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Surface morphology of the ducts of the epididymal region of the drake (*Anas platyrhynchos*) as revealed by scanning and transmission electron microscopy

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INTRODUCTION

The epididymal region of birds has been studied extensively and intensively only within the last decade because of the attention now being focussed on the semen of, and artificial insemination in, birds.

Some illuminating histological and ultrastructural studies have thus been reported in the chicken (Budras & Sauer, 1975; Tingari, 1971; 1972), turkey (Hess & Thurston, 1977; Hess, Thurston & Biellier, 1976), Japanese quail (Aire, 1979*a*) and guinea-fowl (Aire, Ayeni & Olowo-Okorun, 1979). Aire (1980) has also reported an in-depth and comparative study of the ductuli efferentes in some avian species, utilizing both histological and transmission electron microscopical (TEM) methods.

Only one report of a scanning electron microscopical (SEM) study of the reproductive organs of birds (chicken and turkey, Bakst, 1980) is available. The present paper deals with SEM studies of the epididymal region, except the rete testis, of the drake. Attempts are made to correlate the SEM findings with TEM observations, in using a recent classification of the epithelial cell types in the epididymal region of birds (Aire, 1980; Aire *et al.* 1979), and making modifications, where necessary, in the light of new observations.

MATERIALS AND METHODS

Adult, sexually active drakes were purchased locally and perfused intravascularly, using 3 % glutaraldehyde buffered with 0·067 M sodium cacodylate. The reproductive organs were subsequently dissected out and immersed in a fixative similar to the perfusate for varying periods of time before preparation of the tissues for TEM and SEM. For TEM, the tissues from the epididymal region were post-fixed in 2% osmium tetroxide buffered with 0·067 M cacodylate solution, dehydrated in a graded series of ethanol and embedded in Epon. For SEM the tissues were dehydrated, critical-point dried and sputter coated with gold-palladium. Ultrathin sections of TEM materials were viewed and photographed in a Hitachi HS-9 electron microscope while the SEM tissues were viewed and photographed in a Jeol JSM 35 scanning electron microscope.

OBSERVATIONS

The epididymal region of the drake was similar architecturally to that already described for some other avian species. Thus, the compact mass of tissues consisted of rete testis lacunae (linking the seminiferous tubules to the rest of the epididymal

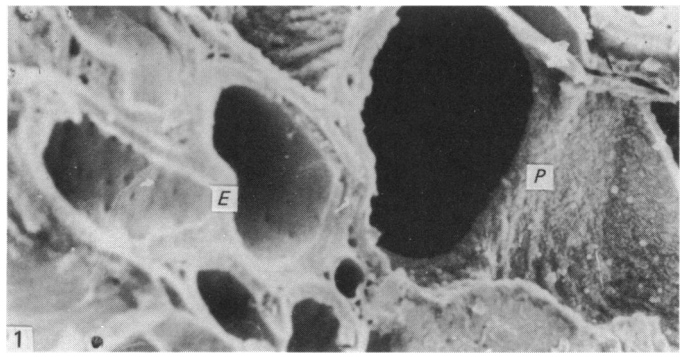

Fig. 1. Scanning electron micrograph of part of the epididymal region of the drake showing the proximal efferent ductule (*P*) and epididymal duct (*E*). × 60.

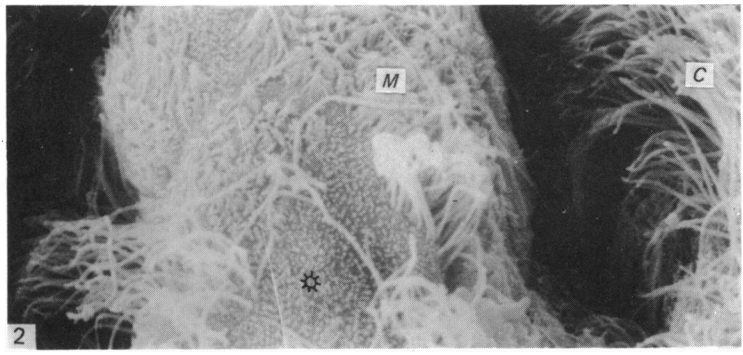

Fig. 2. Scanning electron micrograph of the proximal efferent ductule of the drake. Note an epithelial fold between two grooves. Note also the cilia (*C*) of the ciliated cells and microvilli (*M*) of the non-ciliated Type I cells. Some rete testis cells with stubby microvilli (*) are also present at the boundary between the rete testis and the proximal efferent ductule. × 2700.

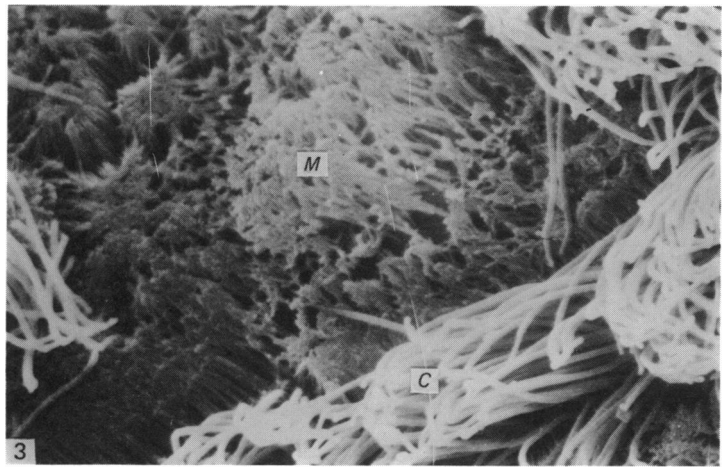

Fig. 3. Higher power view of the surface epithelium of the proximal efferent ductule showing microvilli (*M*) of the non-ciliated Type I cells and cilia (*C*) of the ciliated cells. × 4800.

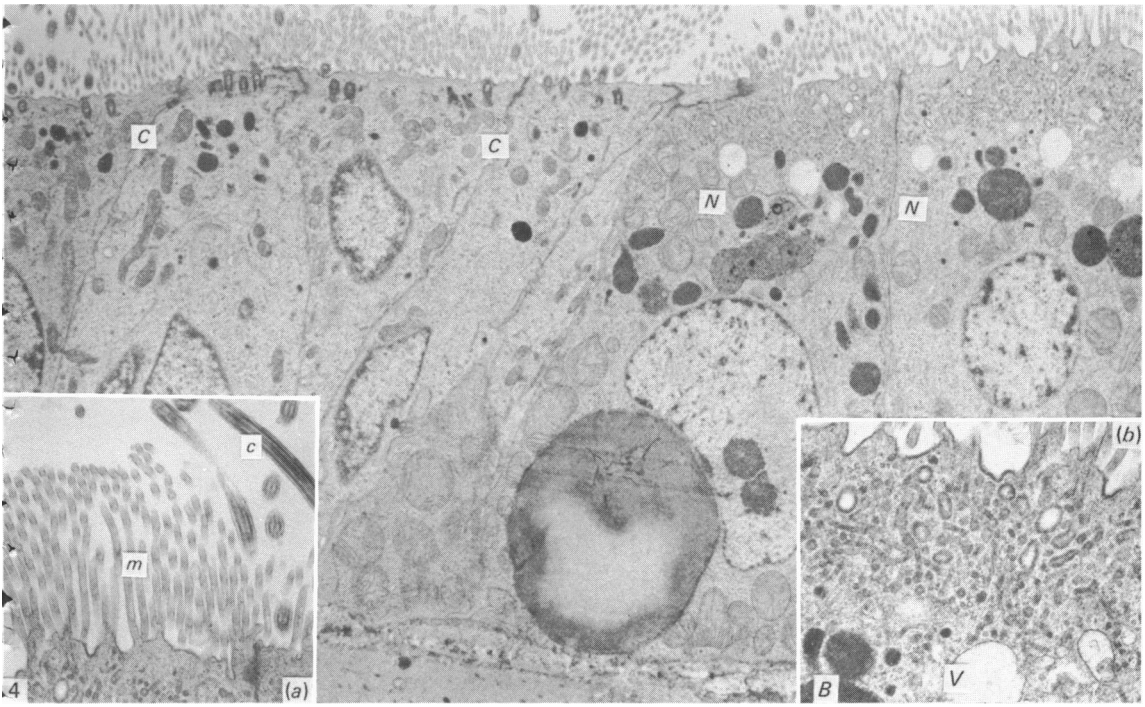

Fig. 4. Survey transmission electron micrograph of the proximal efferent ductule showing ciliated cells (*C*) and non-ciliated Type I cells (*N*). Note that microvilli and cilia, as well as apical cytoplasm of the cells, are regular and normal in shape. × 5000. Inset (*a*) shows the long and regular microvilli (*m*) and cilia (*c*) at higher power. × 9000. Inset (*b*) shows sub-apical cytoplasm of non-ciliated Type I cell at higher power. *B*, dense bodies; *V*, vacuole. Numerous vesicles and canaliculi abound in the cytoplasm. × 12000.

region), the efferent ductules, both proximal and distal, the connecting ducts and the epididymal duct. Figure 1 shows a proximal efferent ductule and portions of the epididymal duct at low SEM power.

Efferent ductules

The proximal efferent ductule showed the characteristic folded epithelium and consisted of ciliated and the preponderant non-ciliated Type I cells (Figs. 2, 4). The epithelium of the distal efferent ductule was less wavy in outline, and contained non-ciliated Type II and ciliated cells, the latter being much the more numerous (Figs. 5, 6). The microvilli were slender and of uniform diameter throughout their length. It is noteworthy that neither club-shaped microvilli nor blebbing of the apical cytoplasm occurred in the epithelia of the efferent ductules in these properly perfused tissues.

Connecting ducts and epididymal duct

The connecting ducts and the epididymal duct had identical surface appearance in both SEM and TEM micrographs (Figs. 7–9). The epithelium of both ducts showed scattered and irregular 'craters' (Fig. 7). Only one cell type was recognised, this being the non-ciliated Type III cell whose luminal surface had outgrowths of microvilli which were shorter than those of the non-ciliated Types I and II cells (Fig. 3) and only a single cilium (Figs. 7–9). In the light of this new observation on SEM and TEM, it is suggested that the Type III cell should be known by a more appropriate and

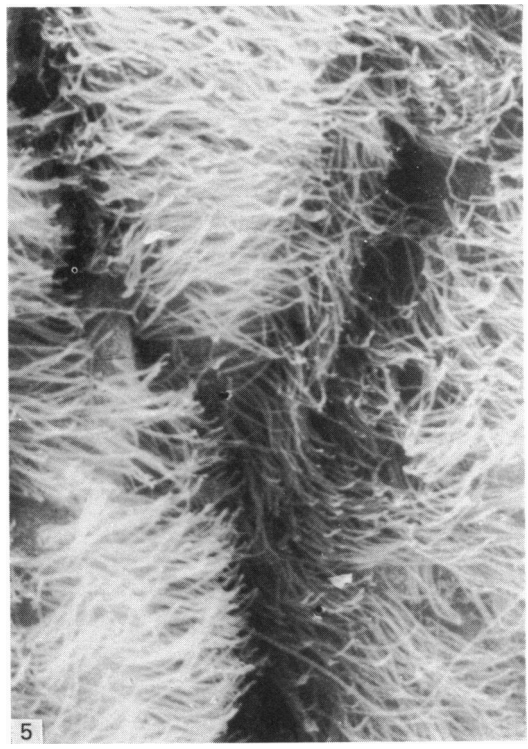

Fig. 5. Scanning electron micrograph of the surface epithelium of the distal efferent ductule. Ciliated cells are predominant in this epithelium. ×2200.

descriptive name – 'uniciliated cell'. The microvilli were short, straight or branched, and of uniform diameter throughout their length. Neither pleomorphic microvilli nor blebbing of the apical portions of the non-ciliated Type III or uniciliated cells occurred normally in well fixed tissues. This cell type in the ductus deferens of perfused birds more commonly showed these artefacts than did the same cell in the epididymal duct, probably because of the poor blood supply to the ductus deferens (Tingari, 1971).

DISCUSSION

The present study confirms observations made in previous studies (Aire, 1979*a*, 1980; Aire *et al.* 1979; Budras & Sauer, 1975) that the efferent ductules of the bird consist of two portions, the proximal and distal. The SEM studies also confirm that there are very many more ciliated cells in the distal efferent ductule than in the proximal efferent ductule where the non-ciliated Type I cells prevail. The connecting ducts and the epididymal duct have an epithelium made up of columnar cells which are now known to bear short microvilli and a single cilium. On the basis of this finding, it is suggested that the non-ciliated Type II (Tingari, 1972) or non-ciliated Type III cell (Aire *et al.* 1979) should be more appropriately known as the uniciliated cell of the connecting and epididymal ducts.

Except by the identification of the ductule on the basis of folding of the epithelium and the relative diameter and by the relative number of ciliated cells present, it is not possible, in SEM studies, to differentiate the non-ciliated Type I cell from the non-

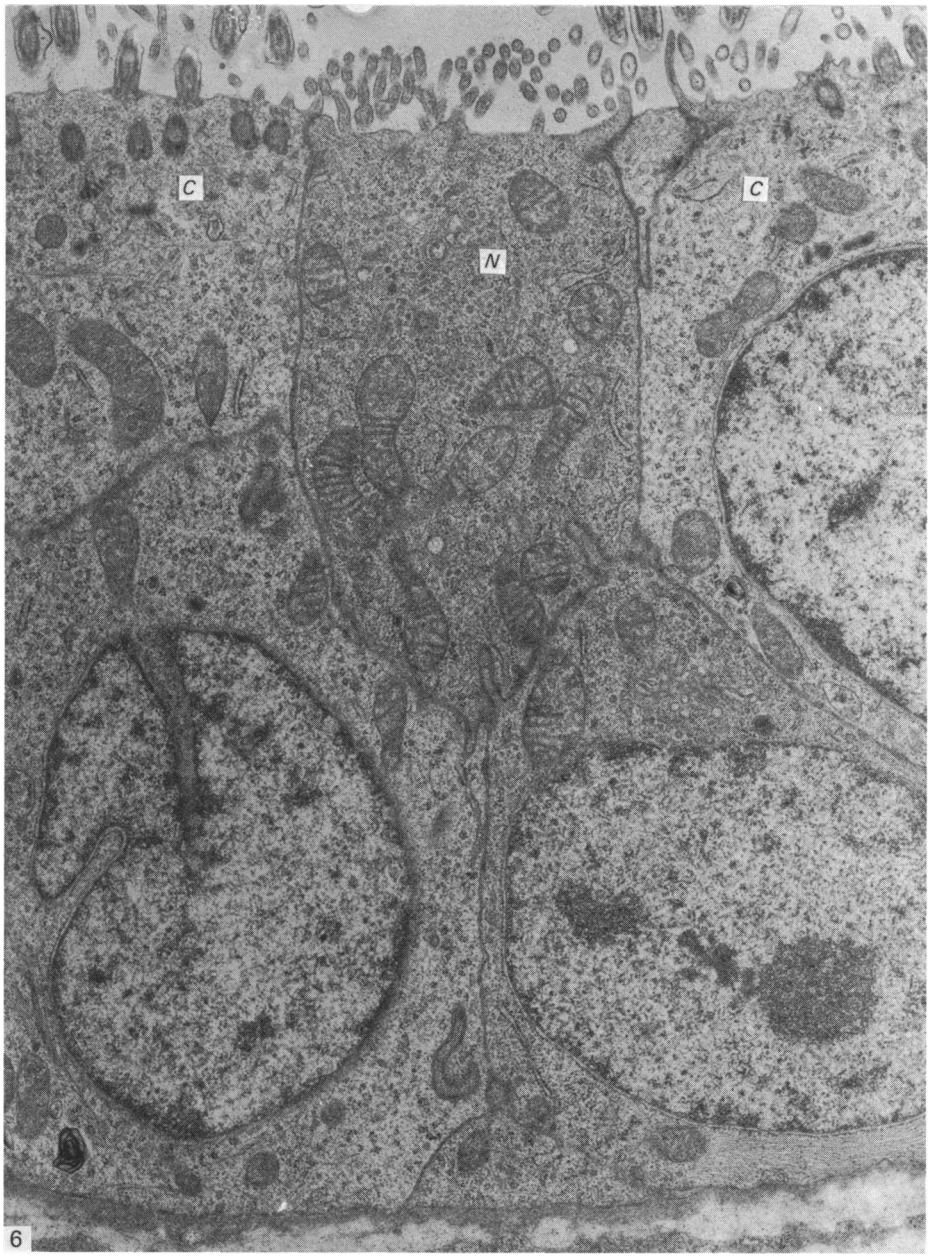

Fig. 6. A transmission electron micrograph of portion of the distal efferent ductule. *C*, ciliated cell; *N*, non-ciliated Type II cell. Ciliated cells are predominant in this epithelium. Note that neither vesicles nor canaliculi are present in the sub-apical cytoplasm of the Type II cell as compared to the Type I cells of the proximal efferent ductule. × 20000.

ciliated Type II cells. Both cells have fairly long microvillous projections of the apical or luminal surfaces. The microvilli are regular and largely of the same height and diameter, as evidenced by both SEM and TEM observations. This is contrary to observations made by Bakst (1980), in the chicken and turkey, where the microvilli in question appeared to occur in variable lengths and shapes. Aire (1979*a*; 1980) and

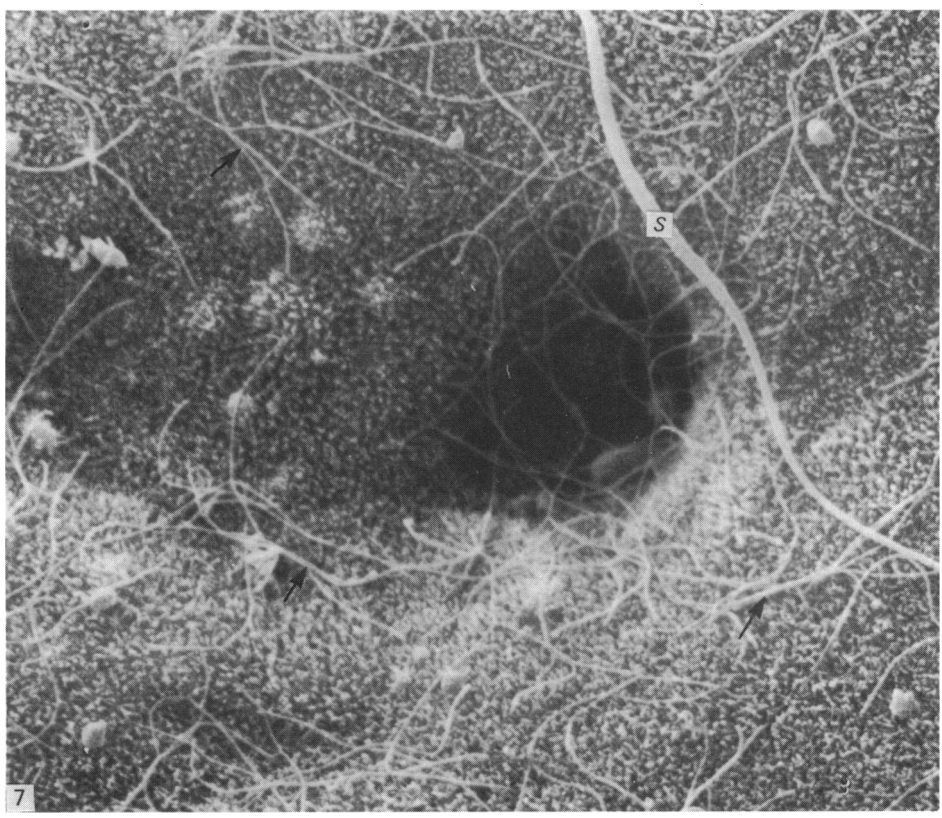

Fig. 7. Scanning electron micrograph of the surface epithelium of the ductus epididymidis. Note the presence of craters in the epithelium. *S*, spermatozoon; filaments (arrows), probably cilia arising singly from the non-ciliated Type III or uniciliated cells. × 3600.

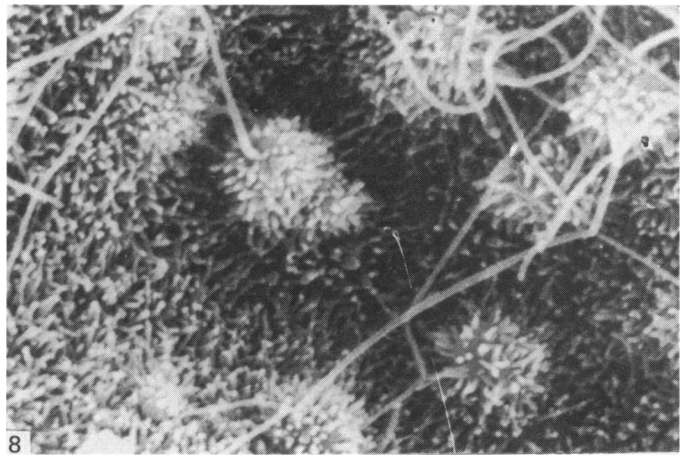

Fig. 8. Higher power view of the surface epithelium of the ductus epididymidis showing short microvilli as well as a cilium projecting from the apical surface of most of the non-ciliated Type III or uniciliated cells. × 4800.

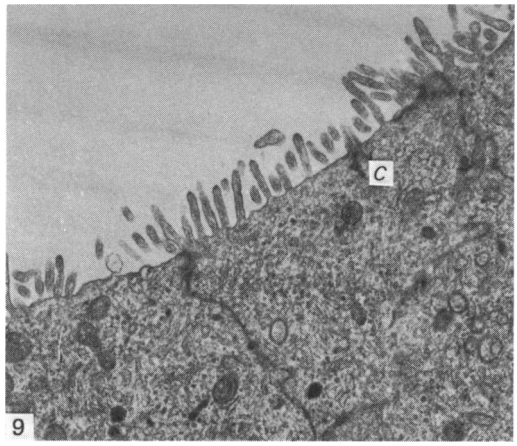

Fig. 9: Transmission electron micrograph of the apical portions of the non-ciliated Type III or uniciliated cells of the ductus epididymidis of the drake. Note presence of short, regular microvilli. The apical plasmalemma is also normally regular. *C*, basal portion of a cilium. × 8000.

Aire *et al.* (1979) considered that such pleomorphic microvilli were the result of improper fixation, and therefore to be regarded as artefacts. A similar explanation is suggested for the appearance of apical blebs or cytoplasmic projections of non-ciliated Type I cells in the proximal efferent ductule. The author's experience with proper and effective perfusion fixation of the epididymal region of birds, as compared to poor perfusion and immersion fixation, has clearly shown that the so-called blebs are fixation artefacts. These cytoplasmic blebs or projections of the apical plasmalemma of non-ciliated Type I cells cannot, therefore, be regarded as evidence of apocrine secretion. Besides, these blebs contain cell organelles such as mitochondria, dense globules (probably lysosomes) and rough endoplasmic reticulum in unaltered forms. Also, a demarcation zone between the bleb and the rest of the cell does not occur, as would be the case in apocrine secretion (Kurosumi, Yamagishi & Sekine, 1961). Instead, it is considered that the structure of the non-ciliated Type I cell clearly indicates that it performs an absorptive function (Aire, 1979*b*; 1980).

The non-ciliated Type II cell, although it cannot be differentiated from the non-ciliated Type I cell in SEM observation, is nevertheless structurally different from the latter cell in transmission electron microscopy (Aire, 1980). The non-ciliated Type II cell also seems to be more resistant to fixation stresses than the Type I cell. Both cell types, however, can be differentiated from the non-ciliated Type III or uniciliated cells by the shorter microvilli which are present on the latter cells. Compared to the Type III or uniciliated cells, the Types I and II cells have microvilli which may be regarded as stereocilia. The Type III or uniciliated cells, in poorly fixed tissues, may also show pleomorphic microvilli and blebbing of the apical portions. These are artefacts due to fixation. The Type III or uniciliated cells were shown to be secretory cells, and in this case displayed merocrine secretion (Tingari, 1972; Aire, unpublished observations).

SUMMARY

Both scanning (SEM) and transmission (TEM) electron microscopic studies of the major ductules and ducts of the perfused epididymal region of the drake were reported. The SEM correlated with TEM studies and confirmed some previous

observations that the non-ciliated Types I and II cells in the proximal and distal efferent ductules, respectively, possessed apical microvilli as distinct from the cilia of the ciliated cells. The relative number of each cell type in each duct was also revealed. All microvilli and cilia were regular in shape. The connecting and epididymal ducts showed 'craters' scattered over their entire epithelial surfaces. Also, a single cilium projected from most of the cells of the epithelial lining into the lumen of these ducts. The name, 'uniciliated cell' has been suggested to describe this cell which has, until now, been referred to as the non-ciliated Type III cell (Aire, 1980; Aire *et al.* 1979). Neither bulbous microvilli nor blebbing of the apical plasmalemma of the cells occurred in properly fixed tissues.

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