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Typology of Ephemerid Chloride Cells

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Summary. The tracheal gills of 16 species of mayfly larvae were studied with regard to the chloride cells. The ephemerid chloride cells occur as two main types: single cells and cell complexes. The single chloride cells are characterized by deep tubular or slit-like infoldings of the apical cell membrane, whereas the chloride cell complexes show numerous intercellular channels resulting from cellular interdigitation at the basolateral side. According to the structural organization of the apices, the ephemerid chloride cells may be classified into caviform, coniform, bulbiform and filiform types. In the caviform type (single chloride cell), the apex retracts to form an apical cavity similar to teleost chloride cells. In the other types (chloride cell complexes), there is a progressive extension of the central cell apex into or beyond the cuticle in the form of cones, bulbs or filaments. The common feature of all types is the differentiation of the cuticle into thin porous plates or envelopes covering or surrounding the various forms of apices.

Histochemical precipitation of sodium and chloride in the apical region suggests that all types have basically the same function of salt absorption. The population of the various types differs with the species. However, there seem to be some taxonomic regularities with respect to the families. No relation was found between the types of chloride cells and habitat of the species.

Key words: Aquatic insect larvae — Tracheal gills: Chloride cells — Fine structure — Taxonomic distribution — Salt absorption, osmoregulation — Histochemical precipitation of sodium and chloride.

Introduction

In mayfly larvae, special cells were found which exhibited the fine structural characteristics of salt transporting cells and, therefore, were termed ephemerid chloride cells (Wichard and Komnick, 1971). These chloride cells were shown to occur either as single cells or as cell complexes. Originally they were observed in the epithelium of the tracheal gills, but in subsequent work they were found distributed on the external surfaces of nearly all body parts (Komnick and Abel, 1971).

As judged from their distribution, the chloride cell complexes appear to be identical with cells formerly described in *Caenis* (Eastham, 1936) and in *Baetis* (Müller-Liebenau, 1969) which were assumed to be mechanical sense organs and, therefore, called campaniform sensillae. They are, however, completely different in fine structure from campaniform sensillae of other insects (Schmidt, 1969; Moran *et al.*, 1971). In addition, it was shown by the use of histochemical techniques and radioactive chloride that the chloride cell complexes are salt absorbing devices and involved in osmoregulation (Komnick *et al.*, 1972).

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During the search for a favorable species to investigate the function of single chloride cells, variations in the structure of chloride cells were observed in different species, and in some species even mixed populations of various types of chloride cells were found. Therefore, representatives of the most important taxonomic groups were studied 1) to investigate the variability of ephemerid chloride cells and 2) to examine whether there were any relations between the types of chloride cells and systematics or environment.

Materials and Methods

This investigation includes several American and European species of *Ephemeroptera*, which are listed in Table 1. The American species were collected from the Cache la Poudre River near Fort Collins, Colorado. The European species were caught in the Fischelbach, a contributory creek to the Ahr river.

For morphological study the nymphs of undefined instar were fixed with 2% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2. The tracheal gills were extirpated in the buffer solution after fixation, stained *en bloc* with 0.5% uranyl acetate and 1% phosphotungstic acid (Wohlfarth-Bottermann, 1957), dehydrated in graded alcohols and embedded in styrene-methacrylate (Kushida, 1961) or Epon (Luft, 1961). For light microscopic examination, 1 μ thick sections were cut and stained with toluidine blue. For transmission electron microscopy thin sections were used with or without staining with lead citrate (Venable and Coggeshall, 1965).

For scanning electron microscopy the fixed gills were dehydrated and transferred into isopentane which was allowed to evaporate (Peters, pers. communication). The dry specimens were mounted on aluminium platelets and shadowed with gold.

For histochemical demonstration of sodium and chloride the potassium hexahydroxo-antimonate and silver lactate methods (Komnick, 1962; Komnick and Bierther, 1969) were employed.

Results

Cytological Observations

The application of the osmium-silver fixative for chloride precipitation to whole nymphs revealed that in all species studied chloride cells occurred in similar distribution over the larval body as described in *Callibaetis* (Komnick and Abel, 1971). However, only the tracheal gills, which are normally very rich in chloride cells, were used for cytological investigation of the chloride cells.

The ephemerid chloride cells display considerable variations in fine structure. Therefore, an attempt to classify the cells into several types appears to be justified for the reason of their brief characterization. The apical differentiations of the cells seem to be an appropriate feature which may be used as a structural basis of classification. Accordingly, the various types observed can be classified into caviform, coniform, bulbiform, and filiform chloride cells.

Caviform Chloride Cells

This type of ephemerid chloride cells which was previously described in *Rhithrogena semicolorata* as single chloride cell (Wichard and Komnick, 1971) forms an apical cavity (Figs. 1a, b, 3b, 5b, c). In most cases, the basal part of the cavity is secondarily confined by projecting microvilli or folds leaving only a small lumen at the level of the cuticle. Above each chloride cell the cuticle has a circular hole (Fig. 4a) which contributes to the cavity. The hole or cavity is

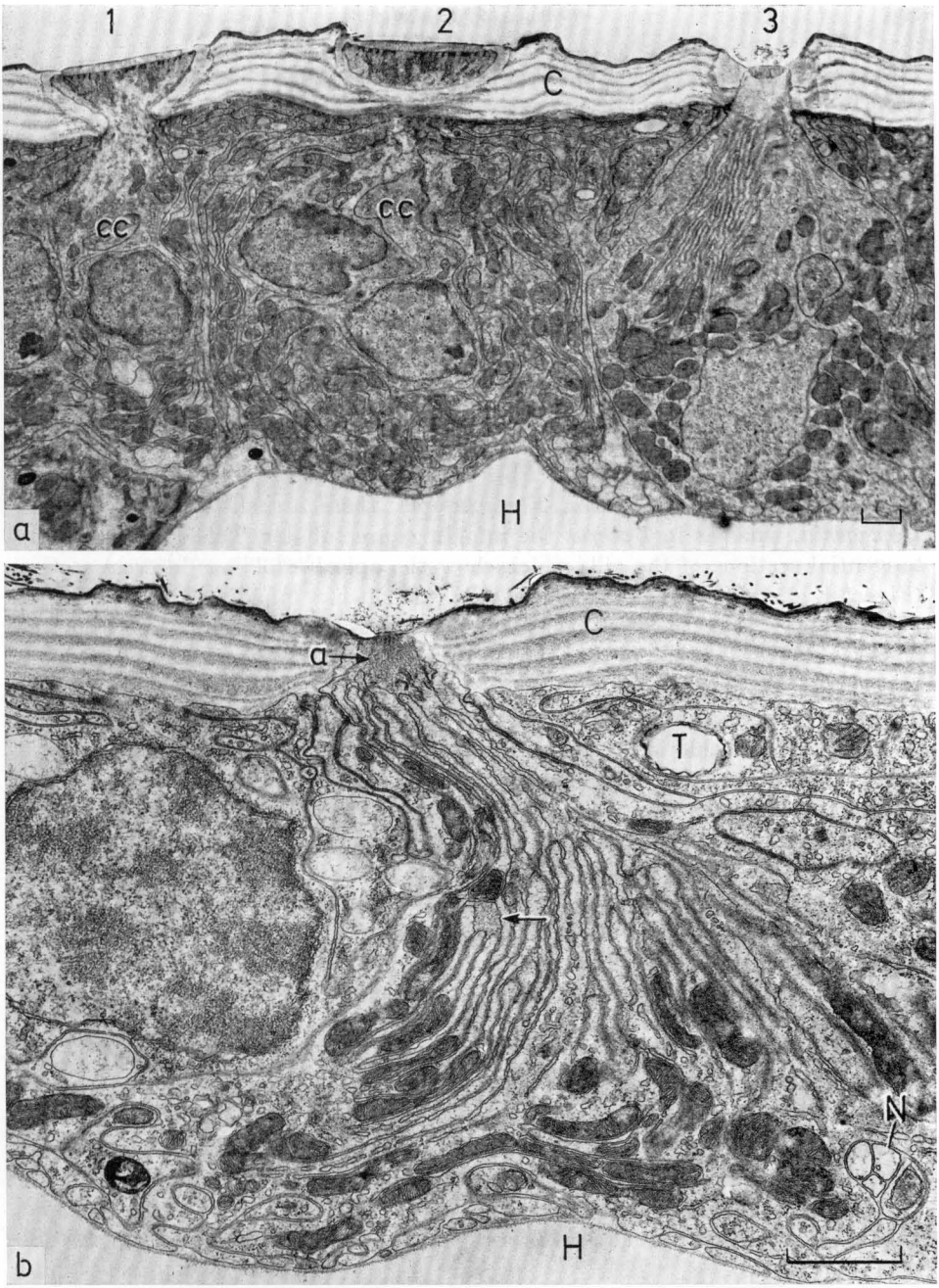

Fig. 1. a Cross section of the tracheal gill epithelium of *Habroleptoides modesta* showing one caviform (3) and two coniform (1 and 2) chloride cells. The apex of the coniform chloride cell labelled 2 is cut through the periphery. *cc* central cell. ×5000 (Scale in all pictures is 1 μm, if not stated otherwise). b Caviform chloride cell in the tracheal gill epithelium of *Heptagenia solitaria*. *a* apical cavity filled with flocculent material. Arrow points to similar material between infoldings of the apical cell membrane in the intermediate region of the cell. *N* unmyelinated nerve within a basal infolding of the chloride cell. *C* cuticle; *H* hemocoel; *T* tracheol. ×15000

separated from the external medium by a porous plate which normally lies in a slightly conical depression of the outer surface of the cuticle. The porous plate is approximately 0.1 μm in thickness and varies around 1 μm in diameter. In cross sections it shows a pattern of fine striations running perpendicularly to the plate surface (Fig. 3b). In tangential sections, the pores are arranged into interwoven hexagonal arrays (Figs. 3a, 5a).

The basal surface of the apical cavity is very complicated. It is defined by microvillous projections and deep infoldings of the apical cell membrane. The infoldings extend into the supranuclear or even basal part of the cell (Figs. 1a, b). Thus, the infoldings bring about a considerable enlargement of the apical cell membrane area and form an elaborate apical labyrinth of channels that communicate with the apical cavity. They contain a flocculent material of moderate electron density that is also observed in the apical cavity and is presumably mucoid in nature (Figs. 1b, 3b, 5b). The form of the infoldings shows some variability in different species. For example, there are slit-like infoldings in *Heptagenia*, whereas in *Epeorus* the infoldings are tubular. This is evident from tangential sections of the gills which show the apical infoldings in cross section (Figs. 5d, e). In cross sections of the gills, the slit-like infoldings are usually cut longitudinally and, therefore, normally extend without interruption from the apical to the basal region of the cell (Fig. 1b), whereas the tubular infoldings often appear as vesicles or rows of vesicles, particularly those which take a tortous course (Wichard and Komnick, 1971).

The caviform chloride cells contain many mitochondria with tightly packed cristae indicative of a high energy metabolism. The mitochondria are predominantly located in the lateral and basal peripheries of the cell as well as in close association with the infoldings of the apical cell membrane (Figs. 1b, 5d, e). In tangential sections, two mitochondria often with a dilated tubular infolding inbetween or one mitochondrion flanked by two infoldings are encountered (Fig. 5f). These arrangements may be considered as small mitochondrial pump units in the sense of Copeland (1964). Occasionally several of these units are grouped together similar to the stacks of mitochondria and membranes in dipteran rectal papillae, but are less organized (Fig. 5g) (Wessing, 1966, 1967; Berridge and Gupta, 1967).

Along the basal face of the chloride cells relatively few interdigitating processes arising from adjacent epithelial cells are encountered, indicating that the caviform chloride cells with the basally located nucleus (Fig. 1a) are essentially single cells interspersed in the epithelium of the tracheal gills. Occasionally, nerve fibers are found close to the base or even enveloped in infoldings of the basal cell membrane (Fig. 1b). This also holds true for the other types of ephemerid chloride cells (Figs. 2a, b).

Caviform chloride cells were observed in all species studied so far, except in those belonging the family *Baetidae*. Furthermore, they were always found in mixed populations with other types of chloride cells (Fig. 1a, Table 1).

Coniform, Bulbiform and Filiform Chloride Cells

These three types differ from the caviform type in that they consist of a small group of cells forming a structural and functional unit, which was previously

Table 1. Taxonomic distribution of the various types of chloride cells in tracheal gills of mayfly larvae

	Single cells	Cell complexes		
	caviform	coniform	bulbiform	filiform
Baetidae				
Baetis rhodani	−	++	−	−
Baetis cf. tricaudatus	−	++	−	−
Callibaetis cf. coloradensis[a]	−	++	−	−
Cloeon dipterum	−	++	−	−
Ephemeridae				
Ephemera vulgata	+	++	−	−
Siphlonuridae				
Ameletus spec.	+	++	−	−
Leptophlebiidae				
Leptophlebia marginata	+	++	−	−
Habroleptoides modesta	+	++	−	−
Choroterpes cf. albiannulata	+	++	−	−
Ephemerellidae				
Ephemerella grandis	+	−	++	−
Ephemerella ignita	+	−	++	−
Heptageniidae				
Heptagenia solitaria	+	−	++[b]	−
Ecdyonurus venosus	+	−	+	+
Epeorus assimilis	+	−	+	+
Rhithrogena doddsi	+	−	+	+
Rhithrogena semicolorata	+	−	+	+

+ present; ++ predominant type; − not observed.

[a] All species were collected from running waters except *Callibaetis cf. coloradensis* and *Cloeon dipterum* which were caught from small ponds (Wichard and Komnick, 1971; Komnick and Abel, 1971).

[b] Some of the bulbiform cells in *Heptagenia* may be likewise considered as transition types of filiform cells.

described as a chloride cell complex (Wichard and Komnick, 1971; Komnick and Abel, 1971). The chloride cell complexes are made up by a central cell which gives rise to the apical differentiations characteristic of the coniform, bulbiform, and filiform types (Figs. 1a, 2a, b). The central cell is surrounded by a variable number of adjacent cells.

Apart from the number of cells involved, the caviform chloride cells on the one hand and the coniform, bulbiform and filiform chloride cells on the other differ in the sites and modes of channel formation and enlargement of the cell membrane area. The adjacent cells of the chloride cell complexes are highly pleomorphic and extensively interdigitated along the basal and lateral circumferences of the complex. There is also some interdigitation between the adjacent cells and the central cell but to a less extent. The cellular interdigitation results

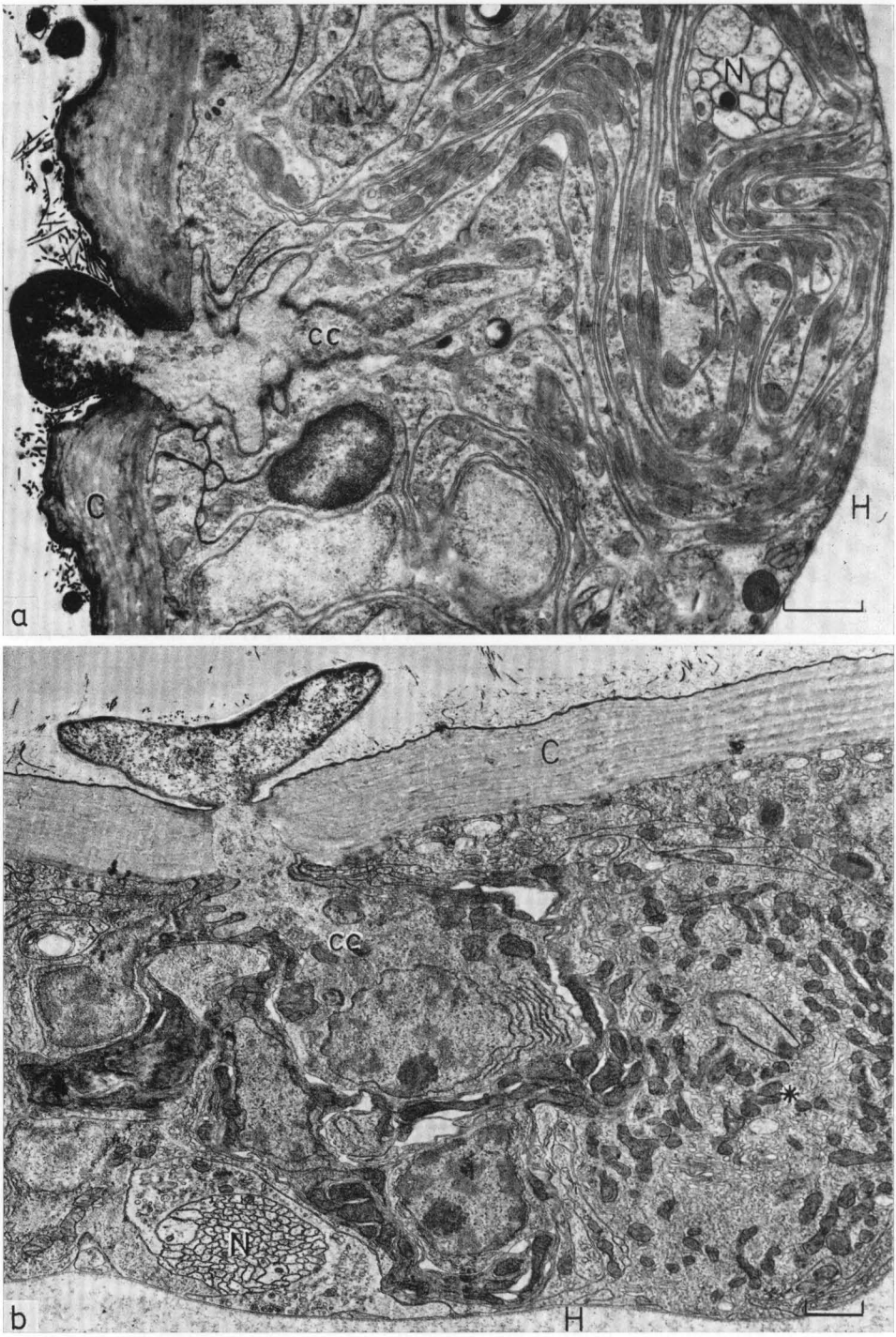

Fig. 2a and b

in a large number of communicating intercellular channels, which open towards the hemocoel and bring about an appreciable enlargement of the basolateral cell membrane area. The slender, interdigitating cell processes are crowded with mitochondria. These organelles are often located in close contact to the cell membranes which line the intercellular channels (Fig. 2a).

In coniform chloride cells the apex of the central cell forms a cone-shaped plug fitting exactly into a funnel-shaped recess of the cuticle (Figs. 1a, 3c). The apex penetrates through a 1 to 2 μm wide hole at the internal face of the cuticle and expands up to 7 μm in width at the external face of the cuticle. The wide, outermost face of the apex is covered by a porous plate which is connected with the cuticle at the periphery. In addition, the porous plate is surrounded by a slight elevation of the cuticle forming a circular rim (Fig. 4b). At the inner side of this ring, there is a small cuticular knob (Fig. 6c). The knobs of adjacent porous plates frequently point into the same direction (Fig. 4b), but the directions may also vary. Furthermore, an estimated 10–20 percent of the porous plates is lacking the knob as judged from scanning electron microscopy.

The porous plates of the coniform chloride cells exhibit the same fine structure in cross (Fig. 3b) and tangential sections (Fig. 6a) as those of the caviform chloride cells, but they are approximately 5 times as large in diameter which corresponds to 25 times in area. An additional cuticular layer possessing wide perforations and overlying the porous plates was not observed in any of species studied. This layer seems to be unique for the coniform chloride cells of *Callibaetis* (Komnick and Abel, 1971).

The apical cell membrane of the coniform chloride cells is also infolded. Unlike in caviform chloride cells, the folds are very short (Fig. 3c). They were never observed extending below the internal level of the cuticle. The exact shape of the folds can be elucidated from tangential sections of the apex. When cut just below the porous plate, circular membrane profiles like those of transversely cut microvilli are found (Fig. 6b). When cut somewhat deeper, an intricate system of winding membranes appears which resembles a finger-print pattern (Fig. 6c). This indicates that there is a tight package of short, irregular folds disintricating into regular microvillous tips underneath the porous plate. In *Choroterpes*, only short microvilli were found suggesting that there is some variation of the apical folds of coniform chloride cells in different species. In addition, variations were also found in coniform chloride cells of *Callibaetis* larvae adapted to different salinities (unpublished observations). The luminal space of the apical folds is hard to visualize at low magnifications because of a dark material contained therein (Figs. 3c, 6b, c).

The apical cytoplasm underneath the membrane infoldings is characterized by numerous small vesicles and stacks of small cisternae apparently representing part of the Golgi apparatus (Fig. 3c). The abundance of these structures is clearly revealed by tangential sections of the apex (Fig. 6d). Since another part of the

Fig. 2. a Bulbiform chloride cell in the tracheal epithelium of *Heptagenia solitaria*. ×11200. b Filiform chloride cell adjacent to an obliquely cut caviform chloride cell (*) in the tracheal gill epithelium of *Rhithrogena doddsi*. *C* cuticle; *cc* central cell; *H* hemocoel; *N* nerve. ×9000

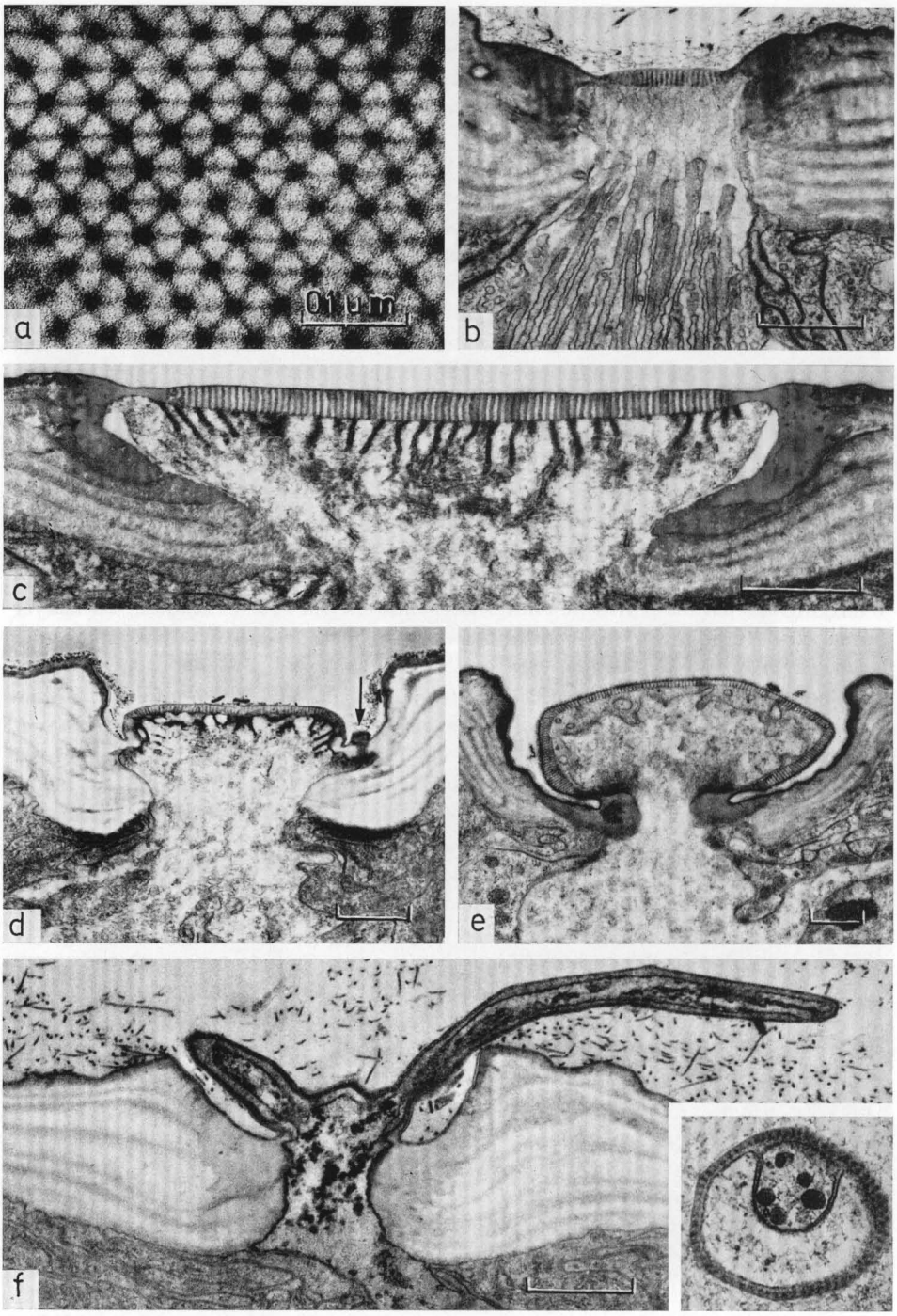

Fig. 3a—f

Golgi apparatus is often found near the nucleus, this organelle occupies two distinct positions within the central cell, the perinuclear region and the apex within the cuticle.

Coniform chloride cells were not found in all species studied. They occur together with other types in several species, but they were the only type observed in members of the family *Baetidae* (Table 1).

The bulbiform chloride cells are characterized by the bulbous apex of the central cell (Fig. 2a). The apex penetrates through an approximately 1 μm wide hole beyond the external surface of cuticle and forms a bulbous expansion outside the tracheal gill. In most cases, the bulbs are partially or totally immersed in depressions of the cuticle (Figs. 2a, 3d, e, 4c). The cytoplasm of the bulbous protrusion is encircled by a thin cuticular envelope which has the same fine structure as the porous plates of the caviform and coniform chloride cells (Figs. 3a, d, e). At the base of the bulbous apex, the porous envelope is continuous with the normal cuticle (Fig. 3e).

The bulbs are of various sizes in different species. The largest ones were observed in *Ephemerella*. In *Epeorus*, the bulbous protrusion is not very pronounced. Only the distal part slightly emerges over the bottom of the cuticular depression (Fig. 3d). At first glance, this type could likewise be taken as a coniform chloride cell. However, tangential sections reveal that unlike coniform cells the outermost apical portion is clearly separated from the cuticle by an empty space indicating a closer relation to the bulbiform cells. Actually, these cells may be considered as transition types between the coniform and bulbiform chloride cells.

The apical cell membrane is irregularly infolded along the circumference of the bulbs (Figs. 3d, e). The lumen of the infoldings contains a flocculent material which is also observed in the small space underneath the porous envelope and in small vesicles. The electron density of this material varies in different preparations (Figs. 2a, 3d, e).

Bulbiform chloride cells are absent in many of the species studied. When found, they were never observed as the only type of chloride cells present (Table 1).

In filiform chloride cells, which consist of cell complexes like the coniform and bulbiform types, the apical cytoplasm of the central cell emerges through a 1 μm wide orifice of the cuticle and forms filaments at the external gill surface (Fig. 2b). When viewed with the scanning electron microscope, the apical filaments arise from funnelshaped impressions of the cuticle (Figs. 4d–f) which are also recognizable in thin sections (Fig. 3f). In addition, scanning electron microscopy

Fig. 3. a Tangential section of the porous envelope of a bulbiform chloride cell of *Ephemerella grandis* showing the pore system at high magnification. ×150000. b Apex of a caviform chloride cell of *Rhithrogena doddsi*. ×15400. c Apex of a coniform chloride cell of *Baetis rhodani*. ×18000. d Apex of a bulbiform chloride cell of *Epeorus assimilis* (transition type). Arrow points to the cuticular knob. ×10000. e Apex of a bulbiform chloride cell of *Ephemerella grandis*. ×7500. f Apex of a filiform chloride cell of *Epeorus assimilis*. ×15000. Inset: Cross section through an apical filament of a filiform chloride cell of *Rhithrogena doddsi* fixed during moult. The filament is surrounded by moulting gel. The width of the inset corresponds to 1μm. ×30000

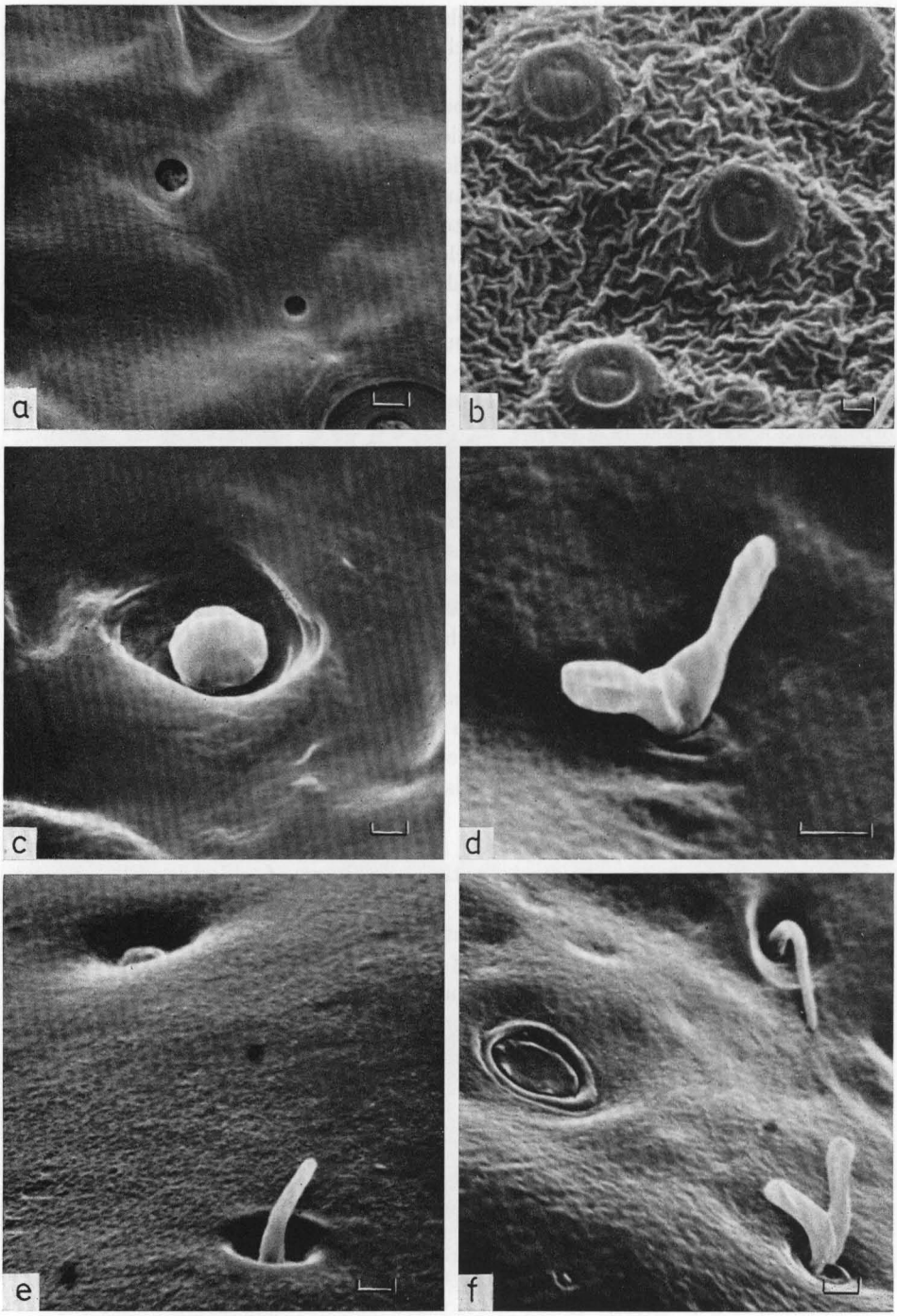

Fig. 4a—f

better reveals the external form of the filaments, which are randomly cut in thin sections. In *Rhithrogena doddsi*, the filaments measure approximately 1 μm in diameter at the base, 0.5 μm near the rounded tip and extend up to 5 μm in length. In addition to single filaments, forked forms occur in this species (Figs. 6d–f). In *Epeorus assimilis*, filaments with up to 11 branches were observed. All branches arise at the same level from a short stalk. The whole structure resembles a minute brush (Figs. 3f, 7).

The apical filaments likewise are enveloped in a porous sheath of the cuticle (Figs. 3f inset, 7a). They also contain vesicles with dense material. However, infoldings of the apical plasma membrane were never observed. In cross sections of filaments of *Rhithrogena doddsi*, several cell processes were occasionally found (Fig. 3f inset), whereas the brush-like filaments of *Epeorus* seem to consist of only one branched cell process (Fig. 7).

Filiform chloride cells were observed only in some species belonging to the family *Heptageniidae*. They always occurred together with other types of chloride cells (Table 1).

Sodium and Chloride Localization

When fixed in the osmium-antimonate or osmium-silver solutions, all four types of chloride cells show a positive reaction for sodium and chloride whereby the sites of sodium deposits coincide with the sites of chloride precipitates (Fig. 8).

In caviform chloride cells, precipitates of both sodium and chloride are concentrated in the apical cavity and associated with the apical infoldings (Figs. 8a, b).

In coniform, bulbiform and filiform chloride cells, dense deposits are located in the apical cytoplasm of the central cells (Figs. 8c–h). In the filiform type the precipitates are densest in or limited to the basal region of the apical filaments (Figs. 8g, h). In the tracheal gills of *Epeorus assimilis*, for example, which have a mixed population of caviform, bulbiform and filiform chloride cells, the three types give positive reactions at the same time (Figs. 8a, b, e–f).

Taxonomic Distribution

Including previous studies (Wichard and Komnick, 1971; Komnick and Abel, 1971), a total of 16 mayfly species have been investigated with respect to the chloride cells. These represent 6 families out of the 19 into which the order of *Ephemeroptera* is subdivided (Illies, 1968). Table 1 summarizes the types of chloride cells observed thus far in different species. Although the small number of species examined per family is still an insufficient base for generalization, certain regularities in taxonomic distribution of ephemerid chloride cells are recognized.

Fig. 4a—f. Scanning electron micrographs showing the cuticular differentiations of the various types of ephemerid chloride cells. a Two caviform chloride cells of *Ecdyonurus venosus*. ×5000. b Four coniform chloride cells with cuticular knobs of *Cloeon dipterum*. ×3500. c A bulbiform chloride cell of *Ephemerella grandis*. ×5000. d A filiform chloride cell with branched apical filiment of *Rhithrogena doddsi*. ×10000. e A filiform and a bulbiform chloride cell of *Rhithrogena doddsi*. ×5000. f Two filiform chloride cells with branched and unbranched filaments, and a bulbiform chloride cell (transition type) of *Rhithrogena doddsi*. Shrinkage due to drying at the base of the branched filament in the lower right corner reveals the orifice of the cuticle. ×5000

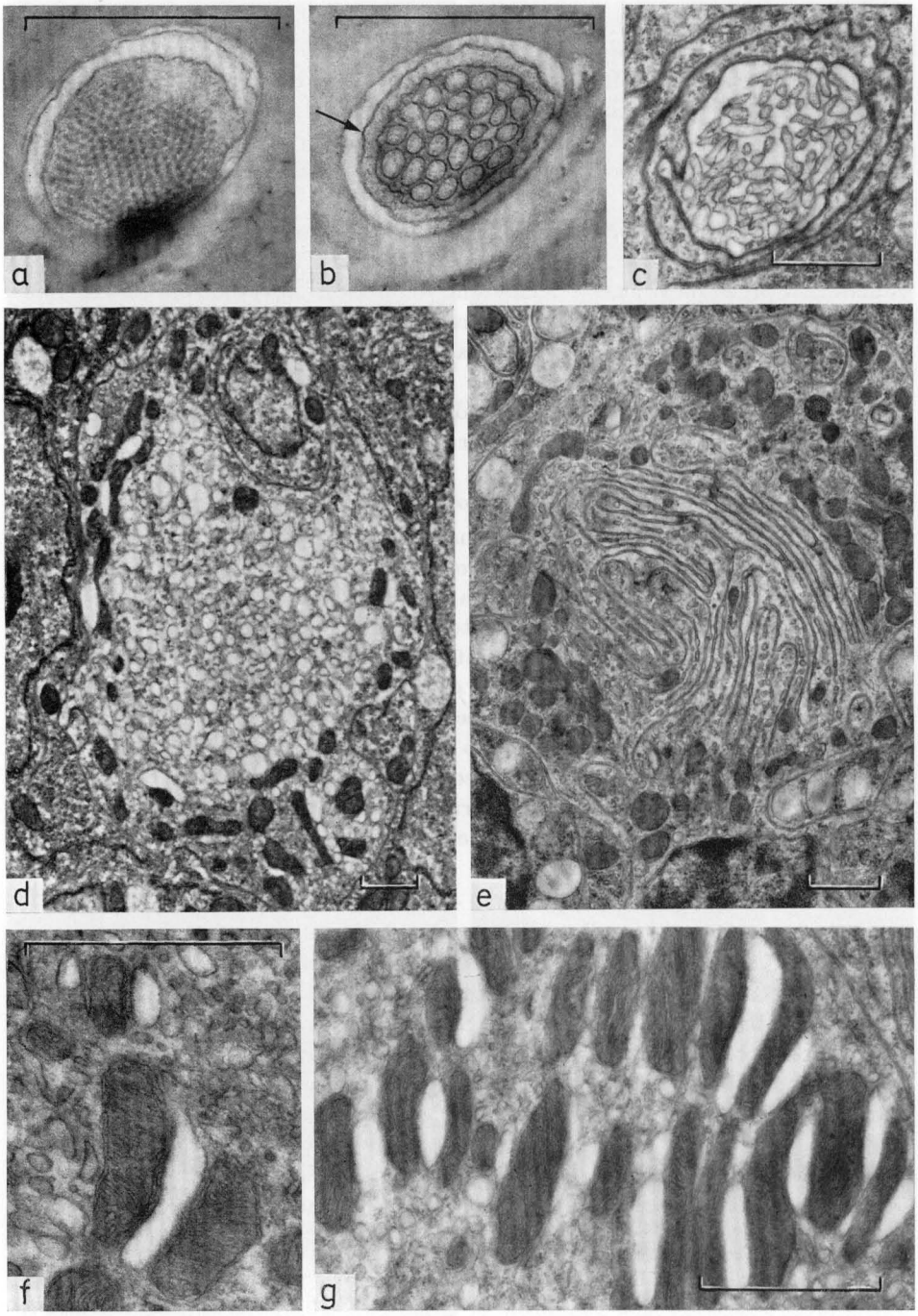

Fig. 5a—g

In the species of all families studied, mixed population of two or more types of chloride cells were observed, except in *Baetidae*, where only the coniform type was found. The coniform chloride cells, therefore, are the only type which can occur alone.

The caviform type was encountered in all families, except in *Baetidae*. The same is true for the coniform type which is lacking only in *Ephemerellidae* and *Heptaniidae*. The bulbiform type was observed only in two families (*Heptageniidae*, *Ephemerellidae*), and the filiform type was present only in one family (*Heptageniidae*). According to Table 1, the caviform and coniform types are the most frequent ones with respect to their occurence amongst the families. In *Heptageniidae*, the chloride cell complexes are often difficult to identify according to the form of their apices, because in addition to clear bulbiform and filiform types there are many transition types from nearly coniform to bulbiform types on the one hand and from bulbiform to nearly filiform on the other. Furthermore, the filiform type undergoes progressive branching from single and forked filaments in *Rhithrogena* to brush-like structures with variable numbers of branches in *Epeorus*. The chloride cell complexes are apparently highly variable with respect to their apices.

The taxonomic distribution of ephemerid chloride cells becames simpler when compared on the basis of their cellular organization: All families studied possess chloride cell complexes. These occur alone in *Baetidae*, and in conjunction with single chloride cells in the remainder of the families, where they still predominate. They mostly occur as the coniform type, which may be replaced by the bulbiform, or the bulbiform and filiform types. Within each family the distribution seems to be uniform.

Relation to Habitat

Mayfly larvae usually live in fresh water, although some species are known to tolerate brackish water (Illies, 1968). This study includes only fresh water species, which live in a hypotonic environment. They normally occur in either still or running water. Since there is a fairly wide range of variation in the quanti tative and qualitative composition of solutes in both still and running waters, the mere distinction between still and running water is a fairly inadequate criterion for the osmoregulatory situation. Nevertheless, the following comparison shows that no specific relation exists between the types of chloride cells and the habitat of the species.

Fig. 5a—g. Caviform chloride cells cut tangentially to the gill surface at various levels. a Porous plate (*Epeorus assimilis*). ×35000. b Apical cavity showing microvilli transversely cut near their apical tips and surrounded by mucosubstances. Arrow points to the peripheral wall of the cavity made up by a circular cytoplasmic sheath (*Epeorus assimilis*). ×35000. c Apical cavity cut near the bottom (*Epeorus assimilis*). ×15000. d Intermediate region of a caviform chloride cell of *Epeorus assimilis* showing transversely cut tubular infoldings of the apical plasma membrane. ×8000. e Intermediate region of a caviform chloride cell of *Heptagenia solitaria* showing transversely cut slit-like infoldings of the apical plasma membrane. ×10000. f and g Relation between mitochondria and infoldings of the apical plasma-membrane in the intermediate region of caviform chloride cells in *Epeorus assimilis*. f ×35000; g ×25000

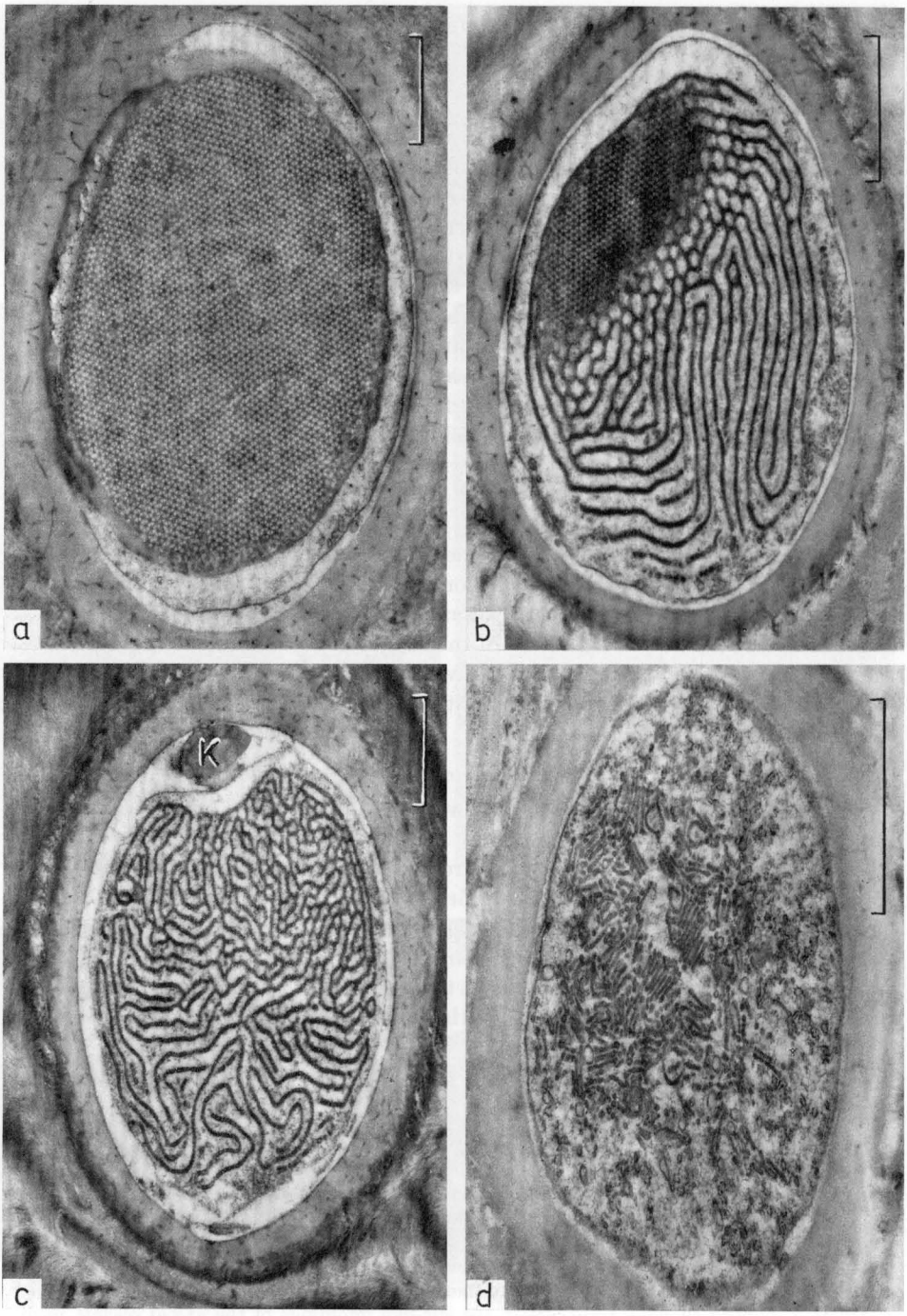

Fig. 6a—d

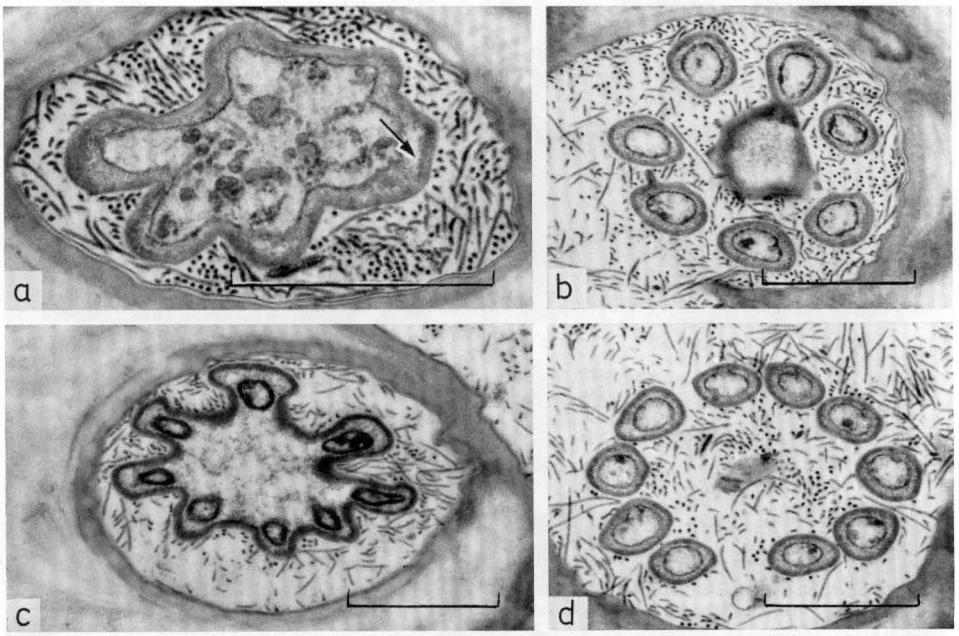

Fig. 7. Cross sections through apical filaments of filiform chloride cells of *Epeorus assimilis* cut near the branching point. Arrow points to pores. a ×35000; b—c ×20000

For example, *Callibaetis cf. coloradensis* and *Cloeon dipterum* both living in stagnant water possess the same type of chloride cells as *Baetis rhodani* and *Baetis cf. tricaudatus* which live in running water. These species belong to the same family. On the other hand, *Baetis rhodani* and *Epeorus assimilis* belonging to different families and living closely together in the same environment, possess different types of chloride cells (Table 1).

Since *Baetis rhodani*, *Habroleptoides modesta*, *Ephemerella ignita*, *Ecdyonurus venosus* and *Epeorus assimilis*, which were collected from a very small part of the same creek, cover all types of chloride cells (Table 1), it appears highly improbable that there is any relation between a given *type* and the habitat. However, preliminary results obtained from adaptation experiments on *Callibaetis* indicate that there is a reciprocal relation between the *number* of coniform chloride cells per animal and the salinity of the environment.

Fig. 6a—d. Tangential sections through the gill cuticle of *Baetis rhodani* showing apices of coniform chloride cells cut at different levels. a Tangential section of the porous plate. ×15000. b Oblique section running through the porous plate, apical microvilli and folds. ×20000. c Slightly oblique section running through the apical folds. *K* cuticular knob. ×15000. d Section through a deeper part of the central cell apex showing apical Golgi apparatus. ×30000

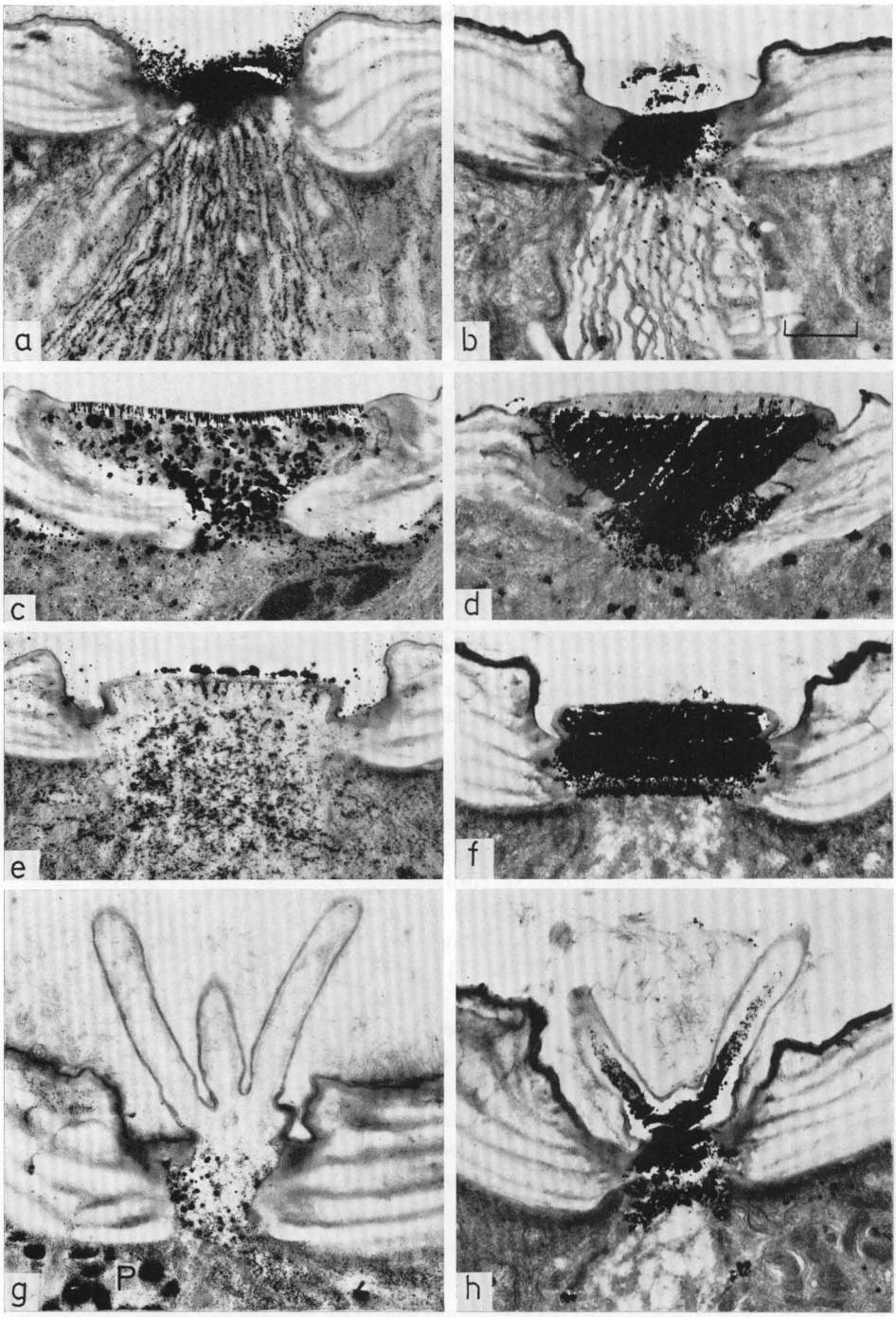

Fig. 8a—h. Sodium (left hand row) and chloride (right hand row) precipitation within the apical region of the various types of ephemerid chloride cells. a and b Caviform chloride cells of *Epeorus assimilis*. ×10000. c and d Coniform chloride cells of *Habroleptoides modesta*. ×10000. e and f Bulbiform chloride cells (transition type) of *Epeorus assimilis*. ×10000. g and h Filiform chloride cells of *Epeorus assimilis*. *P* pigment granules. ×10000

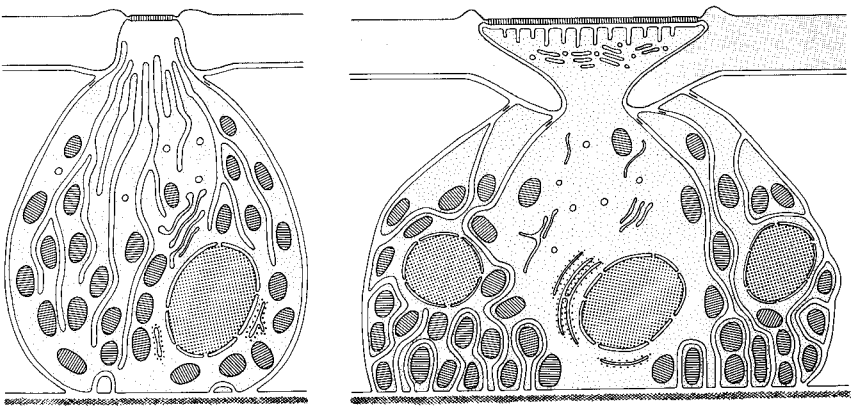

Fig. 9. Diagrammatic representation of the cellular organization of single chloride cells (caviform type) and chloride cell complexes (coniform type). The bulbiform and filiform types have basically the same cellular organization as the coniform type

Discussion

The ephemerid chloride cells are very variable in structure. According to their apical differentiations, they are classified into four types: caviform, coniform, bulbiform, and filiform chloride cells. In the caviform type, the apex retracts to form an apical cavity, whereas in the other types the apices extend into or beyond the cuticle in the form of cones, bulbs or filaments. The cellular organization and the occurrence of transition types suggest that the latter three types are closely related to each other. They may be placed in a row characterized by progressive externalization and articulation of the apices. Since the apices are probably involved in ion trapping and accumulation (Fig. 8; cf. Komnick *et al.*, 1972), the structural variations are possibly related to quantitative differences in ion adsorption.

According to their cellular organization, the ephemerid chloride cells may be classified into two main types: single chloride cells and chloride cell complexes (Fig. 9). The essential difference between these two types in respect to ion transport would be plasma membrane enlargement and channel formation at opposing sites of the epithelium. The infoldings of the apical cell membrane in single chloride cells, regardless whether they occur as tubules or slits, result in an enlargement of the resorptive cell surface and the formation of backward transporting channels in the sense of Diamond and Brossert (1968). This implies an inward ion transport across the apical channel membranes in single chloride cells.

On the other hand, in chloride cell complexes cellular interdigitation provides an enlargement of the excretory cell surface and formation of forward transporting channels (Diamond and Brossert, 1967). Consequently, ion transport across the basolateral channel membranes is directed outwards. These conclusions are based on the assumption that the function of all types of ephemerid chloride cells is the absorption of salt from the external solution which implies transepithelial ion transport from the apical to the basal side. A salt absorptive function has definitely been demonstrated only in the case of coniform chloride cells (Komnick

et al., 1972). Although experimental evidence is still lacking, there are arguments in favor of an absorptive function also in the other types of chloride cells:

Since there is some controversy in the literature (Torack and Lavalle, 1970; Shiina *et al.*, 1970; Clark and Ackermann, 1971; Klein *et al.*, 1972) about the validity of histochemical techniques employed, a brief discussion about the chemical nature of the deposits is required. There is no doubt that the osmium-silver fixative under the conditions applied is specific for chloride. This has been demonstrated by selected area electron diffraction and by autoradiography using $^{36}Cl^-$ (Komnick and Bierther, 1969; Wichard and Komnick, 1971; Komnick *et al.*, 1972). The osmium-antimonate fixative, however, reacts with a variety of inorganic and organic materials present in animal tissue, and is supposed to bind also with mucosubstances (Clark and Ackerman, 1971). Histochemical staining (Komnick *et al.*, 1972) and morphological findings described in this paper point to the presence of mucosubstances at the sites of antimonate deposits. However, it has been demonstrated with shed cuticles of *Callibaetis* (Komnick *et al.*, 1972), that antimonate precipitates were present in the apex of the coniform chloride cells only when sodium was present. When the cuticles were rinsed in distilled water prior to fixation, no deposits formed in the apices with the osmium-antimonate fixative. Therefore, it is concluded that the antimonate deposits in the apical portion of the ephemerid chloride cells indicate the presence of sodium at the same sites where chloride is present. At least in these cases, antimonate precipitates are primarily caused by sodium accumulated in the mucosubstances rather than by the mucosubstances themselves.

1. All types of chloride cells show dense precipitates of sodium and chloride in the apical region (Fig. 8) indicating that there is an accumulation of these electrolytes, although their origin and destination is not revealed by the histochemical technique.

2. The apices of all types of chloride cells are equipped with porous plates or porous envelopes, which are considered as sites of increased cuticular permeability (Komnick and Abel, 1971).

3. Another reason supporting an absorptive function is the observation of different types of chloride cells in different species. In those species lacking the coniform type (Table 1), which has been demonstrated to absorb salt, this function must be performed by the other types present, since the overall osmoregulatory situation is probably the same in these fresh water animals.

However, it is assumed that the different types of chloride cells may differ in their functional effectiveness. The coniform type is the only one which was observed alone in a number of species. This suggests that this type is the most efficient one to cover salt absorption required in osmoregulation. The other types probably are less efficient, because they always occur in mixed populations. If so, the question remains why the species of the family *Leptophlebiidae*, for example, which possess coniform cells, also have the caviform type ? This question is related to the more general question why these aquatic animals have developed different types of cells for the same function if one type alone is capable to meet to osmoregulatory requirements ? Further investigation of the function and taxonomic distribution of ephemerid chloride cells is needed to clarify this point.

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