Presence of Specialized Cellular Complexes in the Tracheal Gills of Stonefly Nymph, *Paragnetina media* (Walker)

Until recently, the tracheal gills of the stonefly were considered to have only a minor respiratory function[1]. However, our experimental studies showed that these gills are of considerable importance as a respiratory organ accounting for about 80 % of the animal's oxygen consumption[2,3]. We were interested in the distribution of tracheoles in the gills, and therefore, an electron and light microscopical examination of the tracheal gill was conducetd. The study of ultra-thin sections of the gill filaments revealed a highly tracheated epithelium and in addition, numerous 'specialized cellular complexes' just below the cuticle. The purpose of this communication is to report these complexes which have not so far been observed in plecopterans.

The tracheal gills of the *Paragnetina* nymph are tubular or filiform evaginations of the body wall. They are present in 2 lateral tufts on each thoracic segment. The gill filaments were fixed in buffered 4 % glutaraldehyde followed by 1 % osmium tetroxide also buffered, dehydrated, with acetone, embedded in Vestopal W and were

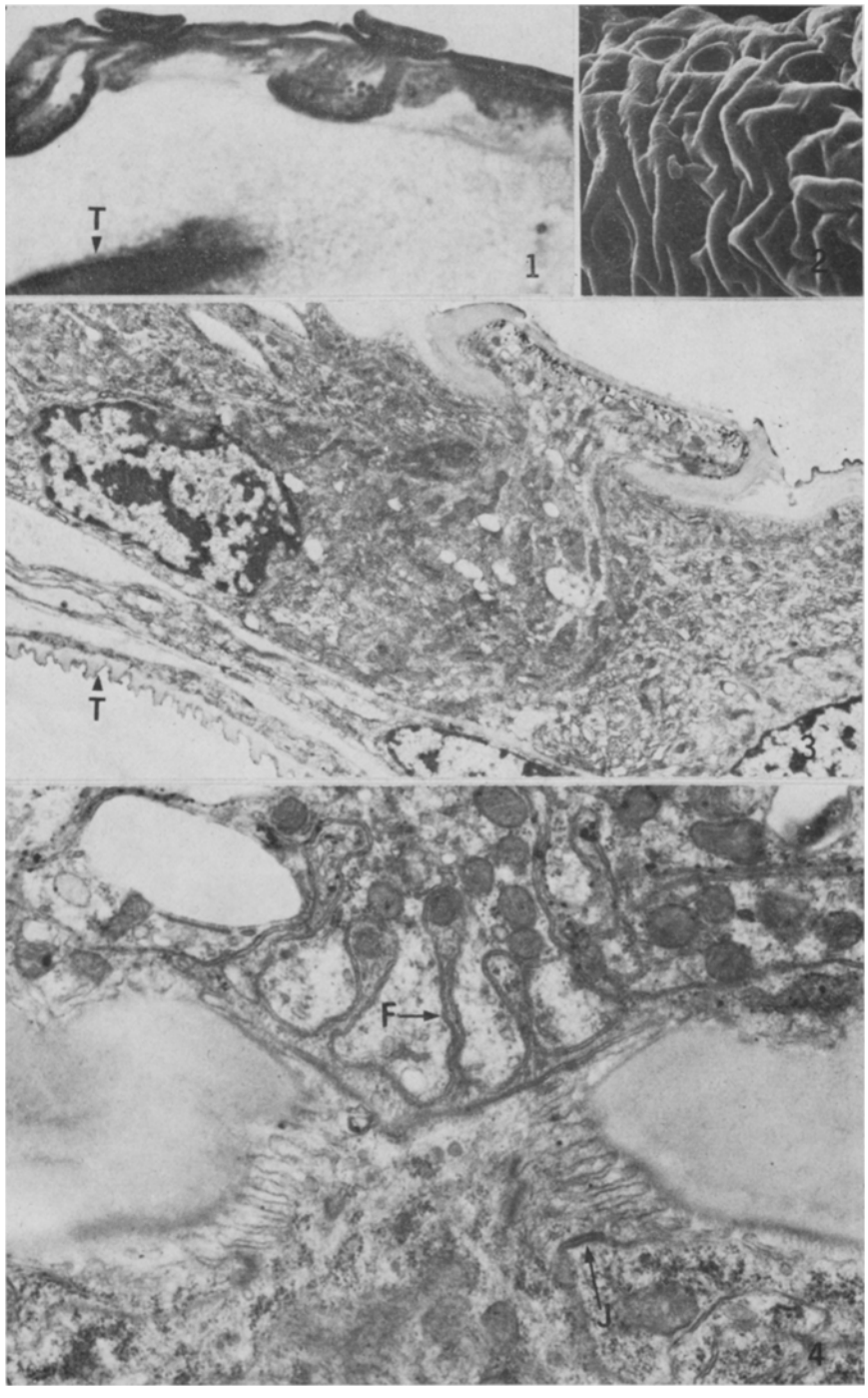

Fig. 1. Light micrograph of section of a tracheal gill filament of *Paragnetina media*, showing 2 specialized cells. T, Trachea. × 2,400.

Fig. 2. SEM picture of the gill surface. × 1,200.

Fig. 3. One complete specialized cellular complex. EM. × 5,300.

Fig. 4. Basal and neck portions of the cellular complex showing membrane folds F and desmosome, J. × 23,900.

sectioned mostly longitudinally. The sections were stained with uranyl acetate and lead citrate. Gill material was also prepared for scanning electron microscopy by the method of WALTER and BUCK[4], and was examined with a Cambridge Stereoscan electron microscope. Material for light microscopy was fixed in buffered 4 % glutaraldehyde dehydrated with cellosolve, embedded in glycol methacrylate, sectioned at 2 µm with glass knives and stained with either 0.01 % acid fuchsin or 1 % toluidine blue.

The epithelium of the gill filament is interspersed with elements of the tracheal system and the specialized cellular complexes are present all along the surface of the filament; their constituent cells are distinct from the adjoining respiratory epithelial cells. These special structures have a cup shaped apical portion, narrow middle and an enlarged basal portion (Figures 1 and 3). The apical portion is covered with a thin cuticular disc with a regularly striated ultrastructure (Figures 3, 5 and 6). These discs are also seen on the surface of the gill filament with the scanning EM (Figure 2). The central disc is surrounded by a thick cuticular rim-like structure. External to the disc, there is a mass of amorphous substance, presumably a secretion product similar to that reported

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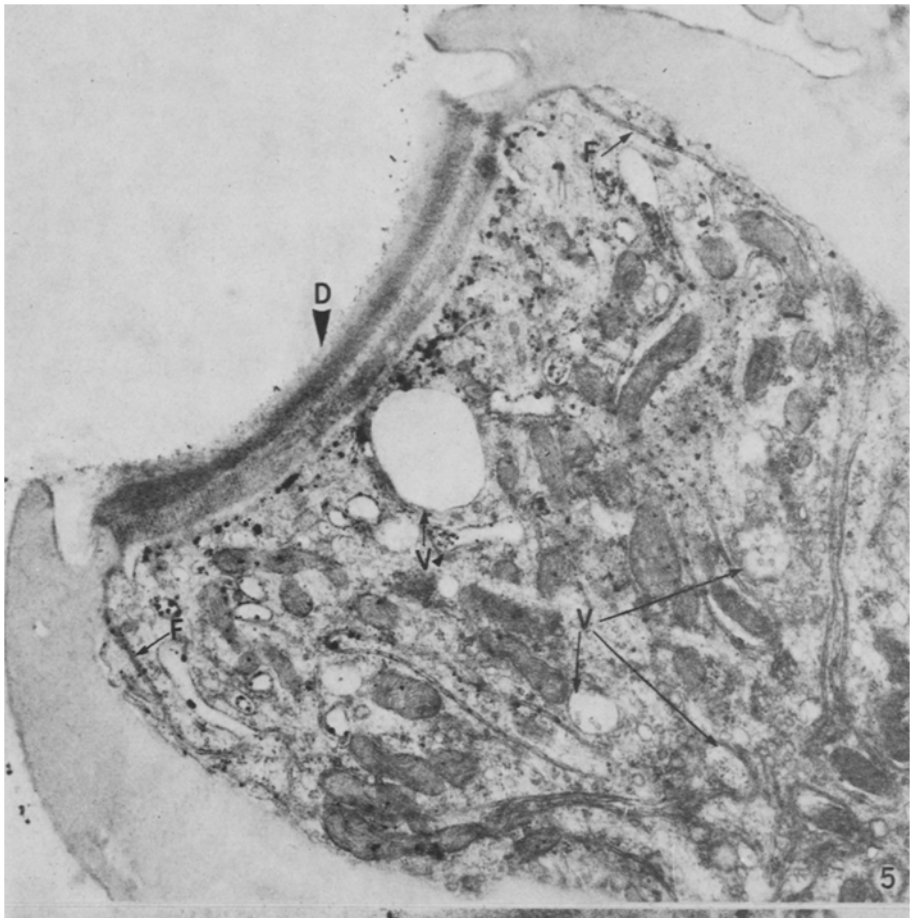

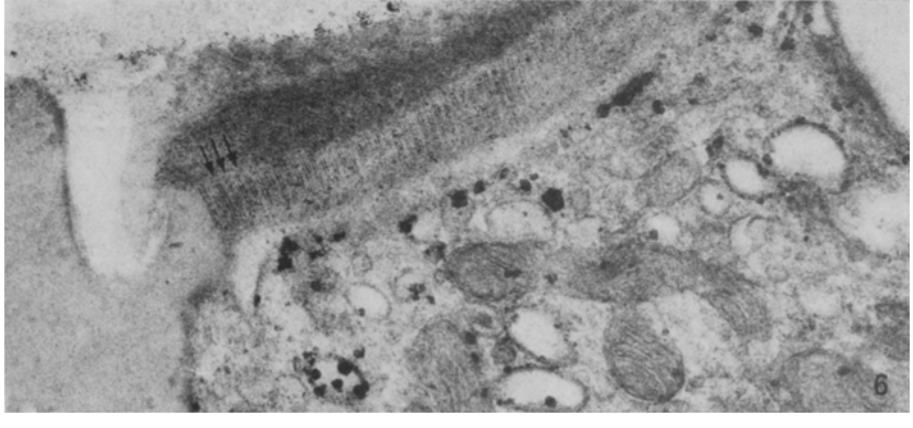

Fig. 5. Cup of the cellular complex. D, disc., F, folds; V, vacuoles. × 28,350.

Fig. 6. Disc of the cellular complex showing striations (arrows). × 65,400.

in fish[5]. Among the other remarkable features of the cells of these complexes are: 1. The vacuolated cytoplasm with rough endoplasmic reticulum restricted to the central and basal portions of the cell. 2. Micro-villi like surface projections intimately associated with the cuticular undersurface in the neck. 3. Substantial deposits of an electron dense material which accumulates preferentially in the cytoplasm immediately below the disc and upon vacuolar and other membranes. 4. Numerous interdigitating membrane folds, containing a single mitochondrion each, and restricted to the cup and neck region.

The distribution of the electron dense material is interesting: it is generally absent in the cytoplasm and within the vacuoles of the lower part of the cup, but invariably occurs as granules coating either the external or internal surfaces of the membrane delimiting the vacuoles of the upper regions of the cup. Conversely, the vacuoles in the lower region contain clusters of small uniform membrane-bounded vesicles similar in appearance to other vesicles in the surrounding cytoplasm. These vesicles are quite absent from the upper vacoules. The relation between the vesicles and electron dense granules if any, is not known.

The interdigitating membrane folds represent deep invaginations of the plasma membranes of cells neighbouring the one which constitutes the bulk of the complex. The invaginations develop symmetrically near the top of and at the sides of the cup (Figure 5), and extend down to the neck region, where their ends are most clearly seen (Figure 4). The location of the cell bodies of the cells which contribute these folds is under investigation; it is most likely that they lie both within the complex as well as in the adjacent respiratory epithelium. The presence of desmosomes in and near the neck region (Figure 4) supports this concept that the complex consists of two or more closely associated cells. Analagous cells, called 'chloride cells' and possibly involved with salt absorption, occur in the gill plates of ephemeropteran nymphs[6–8].

Serveral of the features of the cells of the complex in *Paragnetina*, in particular the membrane folds associated with mitochondria, are shared by the so-called chloride cells of the Ephemeroptera. They suggest that these complexes are capable of providing metabolically active surfaces for the exchange of materials with the environment. However, until definitive physiological experiments have been concluded the exact function of these complexes is in doubt.

Résumé. Les coupes ultraminces d'un filament trachéobranchial d'une nymphe de plécoptère ont rélévé dans l'épithélium branchial des complexes cellulaires spécialisés. Ces complexes sont présents tout le long du bord du filament, juste au-dessous de la cuticule et n'avaient pas encore été observés chez les plécoptères. Ils ressemblen aux «cellules de chlore» des poissons et à celles des nymphes des éphémères.

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Nuclear Multivesicular Bodies in Cultured Hamster Cells

The nuclear envelope is not a static structure separating the nuclear substance from the cytoplasm, but a dynamic membrane which is capable of participating in storage[1] and organelle formation processes[2–4]. Blebbing phenomena taking place from the outer nuclear membrane have been extensively reported in many different cells and species; almost all of these nuclear membrane extrusions have been related to the endoplasmic reticulum system[5,6]. KILARSKI and JASINSKI[7] reported on the formation of multivesicular bodies (MVB) from the nuclear envelope in cells of the gas-gland of the perch *Perca fluviatilis* L. under stimulated physiological activity.

These MVB have been observed in various types of cells; they are saccules containing small vesicles or tubules which most probably represent a particular kind of secondary lysosome[8–12]. In this paper we report on the formation of MVB from the inner nuclear membrane (INM) of BHK 21 cells and its presence in the nucleoplasm.

Material and methods. In the present study, we have used BHK 21 cells, a fibroblastic tissue culture-strain originated in the kidney of a baby hamster[13]. These cells were cultivated in Eagle's basal medium containing hydrolized lactoalbumin and 20% calf serum. Cells were harvested during the logarithmic growth phase. Cell pellets were fixed in 2% osmium tetroxide in Palade's buffer solution for 1 h. After being dehydrated with gradually increasing concentration of ethanol, pellets were embbeded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed in a Philips EM 300 electron-microscope.

Results and discussion. The nuclear envelope of the BHK cells will normally show iregularities and invaginations similar to those described in the bibliography[14]. We have found a particular kind of structure in these cells, consisting of the invagination of the INM. The latter takes on the shape of a baglet which is filled with nume-

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