cells exhibited a well-developed polygonal network which intersected a wide area in a region close to the cloacal lumen. It resembled the “very tight” epithelium, such as that of the gallbladder in fine structure [2]. The physiological significance of the tight zonula occludens was considered to consist in that plugged tightly in the intercellular cleft, the zonula occludens might serve as a barrier to prevent lymph and substance from diffusion through the epithelium via a paracellular route [4, 9, 14]. (2) The zonula occludens in the columnar epithelial cells was composed of particles, rods and short strands to form a few discontinuous layers of network. Morphologically, it resembled the “very leaky” epithelium of paracellular permeability, such as that of the proximal convoluted tubule of the kidney [2–4, 14].

In the female urodeal epithelium, a well-developed polygonal network was found in the zonula occcludens in the columnar epithelial cells, as well as in the mucin-secreting cells. Therefore, the zonula occludens could prevent lymph from permeating through the epithelium. These findings reveal that the leaky zonula occludens is an apparatus
characteristic of the male phallus and urodeal epithelium. The lymph derived from the subepithelial layer is thus secreted by the paracellular route of leaky columnar epithelial cells to play a part in dilution of the ejaculated semen.

In conclusion, it was elucidated that a paracellular route for lymph was present between epithelial cells of the urodeum and phallus in the guinea cock. The route for lymph in the copulatory organs of other domestic birds will need to be examined.

REFERENCES

Abbreviations
AP: Attachment plaque
TF: Tonofilament
ZO: Zonula occludens
D: Desmosome
ZA: Zonula adherens

Figs. 1–3 and 5–7. Transmission electron micrographs of ultra-thin sections.
Figs. 4 and 8–12. Transmission electron micrographs of freeze-fracture replicas.
Figs. 1–4. Tip portion of lateral phallic bodies.
Figs. 5–12. Epithelium of lymphatic folds.
Fig. 1. Stratified squamous epithelium in lateral phallic body. Numerous desmosomes are seen in the adjoining portion of two cells. \( \times 2,000 \).
Fig. 2. Desmosome between two epithelial cells. Parallel and straight cell membranes are separated by an intercellular space about 30 nm in width. 10nm tonofilaments converge on the attachment plaque. \( \times 84,000 \).
Fig. 3. Basal stratum of stratified squamous epithelium. In the region of half-desmosomes, basement membrane (arrow heads) increases in density. \( \times 12,000 \).
Fig. 4. Clustered elevations are seen on a fracture face of desmosomes in phallic epithelium. \( \times 68,000 \).
Fig. 5. Desmosomes link adjoining portions of two cells in the stratified columnar epithelium. Apical region of cells in the outermost layer is connected with junctional complexes. \( \times 12,000 \).
Fig. 6. Many mucin-secreting cells are seen in pseudostratified columnar epithelium. \( \times 3,000 \).
Fig. 7. Junctional complex in the apical region of cells in the outermost layer. It consists of three components, zonula occludens, zonula adherens and desmosome. \( \times 90,000 \).
Fig. 8. Fractured P-face of zonula occludens in mucin-secreting cell. Well-developed network is seen in zonula occludens. \( \times 60,000 \).
Fig. 9. Fractured E-face of zonula occludens in columnar epithelial cell in male lymphatic folds. Grooves give rise to one or three discontinuous layers of network. \( \times 60,000 \).
Fig. 10. Fractured P-face of zonula occludens in columnar epithelial cell in male lymphatic folds, Zone of zonula occludens is studded with particles and rods. \( \times 48,000 \).
Fig. 11. Fractured P-face of zonula occludens in columnar epithelial cell in male lymphatic folds. Ridges form two or three interrupted strands. \( \times 43,000 \).
Fig. 12. Fractured P-face of zonula occludens in columnar epithelial cell in female urodeum mucosa. It appears to be the same portion in male lymphatic folds. Well-developed network of ridges intersects a wide area of apical region of columnar cell. \( \times 58,000 \).

要 約
ホロホロチョウの尿洞およびファーラス上皮細胞間連結装置：佐々木博之，西田隆雄1）、望月公子1）
（日本電子株式会社EM応用研究室，1）東京大学農学部家畜解剖学教室）—ホロホロチョウの尿洞およびファーラス上皮の細胞間連結装置を超薄切片法および凍結割断法を用いて調べた。外側ファーラス体先端部の重層扁平上皮では、上皮細胞間に接着斑のみが認められた。一方、外側ファーラス体基部および周辺のリンパ網は重層および多列円柱上皮によっておおわれ、その最表層には粘液分泌細胞と円柱上皮細胞とが混在した。これらの細胞は閉鎖帯、接着帯および接着斑からなる接着複合体と指状横合によって、密に接着していた。粘液分泌細胞間と円柱上皮細胞間の閉鎖帯を凍結割断法によるレプリカ像として見ると、粘液分泌細胞間のものでは物質の漏出を容易に許さない構造と推定された。これに反して、円柱上皮細胞間のものは障状体の発達が不充分で、網状構造の形成不全のために細胞間の物質通過が可能と考えられ、この部分からリンバが尿洞腔へ漏出し、精巣に加わることができると推定された。
PHALLUS EPITHELIUM OF GUINEA FOWL