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To Michael Hubbard
with best regards
from Hermann Komnick

Chloride Cells and Chloride Epithelia of Aquatic Insects

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I. Introduction

The term aquatic insects describes a rather heterogeneous group of insects which in various orders have independently invaded the aquatic habitat. In some insects aquatic life is restricted to certain developmental stages, and in others it extends throughout the entire life cycle. Accordingly, adaptations to the aquatic environment have been developed to various extents and in partly different, partly parallel ways.

Aquatic life demands special adaptation in three major respects: locomotion, respiration, and osmoregulation. In contrast to our knowledge of various locomotory adaptations and the great variety of respiratory adaptations of these originally air-breathing tracheate animals, relatively little information is available about the osmoregulatory adaptations of aquatic insects.

Generally, aquatic insects occur over a wide range of environmental salinity, from nearly salt-free springs to salt lakes containing sodium chloride at saturation. However, they flourish in fresh water, whereas the number of species tolerating saline environments is relatively small. This ecological dependence indicates that osmoregulatory

adaptation is the main limiting factor in the distribution of aquatic insects. Their osmoregulatory mechanisms apparently can handle low external concentrations of electrolytes better than high ones.

This article discusses recent work on chloride cells and chloride epithelia of aquatic insects, which appear to be functionally analogous to the long-known anal papillae. An attempt is made on the basis of present knowledge to join together the various morphological adaptations into a functional context.

II. Osmoregulatory Background

The necessity for the existence of accessory ion-absorbing sites in freshwater insects can be understood from the osmoregulatory situation of these animals. Therefore a brief discussion of the osmoregulatory principles of aquatic and terrestrial insects is a prerequisite for better understanding (for review, see Stobbart and Shaw, 1974).

In insects as in many other animals, osmoregulation is closely connected with excretion, the Malpighian tubules and the rectum being the most important organs of both functions. Ions and water lost with the urine, as well as water lost by transpiration, are replaced by oral intake and absorption in the gut, and by metabolic water (Fig. 1). Water uptake by cutaneous or rectal absorption of liquid water or water vapor appears to be restricted to ecologically specialized insects. For example, liquid water absorption has been shown in the cutaneous ventral tube of Collembola (Noble-Nesbitt, 1963) and in the eversible preanal (rectal) papillae of terrestrial syrphid larvae (Schueider, 1948). As for the absorption of water vapor, it has not yet been fully clarified whether it also takes place through the body surface as formerly suggested (Beament, 1965), but through the rectum (Noble-Nesbitt, 1970; Noirot and Noirot-Thimothée, 1971), and even through the mouth, as in ticks (Rudolph and Knülle, 1974). The rectum plays a central role in osmoregulation in that it controls by reabsorption the amount of water and ions being excreted. This faculty resides in the epithelial pads of the rectal wall, the fine structure of which has been investigated in various species (for review, see Wall and Oschman, 1975). However, in some, mostly aquatic, species the rectum lacks transporting cells. These are located in the ileum which performs the reabsorptive function instead of the rectum (Goodchild, 1969; Jarial and Scudder, 1970; Marshall and Wright, 1974; Schmitz and Komnick, 1976b). Water balance in terrestrial insects may become a serious problem for those species living under arid conditions, hence subject to high water loss by transpiration in connection with

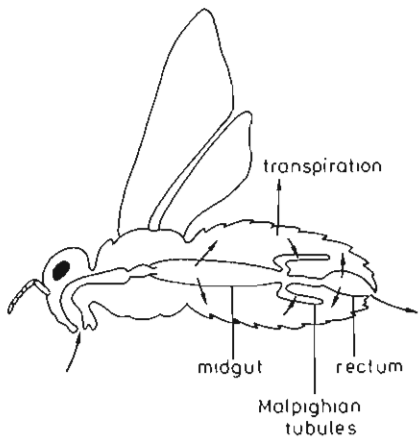

FIG. 1. Osmoregulation in terrestrial insects.

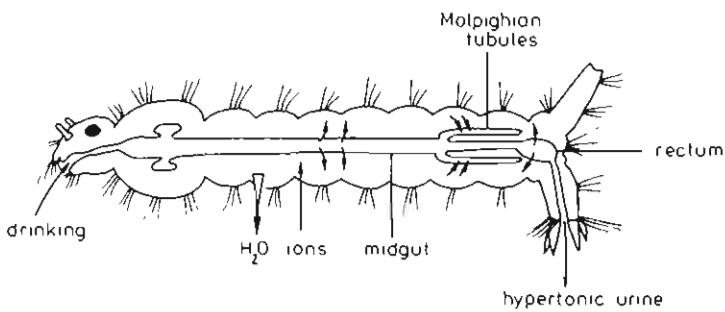

FIG. 2. Hypoosmotic regulation in salt-water insects. (Mosquito larva with small anal papillae.)

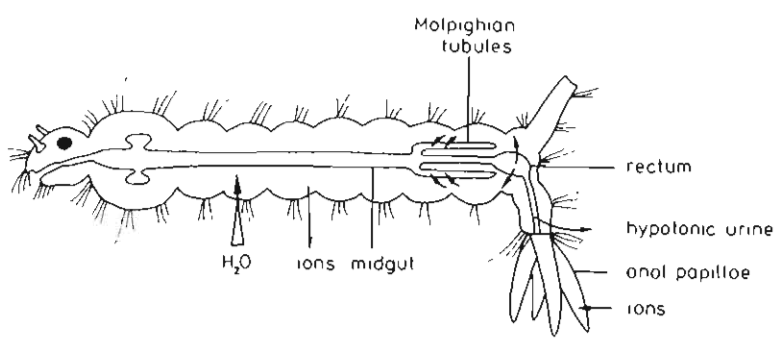

FIG. 3. Hyperosmotic regulation in freshwater insects. Arrows indicate main pathways for water and ions (see text for explanation). (Mosquito larva with large anal papillae.)

limited access to drinking water. They are able to conserve water by rectal reabsorption, resulting in the excretion of concentrated urine.

The osmoregulatory situation of saltwater or brakish-water insects, which are capable of hypoosmotic regulation, is similar to that of terrestrial insects with respect to water deficiency (Fig. 2). The osmotic difference between the external and internal medium favors osmotic outflux of water and influx of ions, which in a given species depend on the permeability of the cuticle. Drinking the hyperosmotic medium in compensation for water loss results in further influx of ions. The main problem of these insects is to conserve water and to eliminate excess ions. Water conservation is achieved by the excretion of hypertonic urine resulting from rectal water reabsorption. In euryhaline species, which are capable of both hyperosmotic regulation in fresh water and hypoosmotic regulation in salt water, different transporting epithelia have been observed in the anterior and posterior regions of the rectum. One is thought to be responsible for ion reabsorption in a freshwater environment, and the other for water reabsorption or possible for ion excretion in a saltwater environment (Goodchild, 1969; Meredith and Phillips, 1973c). In *Ephydrella* the two types of transporting cells are present in the ileum instead of the rectum (Marshall and Wright, 1974). Little is known about accessory sites of ion removal in saltwater insects comparable to the chloride cells of marine fish (Maetz and Bornancin, 1975) or vertebrate salt glands (Peaker and Linzell, 1975). So far, extrarenal ion excretion has been reported only for larvae of the saltwater mosquito *Aedes campestris*, the anal papillae of which appear to be capable of both ion absorption in fresh water and ion excretion in salt water and thus behave like the chloride cells of euryhaline fish (Phillips and Meredith, 1969).

For freshwater insects whose hemolymph osmolarity is in the range of approximately 200–400 mosm, the osmotic gradient is reversed, thus favoring passive water influx and efflux of ions (Fig. 3). The cutaneous water influx amounts to approximately 1–15% of the body weight per day, depending on the species. Removal of surplus water is achieved by the production of hypotonic urine. Although these animals possess effective mechanisms of ion conservation, such as rectal or ileal ion reabsorption and excretion of ammonium bicarbonate as the main osmotic component of the urine, ion loss is inevitable. Thus, for compensation, active ion absorption is necessary for hyperosmotic regulation in freshwater insects.

Stobbart and Shaw (1974, p. 413) wrote in their review: "Very probably ion-absorbing organs of various kinds will be found in many aquatic insects but, in some, such organs are apparently absent." The

only sites generally known to perform this function are the anal papillae. However, more recently, various other sites have been shown to play a role. This article deals exclusively with such ion-absorbing structures of freshwater insects.

III. Survey of the Absorptive Structures

From the theoretical point of view the most favorable site for the location of ion-absorbing structures is the body surface, where they are, without any additional activity of the animal, in direct and permanent contact with the external medium and where the medium is renewed either continuously, as in running water, or intermittently, as in still water, during locomotion. The best position is near the respiratory organs, where the medium is replaced by ventilation in the resting animals as well. This might explain why in numerous species the absorptive structures are indeed located on the body surface and often close to or on the gills. But unlike the cutaneous absorption sites, there are others which are located on the internal body surface, hence require, in addition to osmotic work, energy for making contact with the external medium. This contact can be brought about by eversion of the absorptive structures, as in the case of the preanal papillae, or by ingestion of the medium, which can be achieved by fluid uptake through the mouth or through the anus. Ventilation of the rectum via the anus, as observed in Odonata, exposes only the hind part of the intestinal tract to the external medium and thus appears to be superior to drinking. Unless absorption takes place in the anterior region of the gut and the fluid is regurgitated, which has not yet been shown in freshwater insects, drinking implies that the intestinal epithelium throughout the entire length of the gut is exposed to the highly hypotonic fresh water, which may further add to osmotic water influx and even interfere with the nutritive function by dilution of the food in the gut lumen.

All the possibilities mentioned above are realized in freshwater insects. The presumptive or proven absorptive sites so far identified are representatively shown in Fig. 4. These sites are chloride cells, chloride epithelia, and anal papillae of various types and locations, and the epithelium of the midgut or small intestine in drinking insects. As seen in Table I, which lists the occurrence of these structures according to their taxonomic distribution and which awaits completion by further investigations, convergent and divergent developments of the various absorption structures have taken place. Chloride cells have developed in three different orders, Ephemeroptera, Plecoptera,

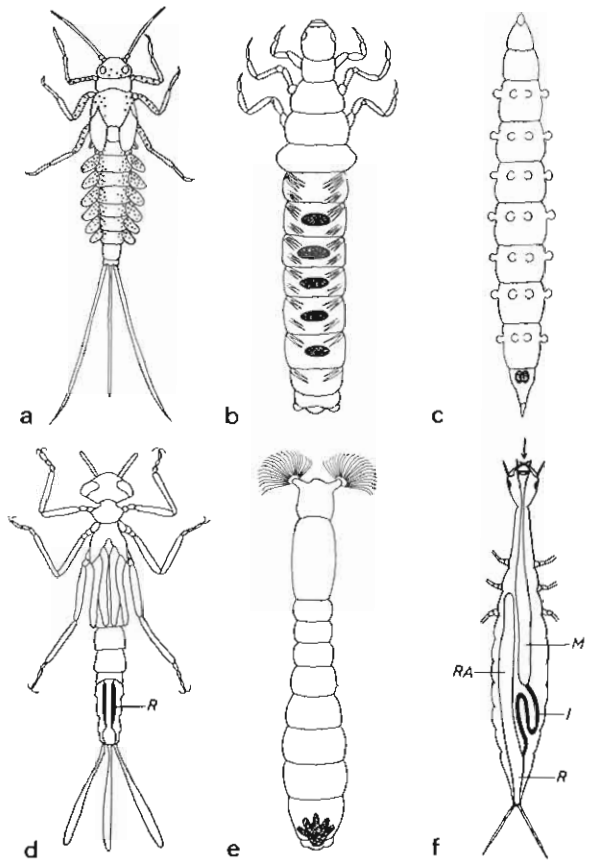

FIG. 4. Examples of the location of ion absorption sites in freshwater insects. (a) Chloride cells in Ephemeroptera, Baetidae. (b) Abdominal chloride epithelia in Trichoptera, Limnophilidae. (c) Anal chloride epithelia in Diptera, Tabanidae. (d) Rectal Chloride epithelia in conjunction with rectal ventilation in Odonata, Coenagrionidae. (e) Everted preanal papillae in Diptera, Simuliidae. (f) Ileum in conjuuction with drinkiug in Coleoptera, Dytiscidae. I, Ileum; M, midgut; R, rectum; RA, rectal ampulla.

and Heteroptera. The same holds true for chloride epithelia, which have an abdominal location in Trichoptera, an anal location in Diptera, and a rectal location in Odonata, as well as for the anal papillae occurring in Diptera, Trichoptera, and Coleoptera. Some of these orders have developed different absorption sites in parallel in different suborders or families. This applies to Trichoptera, some families of which possess abdominal chloride epithelia, and others anal papillae. Among the Diptera, the Nematocera are equipped with anal

TABLE I
OCCURRENCE OF ION ABSORPTION SITES IN AQUATIC INSECTS

Absorption site	Occurrence
Chloride cells	Ephemeroptera
	Plecoptera
	Heteroptera
Chloride epithelia	
Abdominal	Trichoptera: Limnophilidae, Goeridae
Anal	Diptera:
	Brachycera: Tabanidae, Stratiomyidae, Leptidae
	Cyclorrhapha: Ephydridae, Muscidae, Drosophilidae
Rectal	Odonata: Anisoptera, Zygoptera
Anal papillae	Diptera:
	Nematocera
	Cyclorrhapha: Syrphidae
	Trichoptera: Glossosomatidae, Philopotamidae
	Coleoptera: Elodidae
Drinking	
Midgut	Megaloptera: Sialidae
Ileum	Coleoptera: Dytiseidae

papillae and the Brachycera with anal chloride epithelia, whereas aquatic Cyclorrhapha possess either anal papillae or anal chloride epithelia. Among the Coleoptera, the Elodidae have anal papillae, whereas the Dytiscidae drink. Nothing is known about the other aquatic insects.

From the histological point of view, the various absorptive structures, except those connected with drinking, can be arranged in a rational order as follows:

Single cells (unicellular chloride cells)
↓
Cell groups (chloride cell complexes)
↓
Cell fields (chloride epithelia)
↓
Organ formation (anal papillae)

The beginning of organ formation is already detectable in anal chloride epithelia which in some species form eversible, papillae-like appendices. The above arrangement is purely descriptive and does not reflect a developmental relation. The subsequent presentation of the details will mainly follow the classification given in Table I.

IV. Chloride Cells

A. Ephemeroptera

In ephemeropteran larvae chloride cells may be encountered on nearly all parts of the body surface (Komnick and Abel, 1971), However, they are normally concentrated on the tracheal gills as well as on the lateral sides of the abdominal stemites and tergites (Fig. 4a). The distribution pattern of these cells may vary in different species. For example, in *Caenis* they appear to be restricted to the region of the so-called branchial chamber, where they occur in dense populations on the underside of the operculum, on both sides of the gill lamellae and on the tergites underneath the gills. After transition from aquatic to terrestrial life, chloride cells are no longer found in the subimaginal and imaginal stages as reported for *Cloeon* (Komnick and Wichard, 1975a).

The fine structure of chloride cells has been studied so far in numerous species belonging to eight ephemeropteran families (Table II). According to their structural variations, they have been classified into four types termed caviform, coniform, bulbiform, and filiform chloride cells. The caviform type is unicellular, whereas the other three types are small cell complexes consisting of at least two cells (Wichard *et al.*, 1972). Interspecific differences in the occurrence of the four types were observed. These are listed in Table II. The coniform type is the only form found as the exclusive type in certain species, whereas the other types never occur alone, but always with at least one other type.

The unicellular caviform type (Fig. 5a) is characterized by an apical cavity and therefore resembles teleost chloride cells (e.g., Bierther, 1970). The prominent features of this type of chloride cell are the plications of the apical plasma membrane which forms microvillous projections extending into the apical cavity, as well as tubular of slitlike infoldings coursing down into the basal cell region and closely associated with numerous mitochondria (Wichard and Komnick, 1971; Wichard *et al.*, 1972).

The coniform type of chloride cell was first described light microscopically by Eastham (1936) in *Caenis* and interpreted as being an unusual kind of campaniform sensillum. Csoknya and Halasz (1972), in an electron microscope study, favored the same interpretation. However, as judged from their electron micrographs, bacteria adhering to the cuticle and the striations of the cuticular porous plate were mistaken for cilia and the tubular body, respectively. These structures, normally found in sensilla, are definitely absent (Wichard *et al.*, 1972). In addition, the dendritic cell process described by these inves-

TABLE II

ELECTRON MICROSCOPE DEMONSTRATION OF CHLORIDE CELLS IN EPHEMEROPTERAN LARVAE

Family, genus, and species	Type of chloride cell				Reference
	Caviform	Coniform	Bulbiform	Filiform	
Baetidae					
Cloeon dipterum	–	++	–	–	Wichard and Komnick (1971)
Callibaetis coloradensis	–	++	–	–	Komnick and Abel (1971)
Baetis rhodani	–	++	–	–	Wichard *et al.* (1972)
Baetis tricaudatus	–	++	–	–	Wichard *et al.* (1972)
Caenidae					
Caenis diminuta	–	++	–	–	Wichard *et al.* (1975)
Palingeniidae					
Palingenia longicauda	–	++	–	–	Csoknya and Halasz (1972)
Ephemeridae					
Ephemera vulgata	+	++	–	–	Wichard *et al.* (1972)
Siphlonuridae					
Ameletus sp.	+	++	–	–	Wichard *et al.* (1972)
Leptophlebiidae					
Leptophlebia marginata	+	++	–	–	Wichard *et al.* (1972)
Habroleptoides modesta	+	++	–	–	Wichard *et al.* (1972)
Choroterpes albiannulata	+	++	–	–	Wichard *et al.* (1972)
Ephemerellidae					
Ephemerella grandis	+	–	++	–	Wichard *et al.* (1972)
Ephemerella ignita	+	–	++	–	Wichard *et al.* (1972)
Heptageniidae					
Heptagenia solitaria	+	–	+	+	Wichard *et al.* (1972)
Ecdyonurus venosus	+	–	+	+	Wichard *et al.* (1972)
Epeorus assimilis	+	–	+	+	Wichard *et al.* (1972)
Rithrogena doddsi	+	–	+	+	Wichard *et al.* (1972)
Rithrogena semicolorata	+	–	+	+	Wichard *et al.* (1972)

+, Present; + +, predominant type; –, not observed.

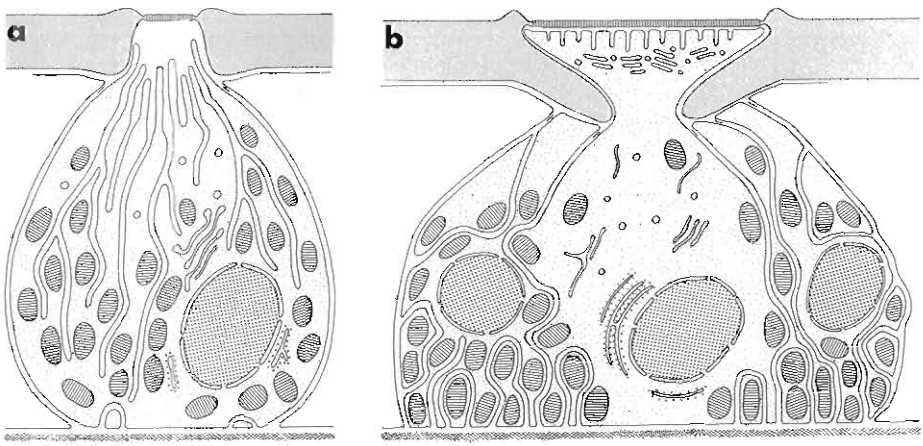

FIG. 5. Caviform (a) and coniform (b) chloride cells of Ephemeroptera. From Wichard and Komnick (1973b).

tigators presumably corresponds to the apical process of the new central cell which grows upward from the epithelial base during the premolting stage (Komnick and Abel, 1971).

The coniform type (Fig. 5b) consists of one central cell and at least one adjacent cell, which are grouped together into a small cell complex clearly distinguishable as a structural unit from the normal epithelial cells of the hypodermis (Komnick and Abel, 1971; Wichard and Komnick 1971; Wichard *et al.*, 1972). The apex of the central cell forms a cone-shaped plug which fits into a recess left by the missing endocuticle. The apical plasma membrane shows short infoldings studded on the cytoplasmic face with small particles, as described in many other transporting cells and possibly containing carbonic anhydrase (cf. Berridge and Oschman, 1972). In tangential sections of the apex these infoldings are partially branched and take a labyrinthine course similar to those depicted in Figs. 10c and 14a in plecopteran and heteropteran chloride cells. However, in contrast to the situation in the caviform type, the main enlargement of the plasma membrane surface is found along the basolateral circumference. This enlargement results from intimate cell interlocking which mainly occurs between the adjacent cells but to a lesser extent also between the central cell and the adjacent cells. The mitochondria are predominantly located in the regions of cellular interdigitation.

The bulbiform and filiform types have the same cellular fine struc-

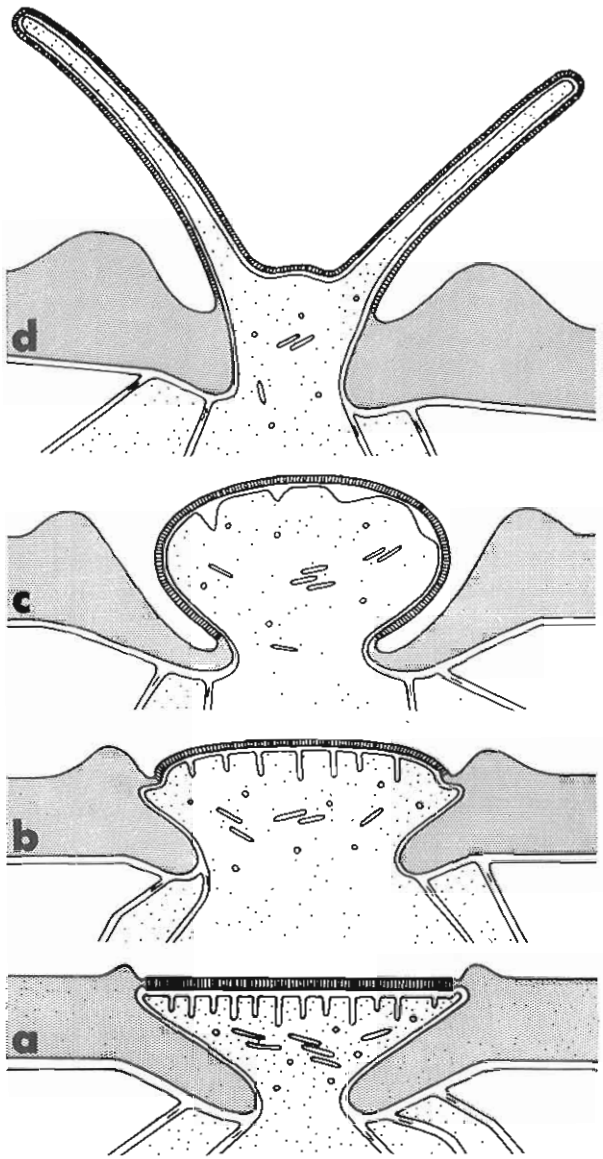

FIG. 6. Diagram of the apical differentiation of ephemeropteran chloride cell complexes. Progressive protrusion and ramification of the central cell apex from coniform (a) to bulbiform (c) and filiform (d) types. From Wichard and Komnick (1973b).

ture as the coniform type. They differ only in the shape of their apical differentiations, which have been studied by both transmission and scanning electron microscopy (Wichard *et al.*, 1972). The central cell of the bulbiform type forms a bulbous projection extending beyond the external level of the cuticle (Fig. 6c), whereas the filiform type possesses a filamentous projection which may be unbranched, bifurcate (Fig. 6d), or even brushlike with up to 11 filaments branching from the top of a short stalk. These two types have possibly derived from the coniform type by progressive protrusion and ramification of the central cell apex (Fig. 6) because, apart from these differences, their fine structure is identical.

A common feature of all types of ephemeropteran chloride cells is the so-called porous plate. However, this structure has a platelike form only in caviform and coniform chloride cells, where it covers the apical cavity and distal face of the cone-shaped plug, respectively. In the bulbiform and filiform types it envelops the apical projections and therefore takes the corresponding form (Fig. 6). These porous plates (Fig. 7a) are local differentiations of the cuticle overlying the chloride cells. The endocuticle is lacking at these sites, and the remaining distal layers of the cuticle form a thin sheet of highly complicated structure which has been studied in detail in coniform chloride cells (Komnick and Stockem, 1973). The material of the dense layer is differentiated into vertical rods arranged in a hexagonal pattern and interconnected by horizontal bars (Fig. 7b and c). In the transcuticular direction this scaffolding leaves triangular and rectangular pores, as shown with the lanthanum infiltration technique. The side dimension of the triangular pores is 20 nm, while the rectangular pores measure 20×4 nm. The porous plate is covered by an additional porous lamina which is continuous with the cuticulin layer. This lamina also contains small pores of 2.5-nm diameter in a hexagonal pattern, which are permeable to colloidal lanthanum hydroxide (Fig. 7d). These porous plates have been interpreted as being sites of high cuticular permeability related to the ion-absorbing function of the chloride cells.

B. Plecoptera

In plecopteran larvae three types of chloride cells have been described so far in a small number of species studied with the electron microscope (Table III). These have been termed caviform, coniform, and bulbiform chloride cells, because they closely resemble the corresponding types of ephemeropteran chloride cells. The last-mentioned two types are already known from light microscope investigations, but they were formerly interpreted as being respiratory in function (Zwick,

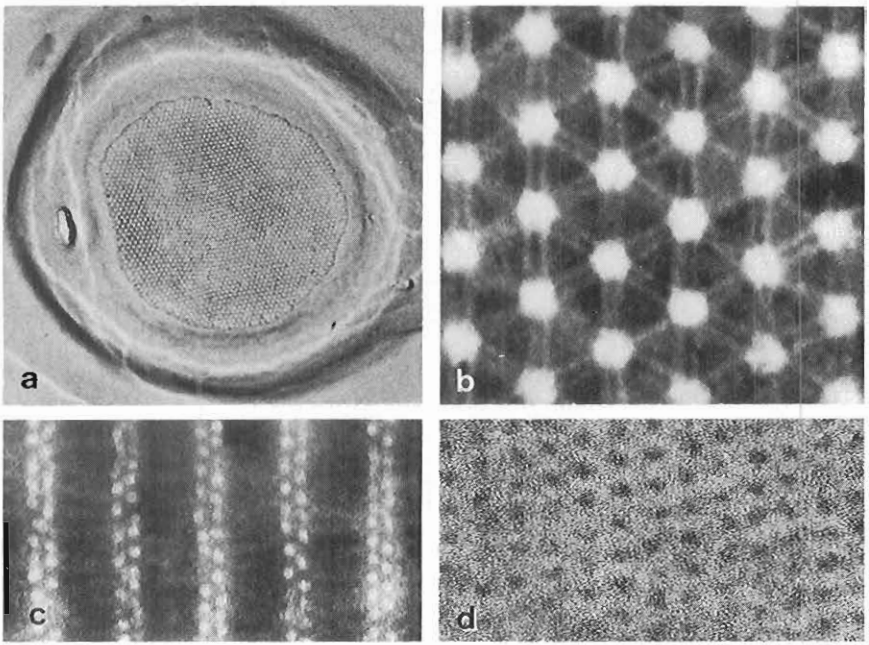

FIG. 7. Fine structure of the porous plate of an ephemeropteran coniform chloride cell. (a) Carbon replica of a chemically etched porous plate. ×12,000. (b) Surface-parallel section of a porous plate infiltrated with colloidal lanthanum hydroxide, showing vertical rods interconneeted by pairs of horizontal bars. ×300,000. (c) Cross section of a porous plate infiltrated with colloidal lanthanum hydroxide, showing double rows of horizontal bars. +300,000. (d) Surface-parallel section of the porous lamina overlying the porous plate, showing hexagonal pattern of 25-Å pores infiltrated with colloidal lanthanum hydroxide. ×600,000. From Komnick and Stockem (1973).

1973). It may be expected that other cells known from light microscopy, to which respiratory or sensory functions have been attributed, will be revealed as chloride cells when studied with appropriate methods.

The distribution of chloride cells on the body surface is species-dependent. The ventral and lateral aspects of the abdomen and thorax, the intersegmental membranes, and the tracheal gills appear to be favorite sites of occurrence (Kapoor and Zachariah, 1973a,b; Wichard and Komnick, 1973c, 1974a). On the proparanota of *Perla marginata*, which are very rich in chloride cells, the population density reaches 3000 cells per square millimeter.

The caviform type found in plecopteran larvae is unicellular and

TABLE III
ELECTRON MICROSCOPE DEMONSTRATION OF CHLORIDE CELLS IN PLECOPTERAN LARVAE

Family, genus, and species	Type of chloride cell			Reference
	Caviform	Coniform	Bulbiform	
Perlidae				
Paragnetina media	–	+	–	Kapoor and Zachariah (1973a,b)
Perla marginata	–	+	–	Wichard and Komnick (1973c)
Perlodidae				
Perlodes microcephala	–	+	–	Wichard and Komnick (1973c)
Leuctridae				
Leuctra sp.	–	+	–	Komnick, unpublished observation
Nemouridae				
Nemoura cinerea	+	–	+	Wichard and Komnick (1974a)
Protonemura auberti	+	–	+	Wichard and Komnick (1974a)

+, Present; –, not observed.

very similar in fine structure to the caviform type in ephemeropterans (compare Fig. 8 with Fig. 5a). Differences exist only in the organization of the extracellular components in the apical region. The cuticular hole contains a spongelike dense material which forms a knob-shaped external protrusion covered with a thin porous layer. From the inner face of this knob a hollow cylinder formed by fine filaments extends down into the apical cavity which occasionally contains a few electron-dense granules (Wichard and Komnick, 1974a).

The coniform and bulbiform types are paucicellular complexes similar to the corresponding types in ephemeropterans. The fine-structural organization of the subcuticular portion is basically the same (compare Figs. 9 and 11 with Fig. 5b). However, in contrast to the respective types in ephemeropterans, the apical portion is also rich in mitochondria and cellnlar interdigitations. In the coniform type, the lateral circumference of the central cell apex is encircled by a thin sheath of adjacent cell cytoplasm, which gives rise to cytoplasmic lamellae radially penetrating into plications of the central cell apex. This mode of interdigitation results in a spokelike pattern seen in cross section (Fig. 10d). The apical plasma membrane shows a labyrinthine pattern of short infoldings (Fig. 10c).

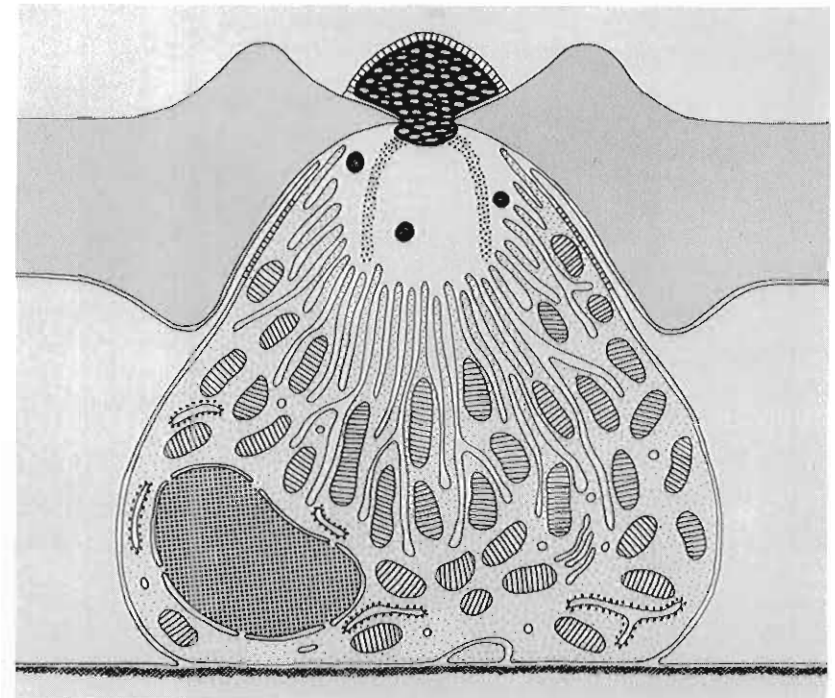

FIG. 8. Caviform chloride cell of Plecoptera. Drawn after Wichard and Komnick (1974a).

The distal, supracuticular portion of the bulbiform type is similar in shape to an acorn (Fig. 11). Apical processes of both the central and adjacent cells penetrate through the narrow stalk into the bulbous dilation, where the cytoplasm of the central cell is arranged in a fountainlike pattern. It extends centrally to the tip of the bulb, bends back along the periphery, and thus overlaps the apical process of the adjacent cell. Additional interdigitation between the central cell and adjacent cell cytoplasm is apparent in this zone of overlap (Fig. 11).

The thin cuticular layer covering the apical part of each type of plecopteran chloride cell is devoid of endocuticle and differentiated into a porous plate or envelope (Figs. 8, 9, 10a and 11). The pores are long, narrow, parallel-arranged slits (Fig. 10b) and therefore distinctly different from the triangular pores in the porous plates of ephemeropteran chloride cells (Fig. 7b). The porous plate of the coniform type consists of several lamellae, and the parallel slit pores run in different directions (Wichard and Komnick, 1973c). The porous envelope of the

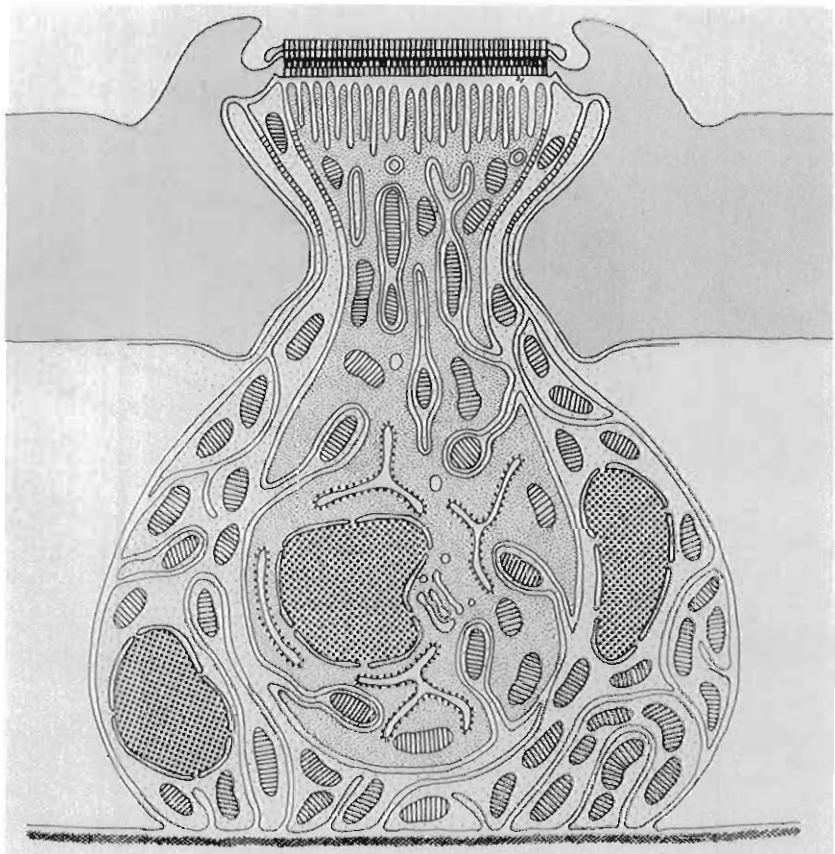

FIG. 9. Coniform chloride cell of Plecoptera. Drawn after Wichard and Komnick (1973c).

bulbiform type is folded. The folds run helically around the bulb and are covered with an outermost layer of lamellate structure (Wichard and Komnick, 1974a).

C. HETEROPTERA

There are several families of aquatic Heteroptera, which are grouped together by some taxonomists as Hydrocorisae. In contrast to the Ephemeroptera and Plecoptera, representatives of the Hydrocorisae normally live in water throughout their whole lives. Therefore chloride cells are present in both larvae and adults. Heteropteran

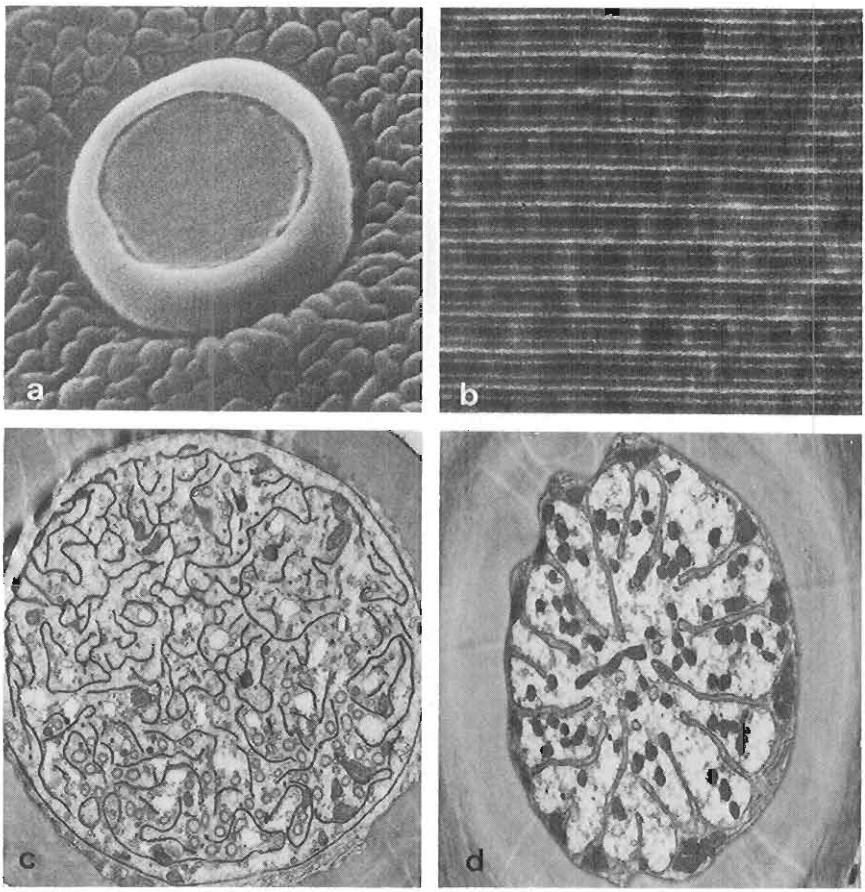

FIG. 10. Coniform chloride cell of Plecoptera. (a) Scanning electron micrograph of a porous plate. ×3200. (b) Surface-parallel section of a porous plate infiltrated with colloidal lanthanum hydroxide, showing the parallel pattern of slitlike pores. ×200,000. (c) Surface-parallel section of the central cell apex directly underneath the porous plate, showing tortuous infoldings of the apical plasma membrane. ×9000. (d) Same plane of section as in Fig. 10c, but somewhat deeper, showing the radial pattern of interdigitation between central and adjaceut cells. ×9000. From Wichard and Komnick (1973c).

chloride cells have been studied fine structurally in representatives of four families (Table IV). However, in addition to the species listed in Table IV, their presence has been demonstrated by histochemical chloride precipitation in *Plea leachi* (Pleidae), *Micronecta wagneri* (Corixidae), and five unidentified corixid species (Komnick and Wichard, 1975a). They normally occur on body sites that are in direct

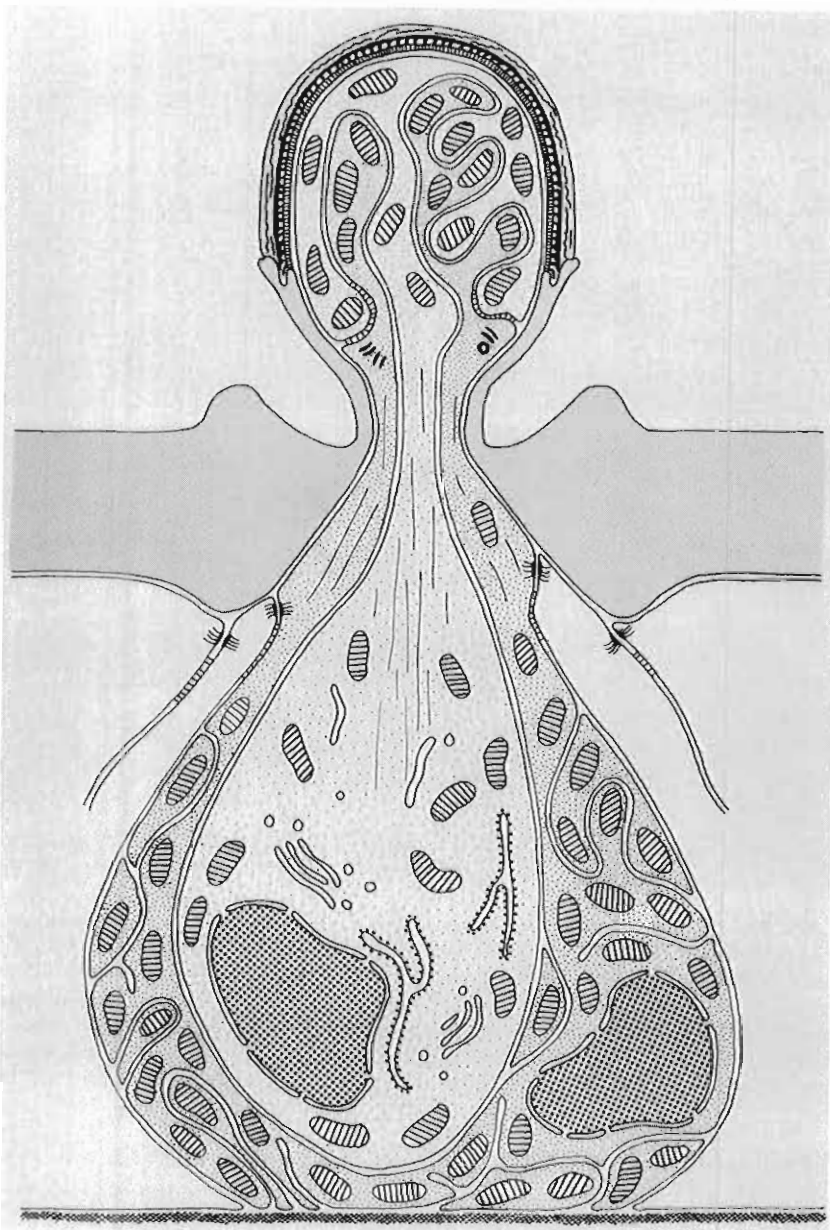

FIG. 11. Bulbiform chloride cell of Plecoptera. Drawn after Wichard and Komnick (1974a).

TABLE IV
ELECTRON MICROSCOPE DEMONSTRATION OF CHLORIDE CELLS IN AQUATIC HETEROPTERA

Family, genus, and species	Type of chloride cell				Reference
	Caviform	Coniform	Bulbiform	Tubiform	
Notonectidae					
Notonecta glauca	+	–	–	–	Komnick and Wichard (1975a)
Naucoridae					
Naucoris cimicoides	+	–	–	–	Komnick and Wichard (1975a)
Corixidae					
Hesperocorixa sahlbergi	–	+	–	–	Komnick and Wichard (1975b)
Corixa punctata	–	+	–	–	Komnick and Schmitz (1976)
Nepidae					
Nepa rubra	–	–	+	+	Komnick (1976)
Ranatra linearis	–	–	+	+	Komnick (1976)

+, Present; – not observed.

contact with the surrounding water, whereas those parts that are covered by respiratory air films or wings are devoid of chloride cells. Their distribution may even vary in a given species during ontogenetic development, depending on the onset of plastron respiration. In *Hesperocorixa* (Table V) there is a stepwise increase in the total number

TABLE V
DISTRIBUTION AND NUMBER OF CHLORIDE CELLS IN THE DEVELOPMENTAL STAGES OF *H. sahlbergi* RAISED FROM THE EGGS IN TAP WATER[a,b]

Stage	Head	Thorax dorsal	Abdomen		$\Sigma\bar{x}$
			Dorsal	Ventral	
Imago	ca. 7000	—	—	—	ca. 7000
5th instar	424.8 ± 72.5	418.2 ± 106.9	3494.0 ± 460.4	—	4337.0
4th instar	233.1 ± 54.0	380.7 ± 51.2	2579.1 ± 392.5	—	3192.9
3rd instar	83.7 ± 14.6	217.9 ± 26.3	1173.5 ± 46.8	—	1475.1
2nd instar	39.4 ± 3.8	116.7 ± 12.8	435.6 ± 31.5	211.1 ± 36.3	802.8
1st instar	—	—	115.5 ± 19.2	30.6 ± 9.7	146.1

[a] Adopted from Komnick and Wichard, 1975a.
[b] Number of chloride cells; $\bar{x} \pm s$, $N = 10$.

of chloride cells with each molt, from originally 146 during the first instar to approximately 7000 in the adult. (These figures do not include the chloride cells present on the legs.) During the first and second instar, they are located on the ventral and dorsal side of the abdomen. After molting into the third instar, when plastron respiration begins, they are no longer observed on the abdominal sternites which are now covered with air. The loss from the sternites is overcompensated for by a large increase in the number on the abdominal tergites. From the second instar to adulthood, the chloride cells spread in increasing numbers over the dorsal side of the thorax and head. In the winged adult, they are no longer present on the dorsal side of the abdomen and thorax, the head and legs now being the only parts exposed to water and the only sites of chloride cell occurrence.

All types of heteropteran chloride cells so far described fine structurally are unicellular. The caviform type found in representatives of

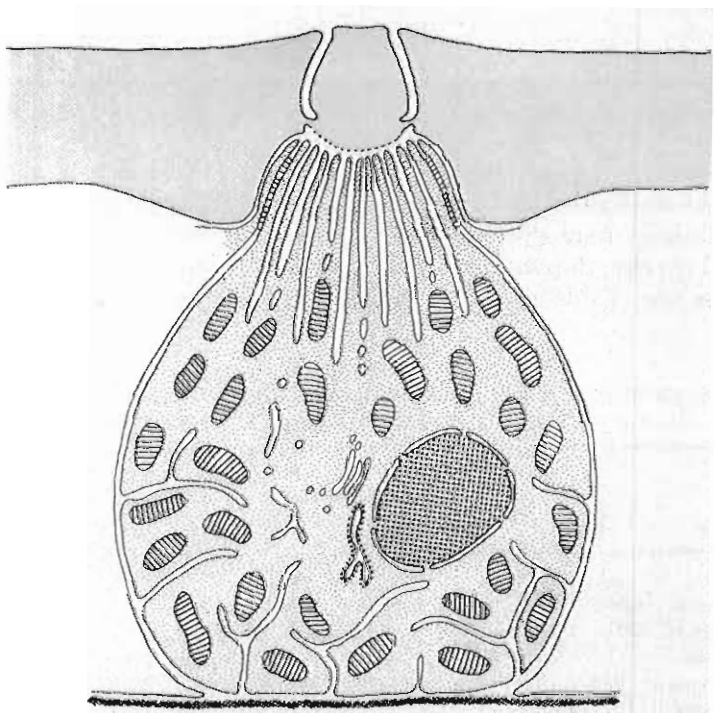

FIG. 12. Caviform cell of Heteroptera, Notonectidae, and Naucoridae. From Komnick and Wichard (1975b).

two different families, Notonectidae and Naucoridae (Table IV), most closely resembles the caviform types in ephemeropteran and plecopteran larvae. However, in addition to the infoldings of the apical plasma membrane, the basolateral cell membrane is also infolded (Fig. 12). The cuticular barrier over the apical cavity is reduced to a thin diaphragm, which at the midlevel of the cuticle occludes an annular cleft indented from the outer surface.

Two slightly different types of coniform chloride cells are present in corixidae (Fig. 13). In *Hesperocorixa sahlbergi* they occur in the numerical relation of approximately 1:1. Both are mitochondria-rich cells which mainly differ in the site and degree of plasma membrane infoldings. One type is infolded along the basolateral side but possesses a nearly smooth apical cell surface with only a few short microvilli (Fig. 13b), whereas the apex of the other type shows deep membrane infoldings (Fig. 13a), which are very densely packed as seen in a cross section of the apex (Fig. 14a). The cytoplasmic face of the infolded membranes is coated with small particles (Fig. 14b). A narrow zone of peripheral cytoplasm is free of folds and contains microtubules which are perpendicularly oriented to the cuticle (Fig. 14b). The possibility that these two cells represent different functional stages of the same cell type rather than two distinct cell types is incompatible with the different structures of the cuticular porous plates, which can be altered only during molt. In one type the circular opening into the cone-shaped hollow of the endocuticle is relatively narrow. The internal side of the porous plate is lined by radially oriented ridges of the epicuticle. Radial rows of oval pores are arranged between these ridges (Fig. 14c). In the other type the opening at the lower level of the cuticle is about three to four times larger in diameter. The internal face of the porous plate is smooth, whereas the external face shows a fingerprint relief. The pores are also arranged in radial rows, but are more circular in outline (Fig. 14d). The perforations are about 0.1–0.2 μm wide and are filled with a flocculent material of unidentified nature.

The conical apex of corixid chloride cells may vary in shape depending on the thickness of the cuticle. The two types of chloride cells present on the front of the head of adult *Corixa punctata* are fundamentally similar to the chloride cells of *Hesperocorixa* larvae with respect to the fine structural organization of the cytoplasm. However, they differ with respect to the shape of the cell apex. Due to the relatively thick cuticle covering the head of the large adults, the apex of the chloride cells is no longer coniform, but cylindrical, measuring approximately 5 μm in diameter and 25 μm in length.

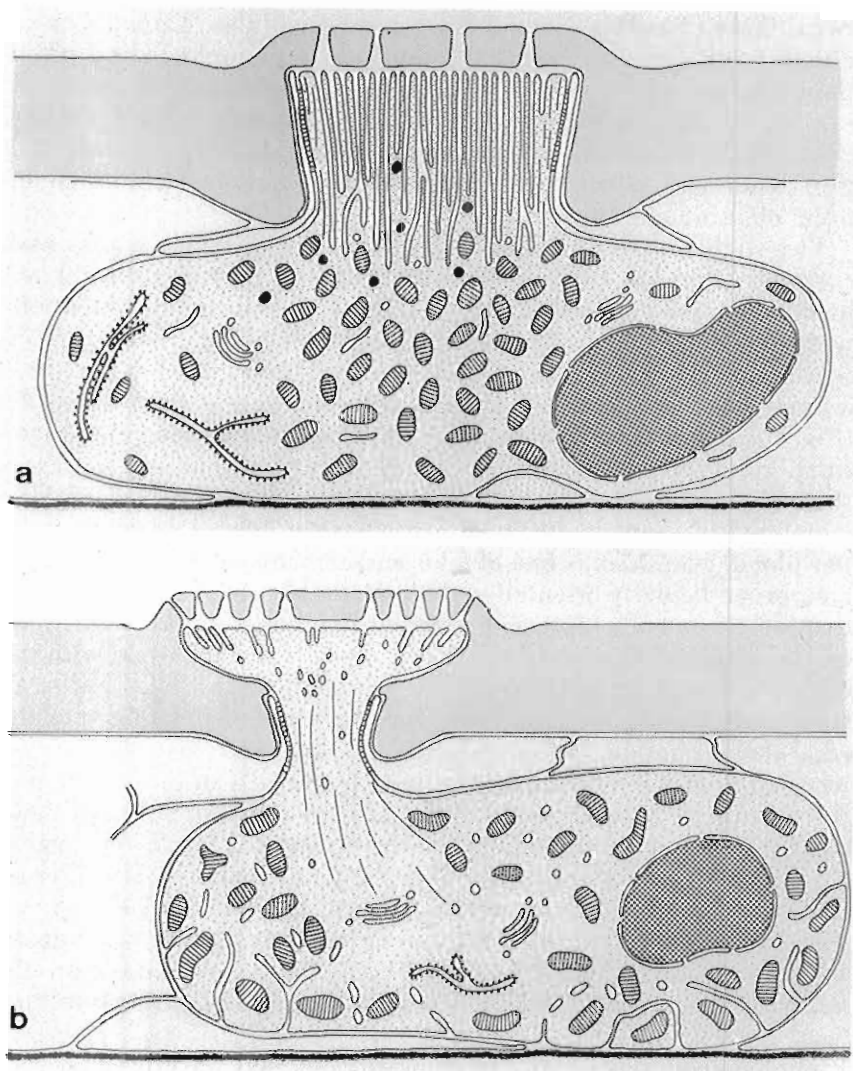

FIG. 13. Two modified coniform chloride cells of Heteroptera, Corixidae. Drawn after Komnick and Wichard (1975a).

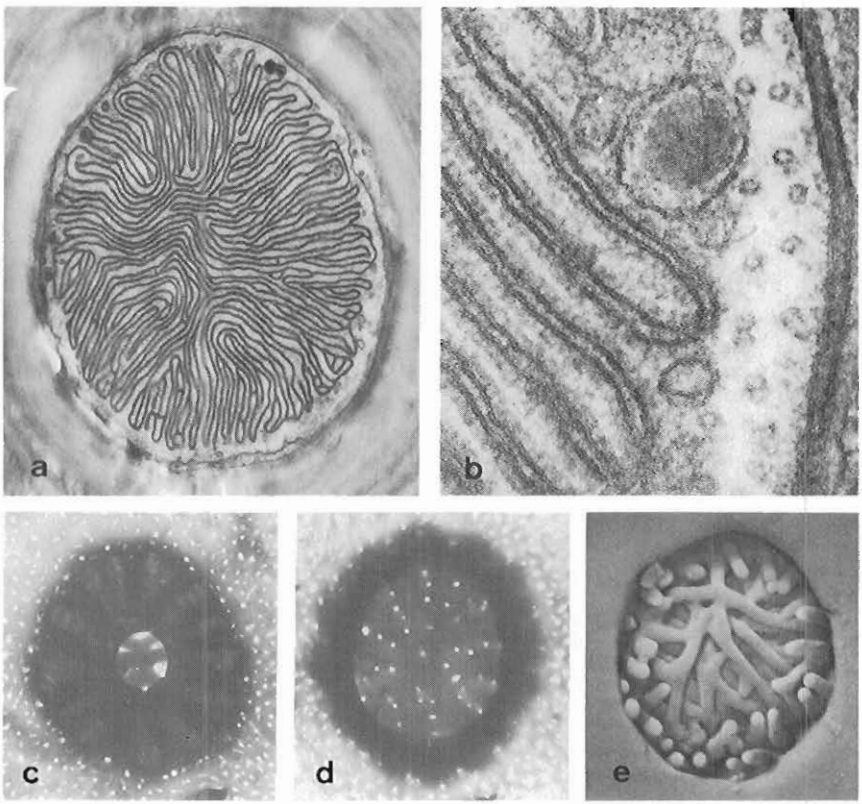

FIG. 14. (a) Surface-parallel section of the apex of a coniform chloride cell as shown in Fig. 13a, demonstrating the densely packed infoldings of the apical plasma membrane. ×15,000. (b) The same section as in Fig. 14a at higher magnification, showing cortical microtubules and the particulate coat on the cytoplasmic face of the infolded membranes. ×125,000. (c) and (d) Transmission electron micrographs of the porous plates of the chloride cells shown in Fig. 13b (c) and in Fig. 13a (d) taken from shed whole-mount cuticle of *Hesperocorixa*. ×6000. From Komnick and Wichard (1975a). (e) Scanning electron micrograph of the porous plate of a tubiform chloride cell of *Nepa*. ×5000. From Komnick (1976).

The bulbiform and tubiform types of chloride cells found in Nepidae are very similar with respect to cellular fine structure. Both types (Figs. 15 and 16) show numerous invaginations of the basal plasma membrane, which are closely associated with mitochondria, and a small apical cavity with short microvilli. They differ, however, in cell shape and cuticular structure. The bulbiform type is a flask-shaped cell, with its neck extending into an external protrusion of the

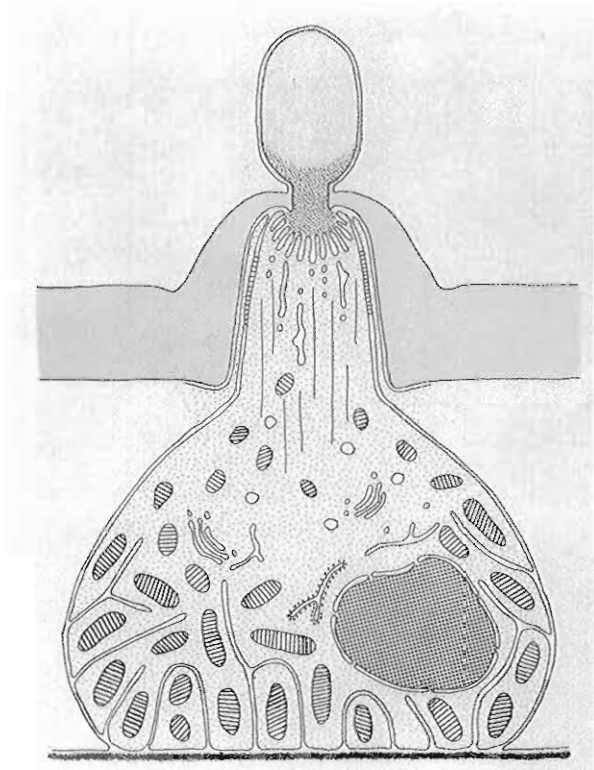

FIG. 15. Bulbiform chloride cell of Heteroptera, Nepidae. From Komnick (1976).

cuticle (Fig. 15). The apical cavity opens through a central hole into a thin-walled cuticular bleb filled with electron-translucent, filamentous, extracellular material which proximally appears more condensed. The tubiform type is nearly disc-shaped, and has a central depression into which a thin-walled cuticular tube penetrates (Fig. 16). Similar to the tracheae, the tube wall is stiffened by a helical fold. The extracellular contents of the tube and its relation to the apical cavity correspond to those of the bulbiform type. However, the tube communicates with the outside via meshes of a porous plate which consists of interlacing rods branching off from the cuticle (Figs. 14e and 16).

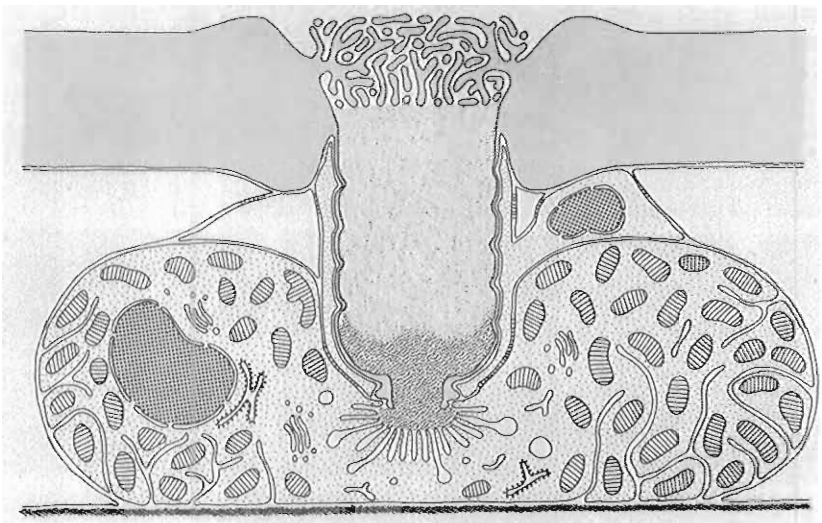

FIG. 16. Tubiform chloride cell of Heteroptera, Nepidae. From Komnick (1976).

D. General Remarks

Chloride cells are readily detectable with the light microscope after appropriate treatment with fixatives containing silver ions for the histochemical precipitation of chloride. They appear as white dots when viewed under reflecting light, provided fixation and subsequent treatment have been carried out under minimum illumination to avoid photochemical reduction of the silver chloride precipitates (Fig. 17a). Good visualization as black dots in the bright-field microscope is also achieved after reduction of the precipitates by light or by reducing reagents. Thus the method of histochemical chloride precipitation is a useful technique for the localization of chloride cells. When examined with the electron microscope (Fig. 17b), the precipitates are specifically localized in the apical region of all types of chloride cells studied so far. There is evidence from selected area electron diffraction and energy-dispersive analysis of x rays (Fig. 17c–e) that the precipitates are silver chloride (Wichard and Komnick, 1971, 1973c).

All types of chloride cells hitherto observed in aquatic insects are fine-structurally characterized by the prevalence of mitochondria and plasma membrane plications. These features are characteristic of cells with active transport functions. Apart from their basic organization as

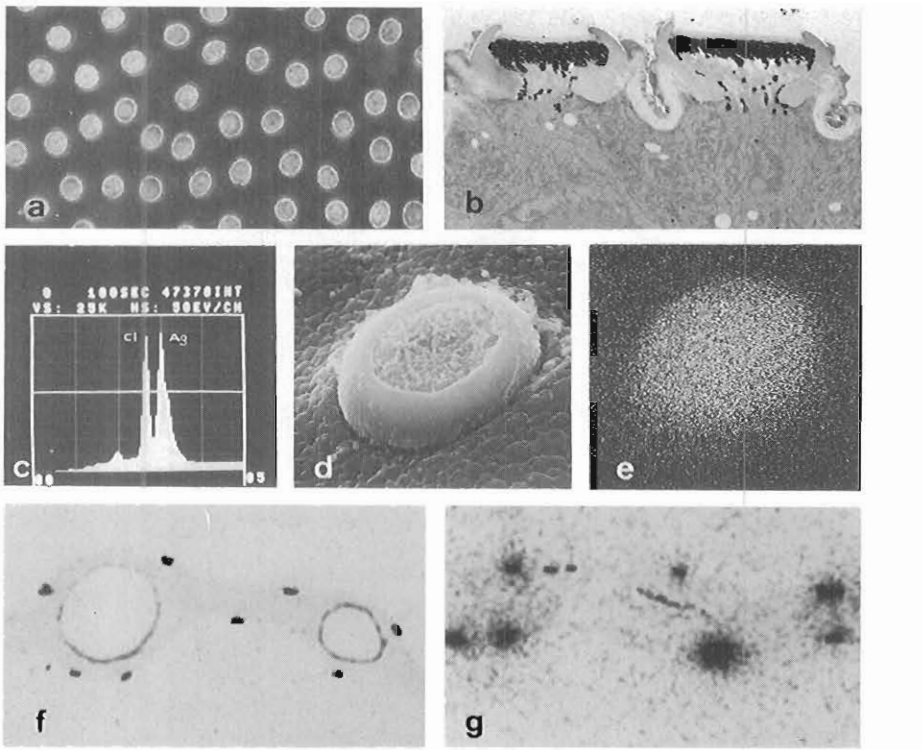

FIG. 17. Coniform chloride cells of Plecoptera fixed in silver salt-containing fixative. (a) Light microscope view of a whole-mount preparation under reflecting light. ×350. (b) Transmission electron micrograph of a cross section showing the apical location of the precipitates in two chloride cells. ×2400. (c) X-ray diagram of a spot analysis in the middle of the porous plate shown in (d). (e) X-ray distribution image of chlorine Kα from the same region shown in (d). (From Wichard and Komnick, 1973c.) (f and g) Autoradiographs of ephemeropteran coniform chloride cells in cross sections of the tracheal gills of *Callibaetis* larvae injected with hypertonic (f) and bathed in hypotonic (g) $Na^{36}Cl$ solution for 1 hour and subsequently fixed for histochemical chloride precipitation. Only (f) shows radioactivity. ×350. From Komnick *et al.* (1972).

transporting cells, the various types of chloride cells may differ in several respects: site and geometry of plasma membrane plications, intracellular distribution of mitochondria, cell shape, number of cells involved (unicellular or paucicellular), and differentiation of the apical cell portion and accompanying cuticle. These structural modifications are possibly related to modified transport functions or mechanisms, but their functional significance remains to be clarified.

Other cytoplasmic components such as dictyosomes, lysosomes,

smooth and rough endoplasmic reticulum, free ribosomes, and microtubules are present, but contribute little to the fine-structural characterization of chloride cells. The presence of occasional secretion granules of an unidentified nature reflects some secretory activity in addition to or related to the transport function.

E. Function

Relatively little is known about the function of the chloride cells of aquatic insects. However, there are biological, morphological, and histochemical criteria which, taken together, strongly support the assumption that they are osmoregulatory cells, their main function probably being the absorption of electrolytes from the external medium:

1. The inevitable loss of electrolytes resulting from the overall osmoregulatory situation in freshwater insects requires specialized sites of active ion absorption.
2. Chloride cells are present in the integument of aquatic insects that lack other external absorptive sites such as chloride epithelia or anal papillae.
3. In amphibiotic ephemeropterans, previously existing chloride cells disappear with the transition to terrestrial life, but persist in the aquatic imagines of Heteroptera (Komnick and Wichard, 1975a).
4. The distribution of chloride cells on the body surface changes during ontogenetic development if they loose direct contact with the external medium by virtue of being covered with respiratory air films or wings (Table V).
5. In a given species the number of chloride cells is related to the external salinity in that it increases with a decrease in the salt concentration (Table VI; see also Wichard *et al.*, 1973; Komnick and Wichard, 1975b; Wichard, 1975; Wichard and Heuss, 1975; Wichard *et al.*, 1975; Komnick, 1976). This adaptive behavior of the chloride cells is comparable with the corresponding size variations of chloride epithelia and anal papillae (See Sections V and VI).
6. The fine-structural organization of chloride cells points to a transporting function.
7. In addition to numerical variation, chloride cells respond to different salinities with fine-structural changes. Short-term exposure of *Callibaetis* larvae to dilute concentrations causes elaboration of the apical cell membrane infoldings of coniform chloride cells, whereas elevated concentrations lead to a smoothing out of the apical cell surface and subsequently to degenerative changes (Wichard *et al.*, 1973).
8. Histochemical demonstration of chloride in the apical region

TABLE VI
NUMBER OF CHLORIDE CELLS ON THE THORAX OF *Notonecta glauca* RAISED FROM EGGS AT DIFFERENT EXTERNAL SALINITIES[a]

Concentration of sea salt (mosm)	First instar	Second instar	Third instar
0.025–0.05	53.4 ± 8.8 (t = 0.4[b])	272.5 ± 61.8 (t = 3.4[c])	345.7 ± 54.1 (t = 3.7[c])
2.5	51.6 ± 7.4 (t = 0.2[b])	195.9 ± 33.1 (t = 3.4[c])	278.1 ± 17.3 (t = 3.1[c])
250	52.3 ± 5.4	145.8 ± 33.6	226.1 ± 17.5

[a] From Komnick and Wichard, 1975b.
[b] Insignificant.
[c] Significant, $P < 0.01$; $f = 18$.

indicates that the cells are somehow concerned with the handling of chlorides, even though some chloride artificially attracted from the hemolymph may contribute to the apical precipitates during prolonged fixation.

9. The combination of histochemical chloride precipitation with autoradiography after the external application of radioactive chloride in a hypoosmotic concentration suggests that the cells absorb chloride from the external solution (Fig. 17f and g) (Komnick *et al.*, 1972; Komnick and Schmitz, 1976).

Direct evidence of the assumed ion-absorbing function is difficult to obtain because, in most insects possessing chloride cells, these cells are more or less scattered over the body surface and therefore, unlike the chloride epithelia and anal papillae, are inaccessible for ligation or exclusion experiments. However, experiments of this kind were successfnlly performed with adult *Corixa punctata,* in which chloride cells appear to be restricted to the legs and head, the latter being densely populated by these cells (Komnick and Schmitz, 1976). When the front part of the head was sealed but the mouth left open, prior to exposure to a radioactive chloride solution, the radioactivity per microliter of hemolymph was drastically reduced (121 ± 39 cpm) in comparison with that in untreated and sham-treated controls (325 ± 55 cpm and 272 ± 28 cpm, respectively). A reverse experiment was performed as follows. In one group of animals a drop of radioactive chloride solution was brought into contact exclusively with the front part of the head after sealing of the mouth and labium. In the control group the drop was exclusively in contact with the pronotum,

which is free of chloride cells. Substantial radioactivity was found only in the hemolymph of the first group (177 ± 31 cpm versus 1 ± 0.2 cpm). The results of the two experiments supplement each other and allow the conclusion to be drawn that, at least in this species, chloride cells are responsible for chloride uptake into the hemolymph. In the light of these findings it remains questionable whether the labium of corixids is also involved in osmoregulatory ion uptake as suggested by Jarial *et al.* (1969).

In spite of this fundamental evidence, chloride cell function in aquatic insects is still far from being fully understood. Some of the important questions calling for elucidation are: Which kinds of ions are absorbed or even released? Which mechanisms and specifications underlie the initial ion trapping from dilute concentrations? What is the cellular transport mechanism? To what extent are the cells involved in water transfer? Do they occur in saltwater insects and are they involved in hypoosmotic regulation by the elimination of ions, similar to the chloride cells of marine fish? What is the significance of the mixed population of various chloride cell types in the same species? How is the functional activity controlled? Most of these questions are also applicable to the chloride epithelia dealt with in the following section.

V. Chloride Epithelia

A. Abdominal Chloride Epithelia

When caddisfly larvae of the families Limnophilidae and Goeridae are immersed in dilute silver nitrate solution, circumscribed areas of the abdominal segments show strong argyrophilia (Fig. 4b). Since the reduction of silver ions was attributed to the activity of respiratory cells, these areas were regarded as respiration fields or gill plates (Krawany, 1935). However, Boné and Koch (1942) interpreted these areas as being presumptive ion absorption sites. Later, analytical specifying reactions and selected area electron diffraction revealed that the argyrophilia resulted from the primary histochemical precipitation of chloride followed by the photochemical reduction of the silver chloride precipitates when the specimens were exposed to light (Wichard and Komnick, 1973a). This led to the designation of these areas as abdominal chloride epithelia. The number and location of the chloride epithelia may vary with the species or genus. For example, *Anabolia nervosa* possesses a total of 10 chloride epithelia which are present on both the dorsal and ventral sides of the third to seventh abdominal segments, whereas *Limnephilus stigma* has only six chloride

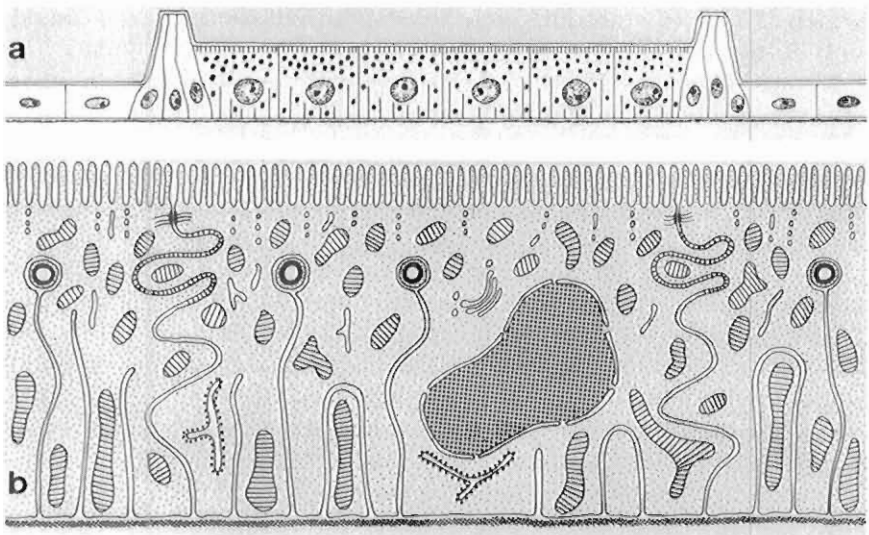

FIG. 18. Abdominal chloride epithelium of Trichoptera. (a) Showing the outline against the normal integument. (b) Showing the fine structure of the transporting epithelium. Drawn after Wichard and Komnick (1973a).

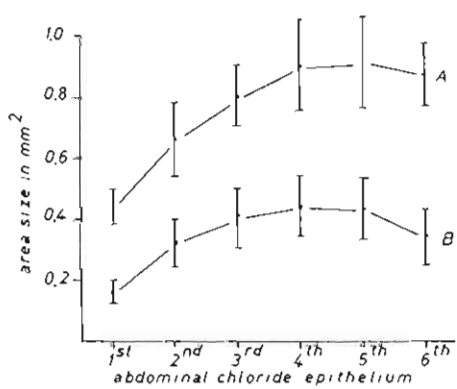

FIG. 19. Area size of the six abdominal chloride epithelia (counted in the caudal direction) in the fifth instar of *L. stigma* raised from the first instar in different salinities. (A) Demineralized water; (B) 250 mosm, sea salt solution. ($\bar{x} \pm s$, $N = 20$.) Adopted from Wichard (1975).

epithelia which are restricted to the ventral side of the second to seventh abdominal segments.

The structure of the abdominal chloride epithelia has been studied in *A. nervosa*. In comparison with the adjacent normal integument, the cells of the chloride epithelia are about twice as high, whereas the thickness of the overlying cuticle is reduced to about half. High, slender cells with their apices inserted into an internal incision of the cuticle form a clear-cut demarcation line around the chloride epithelia (Fig. 18a). The chloride epithelium itself consists of mitochondria-rich cells with numerous plications of the apical and basolateral plasma membranes (Fig. 18b). Tracheoles are inserted in the apical region but also occur in similar distribution within the surrounding normal hypodermis.

On the basis of their fine structure, the abdominal chloride epithelia can indubitably be regarded as transporting epithelia. Comparative experiments on *Glyphotaelius pellucidus* with hypoosmotic, radioactive sodium chloride solutions externally applied to larvae with untreated and sealed chloride epithelia have shown that the major part of both sodium and chloride ions absorbed into the hemolymph is taken up through the abdominal chloride epithelia (Schmitz and Wichard, 1975). In addition, the size of the abdominal chloride epithelia of *L. stigma* collected from different environmental salinities or experimentally adapted to different osmotic concentrations varies significantly and is inversely related to the external salinity (Fig. 19) (Wichard, 1975). This observation is consistent with the osmoregulatory role attributed to the abdominal chloride epithelia.

B. Anal Chloride Epithelia

The anal chloride epithelia, also known as the anal organ, are located on each side of the anal opening (Fig. 4c). They occur in several families of the suborders Brachycera and Cyclorrapha, mostly in aquatic but also in terrestrial larvae (Stoffolano, 1970). In other families they are absent or have been replaced by anal papillae (Wichard and Komnick, 1974e; Komnick *et al.*, 1975). Their relation to habitat and taxonomy has not yet been completely clarified.

Reviews of previous literature on the anal organ have been given by Quintart (1961) and Stoffolano (1970). Gloor and Chen (1950) were the first to ascribe an osmoregulatory function to the anal organ, based on their observation of selective darkening in a silver nitrate solution similar to the reaction seen with the anal papillae of aquatic Nematocera larvae. Subsequent studies contributed little to clarification of its function, until the anal organ was investigated with the electron

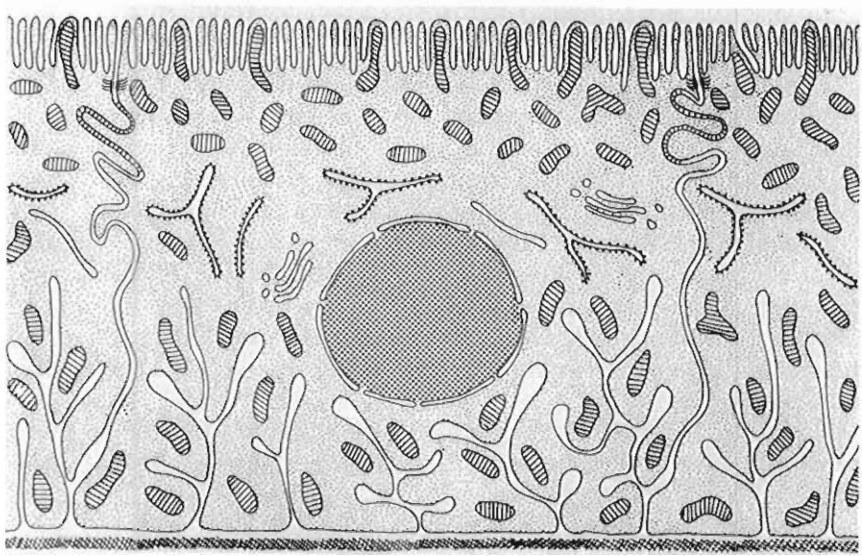

FIG. 20. Anal chloride epithelium of Diptera (*Stratiomys*). From Komnick *et al.* (1975).

microscope by Eichelberg *et al.* (1972), Chen and Brugger (1973), and Komnick *et al.* (1975). According to these workers, the epithelial fine structure of the anal organ supports the assumed transport function. Two slightly different transporting epithelia were observed in the species studied. In *Drosophila* (Eichelberg *et al.*, 1972) and *Stratiomys* (Komnick *et al.*, 1975) the epithelial cells form a continuous layer of uniform thickness (Fig. 20), whereas in *Tabanus* and *Atherix* (Komnick *et al.*, 1975) the epithelial cells cohere only in the apical region (Fig. 21). The resulting continuous zone measures only about 10 μm in thickness. The major part of the cell body, which contains the nucleus and is surrounded by the basal lamina, hangs free in the hemolymphic space with more-or-less wide interspaces between the cells. These columnar basal parts of the cell bodies may reach 180 μm in length. A similar differentiation with respect to cell shape was observed in the transporting epithelium of *Astacus* gills (Bielawski, 1971) and in the ventral tube of Collembola (Eisenbeis, 1974; Eisenbeis and Wichard, 1975a,b), which functions in ion and water uptake (Noble-Nesbitt, 1963). This indicates that this kind of transporting epithelium is of wider occurrence. In spite of the differences in cell shape and

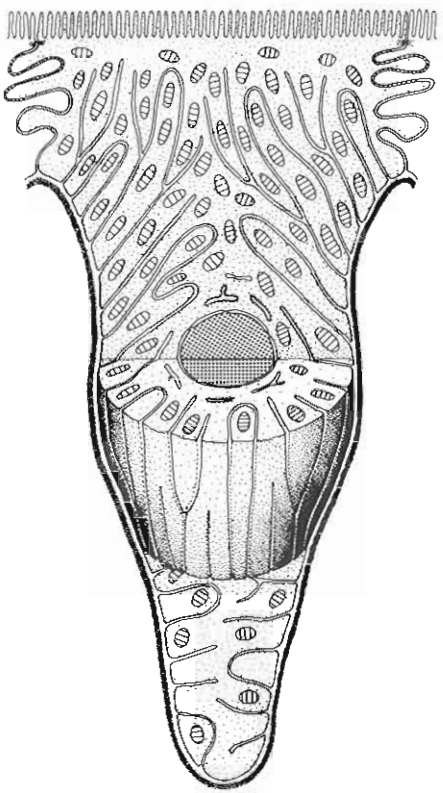

FIG. 21. Anal chloride epithelial cell of Diptera (*Atherix* and *Tabanus*). From Komnick *et al.* (1975).

coherence, the cellular fine structure of the two types of anal chloride epithelia is fundamentally similar with respect to the abundance of mitochondria and plasma membrane infoldings along the apical side and along the cell surface facing the hemocoel (Fig. 20 and 21).

Eichelberg *et al.* (1972) were the first to show that the silver staining of the anal organ in *Drosophila* larvae was due to the histochemical precipitation of chloride in the cuticle and within the epithelium. They also showed precipitates with the osmium-pyroantimonate technique, which is indicative of cations. In aquatic *Tabanus* larvae the chloride nature of the precipitates was unequivocally established by microprobe analysis and autoradiography. The latter technique simultaneously revealed that the chloride ions specifically precipitated

along the anal organ were absorbed from the external medium. Definite proof for the absorptive function was provided by experiments with radioisotopes on normal and appropriately ligated *Tabanus* larvae, showing that the anal chloride epithelia are the main site of sodium and chloride uptake into the hemolymph from hypoosmotic external solutions (Komnick *et al.*, 1975).

C. Rectal Chloride Epithelia

Larval Odonata lack specialized sites for ion absorption on the body surface. Instead, certain regions of the rectal wall are differentiated for this function. The necessary exposure of these internally located absorptive sites to the external medium is ensured by ventilation of the rectum through the anal opening. Since the rectum of anisopterous larvae is modified into a highly differentiated branchial chamber (Sadones, 1896; Tillyard, 1917), the ingested medium primarily serves the respiratory activity of the rectal tracheal gills, but is also utilized for osmoregulatory purposes and for swimming by jet propulsion.

The rectal ventilation of zygopterous larvae has also been regarded as an indication of rectal respiration. Since a true branchial chamber is absent, certain regions of the rectal wall have been interpreted as being blood gills or tracheal gills (Gericke, 1917). However, Pennak and McColl (1944) rejected this interpretation, because they did not find any correlation between the frequency of rectal ventilation and the oxygen concentration of the medium. According to their observations, the respiratory function resides in the general body surface and in the tbree caudal gill lamellae, which also serve as rudders. Furthermore, there are two zygopterous families, Epallagidae and Polythoridae, in which several pairs of tracheal gills are present on the abdomen in addition to the caudal appendices. Nevertheless, the rectum of these animals is ventilated (Wicbard, 1976).

As shown for *Coenagrion puella* larvae, the frequency of rectal ventilation depends on the external salinity. At a 100 m*M* sodium chloride external concentration, there was only sporadic ventilation (0–18 cycles per 2 hours of observation time), whereas at 10 μM sodium chloride 837–2524 cycles were registered within 2 hours from 10 different larvae. This relationship is completely reversible on exchange of the external medium (Wichard and Komnick, 1974b). The osmoregulatory role of the zygopterous rectum, first assumed by Koch (1934) and later by Pennak and McColl (1944), was finally established with the aid of radioactive sodium and chloride. Comparative data obtained from normal and ligated larvae clearly show that the uptake of both ions from hypoosmotic concentrations is almost exclusively gov-

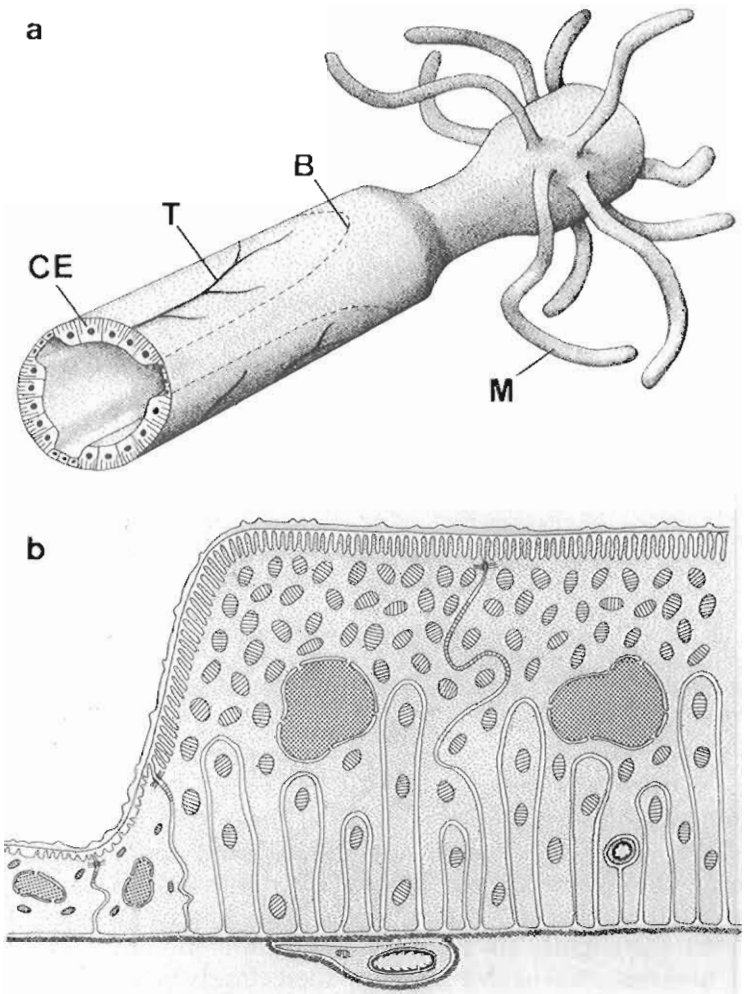

FIG. 22. Rectal chloride epithelia of Zygoptera. (a) Anatomical view. CE, Chloride epithelium; B, border of one chloride epithelium; T, tracheae; M, malpighian tubules. (b) Cytological view. From Schmitz and Komnick (1976a).

erned by the absorptive function of the rectum (Schmitz and Komnick, 1975, 1976a).

This function apparently resides in the three rectal chloride epithelia. These are thickened longitudinal lobes of the rectal wall separated by regions of thin epithelium (Fig. 22a). Their fine structure attests to their functional role in ion transport (Fig. 22b).

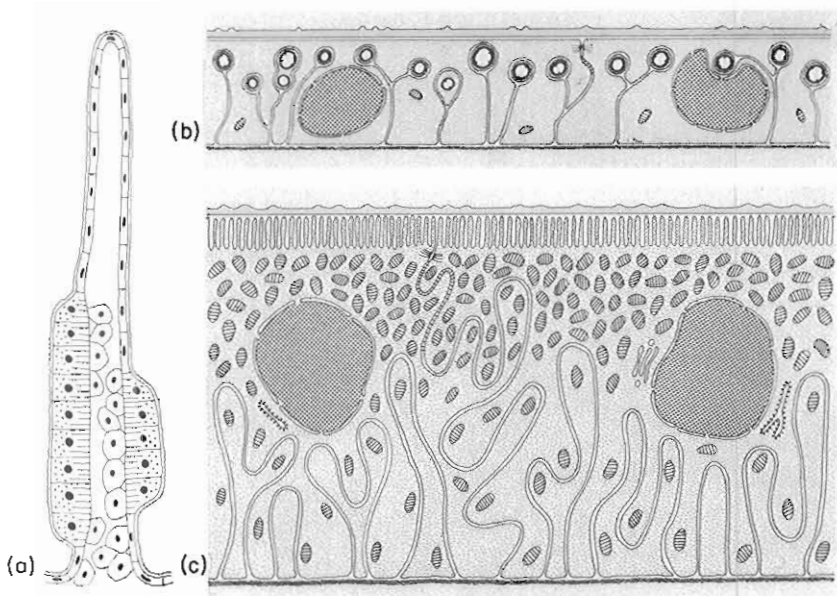

FIG. 23. Rectal chloride epithelia of Anisoptera. (a) Cross section of a gill leaflet with basal chloride epithelia of different sizes on each side. (b) Fine structure of the distal respiratory epithelium. (c) Fine structure of the basal chloride epithelia. From Schmitz and Komnick (1976a).

The same holds true for the rectal chloride epithelia of anisopterous larvae (Fig. 23) (Greven and Rudolph, 1973; Wichard and Komnick, 1974c,d). These transporting epithelia constitute the basal parts of the gill lamellae, the so-called basal pads, according to Sadones (1896), while the distal parts are differentiated into respiratory epithelium, where numerous tracheoles are embedded closely beneath the cuticle (Fig. 23). The number of chloride epithelia far exceeds the constant number of three present in zygopterous larvae, and depends on the body size or larval stage. In *Aeshna cyanea,* for example, six chloride epithelia independent of the gills and located at the posterior end of the rectum were consistently observed in larvae of diverse sizes. They have been homologized with the rectal pads of terrestrial insects by Faussek (1887). The number of additional chloride epithelia associated with the tracheal gills increases with growth and the concomitant increase in the number of gills. In larvae up to 10 mm in length they are present only on one side of the gills. Thereafter, small chloride epithelia of irregular number also appear on the contralateral

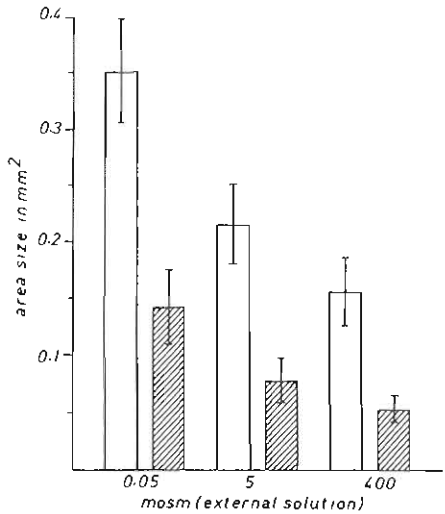

FIG. 25. Area size of gill-associated large (open columns) and small (striated columns) chloride epithelia in the midregion of the rectum of *A. cyanea* larvae of 35-mm body length grown in different salinities for 1 month. Mean ± standard deviation of 10 to 15 chloride epithelia of 15 larvae. (H. Komnick, unpublished.)

tation of silver chloride with the chloride ions normally absorbed to the epithelium. His interpretation proved to be absolutely correct, as shown by x-ray spectrometry of the precipitates and by chloride autoradiography (Fig. 26). In addition, liquid scintillation counting of the redissolved silver chloride precipitates of the rectal chloride epithelia revealed that the chloride ions were absorbed from the external medium ingested into the rectum via the anus and not from intestinal fluid resulting from possible drinking or from secretion through the Malpighian tubules. The uptake of both sodium and chloride into the hemolymph was drastically reduced when rectal ventilation was prevented in larvae, whereas chloride uptake remained unaffected by occlusion of the mouth. The reduction in rectal absorption did not appear to be due to interruption of the oxygen supply, since occlusion of the anal opening during the chosen duration of the experiment was tolerated without any detectable loss of vitality (Schmitz and Komnick, 1976a).

VI. Anal Papillae

Anal papillae, formerly regarded as blood gills or tracheal gills, have developed in parallel in aquatic insects of diverse orders. They are

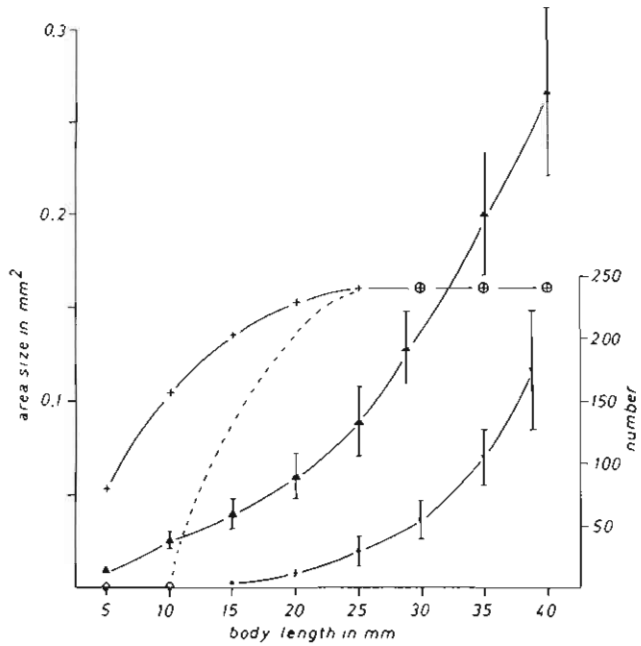

FIG. 24. Number and area size of the gill-associated rectal chloride epithelia of larval *A. cyanea* of different body lengths. Open circles, Number of small chloride epithelia; +, number of contralateral large chloride epithelia; solid circles, size of small chloride epithelia; triangles, size of contralateral large chloride epithelia. Measurements were made from 10 to 15 chloride epithelia in the midregion of the rectum of 10 larvae. The figures represent the mean ± the standard deviation. (H. Komnick, unpublished.)

side, until at 25- to 30-mm body length each gill leaflet carries a large and a small chloride epithelium, their total number amounting to 480 and remaining constant during further growth of the larvae (Fig. 24). The size of both the large and small chloride epithelia is greatest in the midregion of the rectum and decreases in the anterior and posterior directions, but increases with larval growth (Fig. 24). According to our unpublished measurements, the area size of both small and large gill-associated rectal chloride epithelia varies with the external salinity, their growth being accelerated in nearly salt-free solution, whereas the total number is not affected (Fig. 25).

Koch (1934) was the first to show that the rectal chloride epithelia of *Libellula* and *Aeshna* are selectively stained by the absorption of silver ions. He interpreted the underlying reaction as being a precipi-

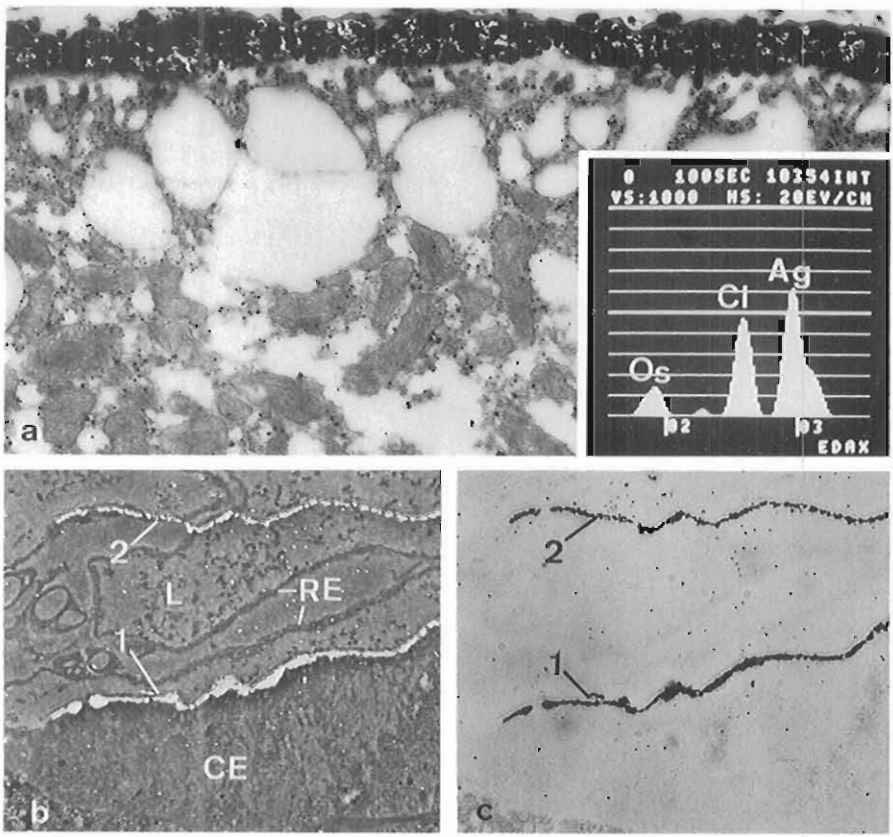

FIG. 26. Apical region of a rectal chloride epithelium of *A. cyanea* showing dense silver chloride precipitates along the luminal surface of the chloride epithelium. ×23,000. Inset: X-ray diagram of the precipitates. (b and c) Light microscopic pictures of an autoradiograph made from a cross section of the rectum of an *Aeshna larva* which had been exposed to 1 m*M* $Na^{36}Cl$ (0.1 μCi/ml) for 1 day and fixed for histochemical chloride precipitation. As the result of a relative shift between the photoemulsion and the section, the autoradiographic silver grains (2) are easily differentiated from the silver chloride precipitates (1) both appearing in separate lines of identical curvature. CE, Chloride epithelium; RE, respiratory epithelium; L, rectal lumen. (b) Phase-contrast. (c) Bright-field. ×450. From Schmitz aud Komnick (1976a).

present in several families of the Trichoptera and in Elodidae (Coleoptera), and among the Diptera they occur throughout the Nematocera but also in Syrphidae (Cyclorrapha). They occur in a postanal position as permanent or retractile cutaneous appendices, or in a preanal position as evaginations of the rectal wall, intermittently projecting out through the anus. These eversible preanal papillae are presum-

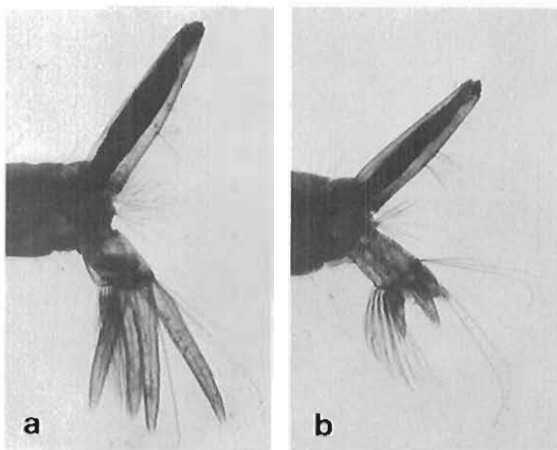

FIG. 27. Anal papillae of *Culex pipiens* larvae of equal body size, raised in different salinities. (a) Distilled water. (b) Forty percent sea water. ×30. Courtesy of Dr. H. Nüske.

edly homologous with the rectal papillae of other insects. Numbers of 2, 3, 4, 5, 6, 8, and 12 anal papillae have been reported in different species. Although normally unbranched, dichotomous ramifications also exist.

An indirect indication of the osmoregulatory role of the anal papillae was provided by Martini (1923), who found that during larval development the papillae of two species of *Aedes* responded to variations in external salinity by altered growth; the lower the salt concentration, the larger the anal papillae. Corresponding results were also reported for *Culex* (Fig. 27) and *Chironomus* (Wigglesworth, 1938; Haas and Strenzke, 1957). The external salinity also affects the epithelial fine structure of the anal papillae, the infoldings of the apical plasma membrane becoming more elaborate in dilute concentrations (Sohal and Copeland, 1966). Koch and Krogh (1936; Koch, 1938) showed with a series of experiments on *Culex* and *Chironomous* , that osmoregulatory uptake of chloride takes place exclusively through the anal papillae. Subsequent investigations have confirmed and extended our knowledge about the function. Although their physiology has not yet been fully clarified, the anal papillae of mosquito larvae are among the best known osmoregulatory adaptations of aquatic insects (for review, see Stobbart and Shaw, 1974). In comparison to studies on mosquito larvae, relatively little work has been devoted to

the physiological investigation of the anal papillae of other aquatic insects, but there is indirect and direct evidence that they are also endowed with the faculty to absorb mineral ions in Syrphidae (Krogh, 1943), Elodidae (Treherne, 1954), and Trichoptera (Wichard and Schmitz, 1976).

As in physiological investigations, the fine structure of the anal papillae has mostly been studied in Culicidae (Copeland, 1964; Sohal and Copeland, 1966; Mashiko and Asakura,. 1968; Meredith and Phillips, 1973a,b), but it has also been investigated in representatives of nine other nematoceran families (Komnick and Wichard, 1975c), as well as in Syrphidae (Wichard and Komnick, 1974f), Elodidae (Wichard and Komnick, 1974e), and Trichoptera (Nüske and Wichard, 1971, 1972). The anal papillae of all species studied are invested with a highly differentiated transporting epithelium. An example is given in Fig. 28 for *Eusimulium*. Characteristic of the preanal papillae of this species is the abundance of mitochondria-associated stacks of inpocketings of the lateral plasma membrane similar to those described in the rectal papillae of other insects (Berridge and Gupta, 1967;

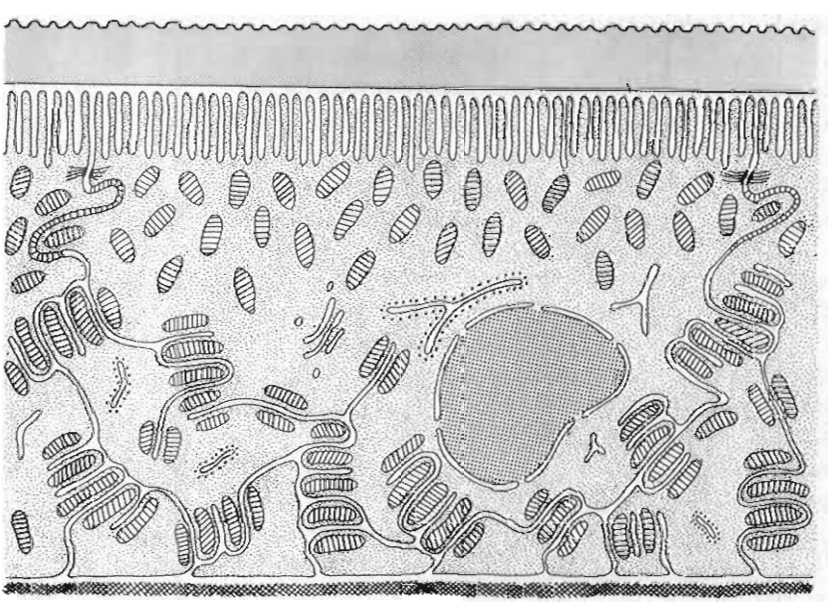

FIG. 28. Epithelial fine structure of the preanal papillae of a nematoceran larva, *Eusimulium verum*. Drawn after Komnick and Wichard (1975c).

Wessing and Eichelberg, 1973). However, this similarity cannot be taken as a mere sign of homology between preanal and rectal papillae, because the preanal papillae of other aquatic Diptera, such as Syrphidae and Ceratopogonidae, lack these stacks (Wichard and Komnick, 1974; Komnick and Wichard, 1975c). There are species-dependent variations in the details of the anal papillar fine structure, such as the location and membrane association of the mitochondria, the extent and geometry of the apical folds and the basal labyrinth, and the presence or absence of tracheoles. However, the basic structure is fundamentally similar, and is indicative of the transport function in all cases. Basically, it is unknown whether these minor structural modifications, which are also observed in chloride cells and chloride epithelia, are related to modified functions or whether they reflect different possibilities for attainment of the same goal, thus merely representing a further example of biological variability, which is particularly fascinating among insects.

VII. Intestinal Absorption Sites

In contrast to the situation with brackish water and saltwater insects which drink to compensate for osmotic water loss, drinking of the external medium for the enteric uptake of ions is rather exceptional in freshwater insects (Stobbart and Shaw, 1974) and appears to be the most primitive mode of osmoregulatory adaptation to the freshwater habitat. Drinking implies that the insect is exposed to the possibility of osmotic water influx on both the external and the internal sides of the body the latter even being devoid of a protective cuticle in certain regions. A low permeability of the cuticle to water and sodium chloride, and a high retention of mineral ions, as shown for *Sialis* (Shaw, 1955a,b), reduce the need for drinking. Therefore it is conceivable that drinking in *Sialis* was not normally observed but postulated from the results of enteric water and ion uptake and was experimentally induced by the reduction in blood volume (Shaw, 1955a,b). From potential measurements Shaw (1955b) concluded that sodium is actively absorbed and chloride passively absorbed, presumably through the midgut wall. To our knowledge, the epithelial fine structure has not yet been described.

In the larvae of the water beetles *Dytiscus* and *Acilius*, drinking of fresh water was observed with the use of dilute amaranth solutions, as was the enteric uptake of sodium and chloride in radioactive tracer experiments. Electron microscope investigation of the intestine suggests that in Dytiscidae, which lack rectal pads, the ileum is the

main site of ion absorption from water that has been drunk and from the renal fluid (Fig. 4f). It is relatively long and lined by a highly developed transporting epithelium throughout its entire length (Schmitz and Komnick, 1976b).

Elodes larvae belonging to another coleopteran family and possessing anal papillae take up chloride from dilute media through both the mouth and the anal papillae (Treherne, 1954). However, the exact intestinal absorption site is not known.

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