CYTOBIOLOGIE
VOLUME 4 · NO. 3
Pages 467–479 · 1971

Location and fine structure of the chloride cells and their porous plates in Callibaetis spec. (Ephemeroptera, Baetidae)

Lage und Feinstruktur der Chloridzellen und ihrer Porenplatte bei Callibaetis spec. (Ephemeroptera, Baetidae)

HANS KOMNICK [1]) and JOHN H. ABEL, JR.

Department of Physiology and Biophysics and Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado, USA

Received August 2, 1971

Abstract

In *Callibaetis* larvae *(Ephemeroptera, Baetidae)* chloride cells are located at both sides of the trachenal gills, at the lateral parts of the tergites and sternites of the first to ninth abdominal segments, at the thoracic sternites, in the first four segments of all legs, and at the dorsal part of the head. Their fine structural organization is very similar to that of the chloride cell complexes of *Cloeon dipterum* [12]. The porous plates are covered by the exocuticle and epicuticle, which show 0.1 to 0.25 µm wide perforations, and possess 300 Å wide pores, which are about 200 Å apart and arranged in a hexagonal pattern. The pores are permeable to colloidal lanthanum hydroxide and seem to contain some electron dense material, which presumably is able to accumulate sodium and chloride.

Introduction

Recently single chloride cells and chloride cell complexes were found in the epithelium of the tracheal gills of mayfly larvae [12]. The chloride cells of *Callibaetis* larvae have been shown to absorb salt from the external solution and to be involved in osmoregulation [4], similar to the chloride cells in the gills of fresh water fish. These cells are easily identified by their fine structure and by a dense silver chloride precipitation when fixed with the histochemical chloride reagent. Since the silver chloride precipitates are also detectable with the light microscope, they can be used as a simple means to study the location and distribution of the chloride cells in whole animals.

One additional feature is the so-called porous plates, which are special differentiations of the cuticular area overlying the chloride cells and which were presumed to represent

[1]) Permanent address. Dr. H. KOMNICK, Institut für Cytology und Mikromorphologie der Universität, 53 Bonn 1, Gartenstrasse 61 a, Germany. – Supported by the National Science Foundation, Washington, D. C.

areas of locally increased cuticular permeability [12]. The assumption of pores was based mainly on the striated appearance of the plates in thin sections. Therefore, further investigation of the porous plates was needed to prove that they were porous in nature in both, structural and functional respects.

Materials and methods

Larvae of *Callibaetis spec. (Ephemeroptera, Baetidae)* were collected from a small pond near Fort Collins and maintained in aerated aquaria.

For cytological study, the animals were fixed with 2 % OsO_4 in 0.1 M cacodylate buffer, *p*H 7.4, dehydrated in graded alcohols and embedded in Epon [6]. Thin sections were cut with a LKB-ultramicrotome and stained with uranyl acetate and lead citrate [11]. Some of the larvae were treated with 1.5 % colloidal lanthanum hydroxide [9] before, during or after fixation and embedded in styrene-methacrylate [5, 10].

For localization of the chloride cells, whole larvae and shed cuticles, collected immediately after moult, were fixed in the histochemical chloride reagent [3] and studied with the light microscope in whole-mount preparations.

For study of the porous plates by transmission and scanning electron microscopy, unfixed shed cuticles were washed for several days in distilled water to remove the adhering central cell apices [4, 12], mounted on grids and dried. For transmission electron microscopy the cuticles were used without staining; for scanning electron microscopy the preparations were shadowed with gold.

Results

Location of the chloride cells. When the larvae are fixed with the histochemical chloride reagent, a dense precipitation of silver chloride forms in the central cell apex of the chloride cells beneath the porous plates [12]. The same is true in shed cuticles [4], which retain the apical portion of the central cell after moult. In the light microscope the precipitates appear as round dots, which are white, when viewed with reflecting light (Fig. 1 c and d) or are brownish black, when transparent body parts such as the tracheal gills and shed cuticles are viewed in the bright field (Fig. 1 a and b). Since each of these dots indicates the location of one chloride cell in the epithelium beneath the cuticle [4, 12], the distribution of the chloride cells can easily be studied in whole larvae with a dissecting microscope or in shed cuticles with a normal bright field microscope. In *Callibaetis* larvae chloride cells are detected on both sides of all tracheal gills, where they are concentrated mainly in the proximal and middle parts (Fig. 1 a). The gills of the first 3 abdominal segments are bipartite. One lobe is large and the other is rudimentary. Chloride cells are found in both lobes, but in the smaller lobe they are fewer in number and widely dispersed.

Furthermore, large numbers of chloride cells are found on both the ventral and dorsal sides of the abdominal segments, where they form for the most part a single or double cluster of cells on the lateral parts of the sternites and tergites (Fig. 1 c and d). Their number gradually decreases in a caudal direction; few are observed on the 9th abdominal segment, and they are completely absent from the 10th segment.

On the thoracic sternites chloride cells also occur, but in smaller numbers. From here they extend into all 6 legs, where they are found in the coxa, trochanter, femur and there are even a few in the tibia. They normally form two rows along the frontal and back edges of the femur (Fig. 2 b).

In the head region chloride cells were observed on the dorsal capsule.

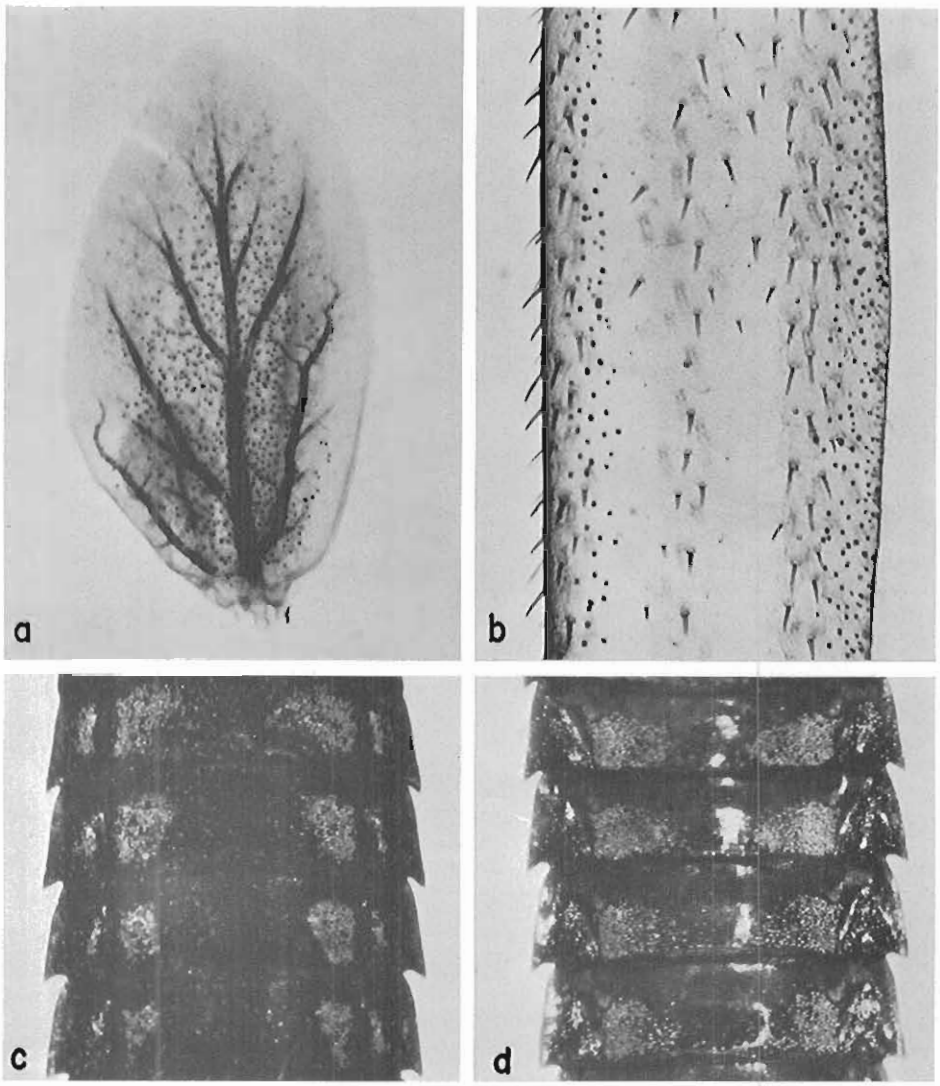


Fig. 1. Parts of *Callibaetis* larvae fixed in the histochemical chloride reagent. – **a.** Tracheal gill. Bright field. 75 ×. – **b.** Exuvia of the proximal part of a femur. Bright field. 125 ×. – **c.** Ventral view of the 2.–5. abdominal segments and **d.** dorsal view of the 3.–6. abdominal segments of the same animal. Dissecting microscope with reflecting light. 40 ×. – The dark dots in a and b, and the light dots in c and d are silver chloride precipitates and indicate the location of chloride cells in the epithelium beneath the cuticle.

Fine structure of the chloride cells. The chloride cells of *Callibaetis* are very similar to the chloride cells complexes in the tracheal gills of *Cloeon* [12]. They also consist of a central cell surrounded by several abjacent cells. The central cell is easily identified by its roughly cone-shaped apex, which extends into a recess of the cuticle (Fig. 2 and 3 a). The funnel-shaped recess of the cuticle enclosing the central cell apex can

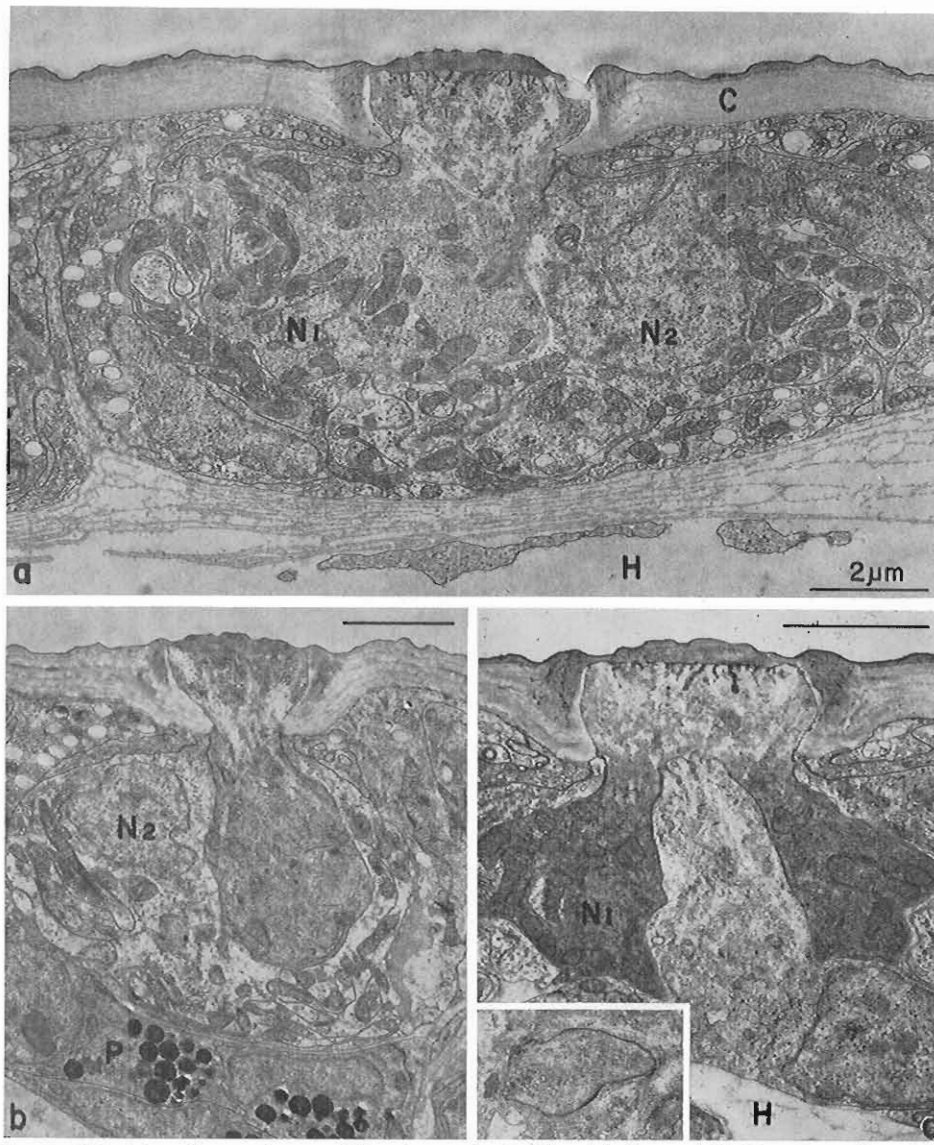


Fig. 2. Chloride cell complexes within the tracheal gill epithelium of *Callibaetis*. – C cuticle; H hemocoel; N_1 nucleus of the central cell; N_2 nucleus of an adjacent cell; P pigment cells. – **a.** 8000 ×; **b.** 7500 ×; **c.** 10 000 ×; **inset.** 12 000 ×.

be visualized in the thicker abdominal cuticle with the transmission electron microscope (Fig. 7 b) or with the scanning electron microscope by viewing the lower surface of shed cuticles (Fig. 7 d). The apical plasma membrane beneath the porous plate often shows irregular and short unfoldings which contain an electron-dense material (Fig. 2 a, c, 5 a). The cytoplasmic leaflet of the unfolded membrane is coated with small granules (Fig. 5 a) similar to those described in the rectal papillae of termites [8].

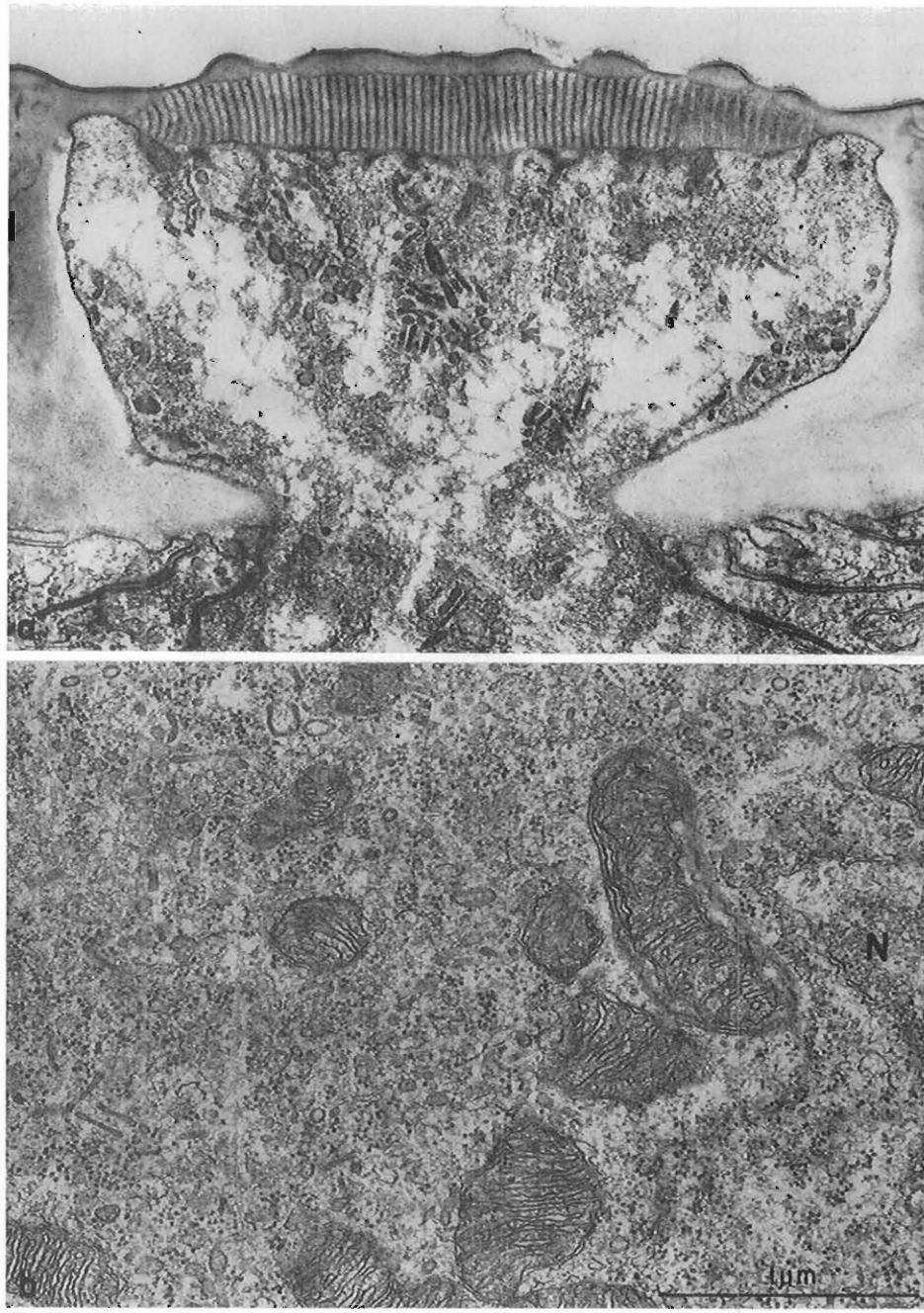


Fig. 3a. Porous plate and apex of the central cell of a chloride cell complex in the tracheal gill epithelium of *Callibaetis*. 35 000 ×. – **b.** Cytoplasm of the intermediate part of a central cell. N nucleus. 35 000 ×.

The apical cytoplasm is normally characterized by a large number of small vesicles, small Golgi-like cisternae, some microtubules running more or less perpendicularly to the porous plate, and electron-translucent areas (Fig. 3 a).

The intermediate and basal part of the central cell, which contains the nucleus, is relatively electron dense (Fig. 2 b, c). This is partly due to the large number of ribosomes in this region (Fig. 3 b). Rough endoplasmic recticulum is occasionally abundant in the basal part of the cells. The ribosomes and Golgi cisternae are presumably involved in the formation of the glycoprotein and acid mucopolysaccharide contained in the central cell apex [4].

Furthermore, a moderate number of mitochondria, and occasionally multivesicular bodies and dense bodies resembling lysosomes are also present in the basal part.

Whereas the apex of the central cell always has the same shape, the form of the basal part is variable, often highly irregular and forms processes, which interdigitate

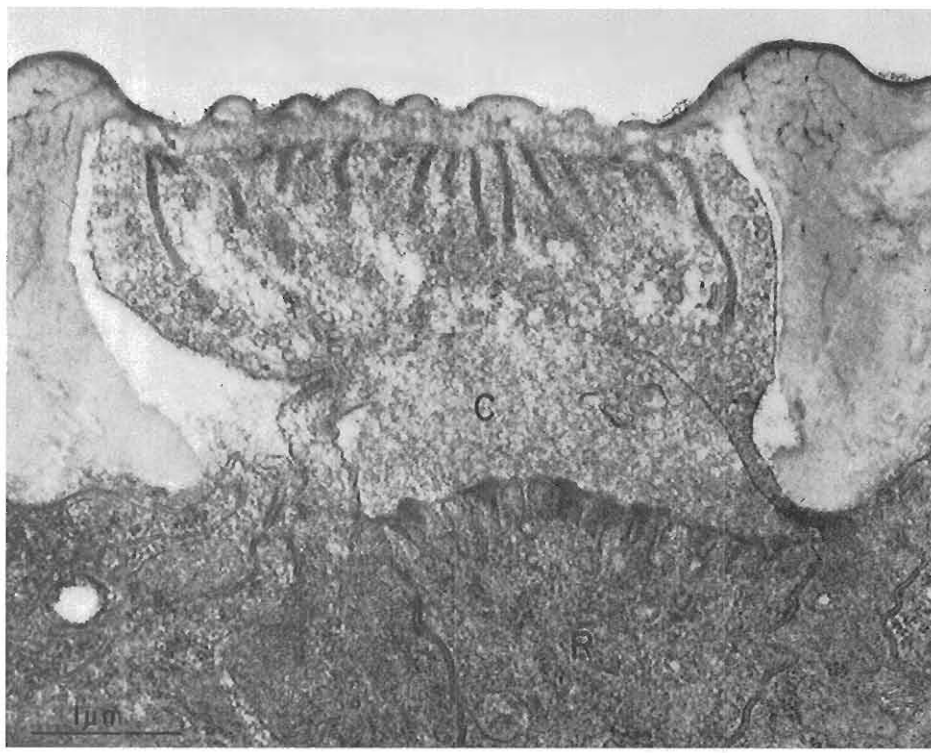
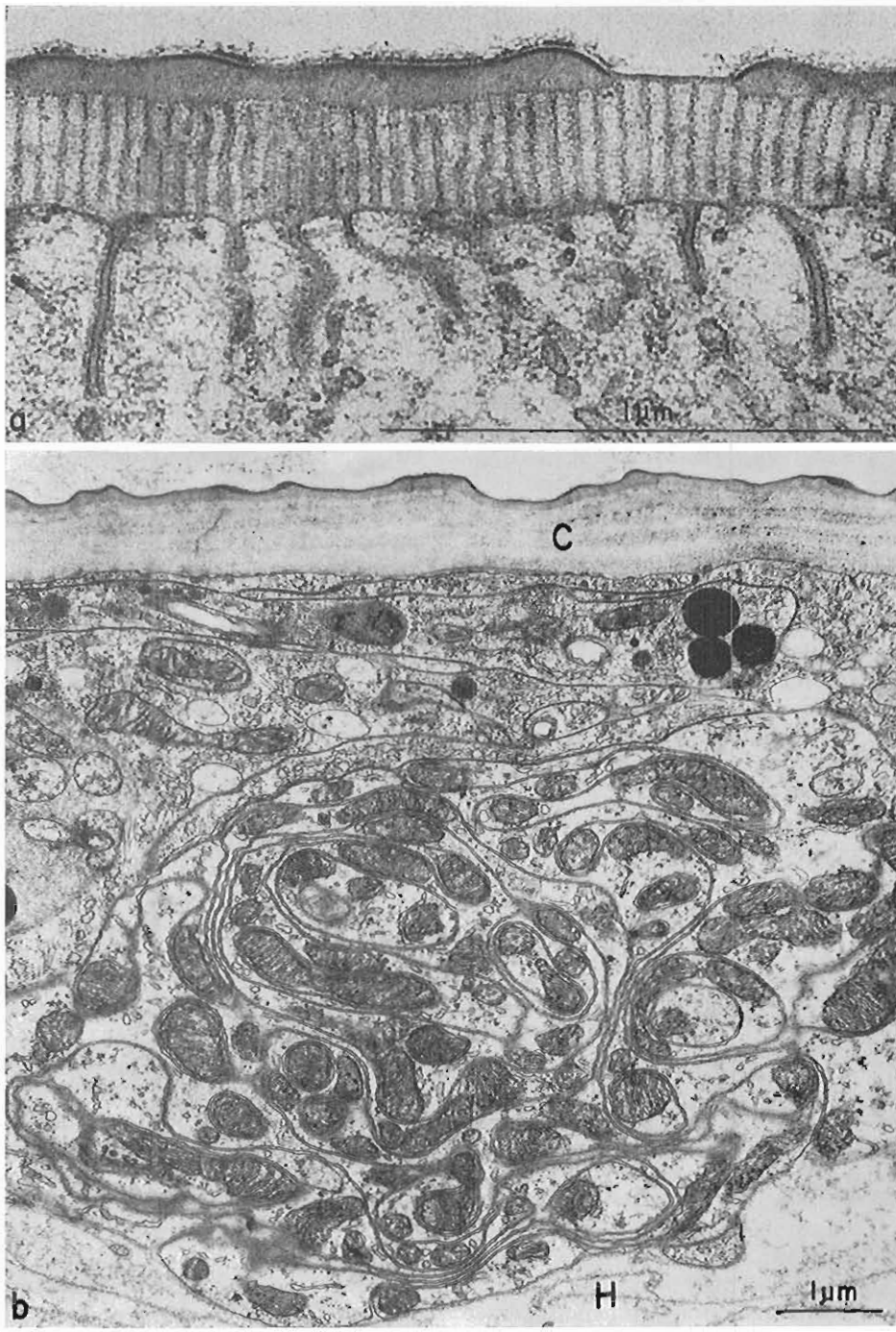


Fig. 4. Apex of the central cell of a chloride cell complex in the tracheal gill of *Callibaetis* fixed in a pre-moulting stage. – C intercellular cavity filled with moulting gel between the apices of the old and replacing central cell (R). – 20 000 ×.

Fig. 5 a. Part of the porous plate and central cell apex of a chloride cell complex in the tracheal gill epithelium of *Callibaetis*. 70 000 ×. – **b.** Section through the periphery of a chloride cell complex showing interdigitated processes of adjacent cells. – C cuticle; H hemocoel. 16 000 ×.


a
1µm
C
b
H
1µm

with the adjacent cells of the complex (Fig. 2 a). There are two basic forms of the basal cell body. In one the basal part is fairly compact and flask-shaped (Fig. 2 b), while in the other it seems to be split and to consist of two lobes with a thick process of another cell located between (Fig. 2 c). Since in cross section this process is completely surrounded by the cytoplasm of the central cell (Fig. 2 c inset) without any intercellular cleft running to the periphery, the central cell in these cases apparently has a deep, cone-shaped impression like the bottom of a champagne bottle. The plug-like process of the second cell with its nucleus located closely to the base of the epithelium normally extends up to the lower level of the cuticle (Fig. 2 c). It was never observed to reach the porous plate. The apical tip of this process possesses fine short and irregularly shaped microvilli (Fig. 2 c). The overall impression of this structural relationship is that two central cells are piled on top of one another, but only one cell forms the apex typical for the chloride cell complexes. Furthermore, this relationship apparently represents an early pre-moulting srage. During preparation of moult, before the new cuticle is formed, the apex of the inner cell widens and the microvilli become more pronounced (Fig. 4). An intercellular cavity filled with moulting gel [7] appears between the apices of the two cells. The apex of the old central cell, which is going to be shed with the cuticle [4, 12] is connected with the subcuticular cell body by only a thin cytoplasmic sheet around this cavity and the apex of the new central cell (Fig. 4). Therefore, it is concluded that the inner cell represents a replacement cell. It is not known whether the remaining basal cell body of the old central cell dies or differentiates into an adjacent cell after moult and contributes to the growth of the chloride cell complex.

The adjacent cells are highly pleomorphic and highly interdigitated along the periphery of the chloride cell complex (Fig. 5 b). The interdigitated cell processes contain numerous mitochondria in close association with the plasma membrane. Since the function of the chloride cells complexes of *Callibaetis* is the absorption of salt [4], the interdigitation of the adjacent cells provides a large membrane area of the basal, secretory face as well as numerous "forward transporting channels" in the sense of DIAMOND and BROSSERT [1, 2].

The porous plates. The porous plate is a special differentiation of the cuticle overlying the central cell apex of the chloride cell complex. Cross sections of the plates are spindle-shaped and show regular striations oriented perpendicular to the surface (Fig. 3 a). The central part of the plate is 0.2 to 0.3 µm thick, whereas the normal gill cuticle measures about 1 µm across. Similar to other soft-bodied insects [7], the normal gill cuticle consists of three layers (Fig. 2, 5 b). The inner lamellate endocuticle makes up the largest part of the total cuticle thickness. The outer electron-dense and homogeneous exocuticle is very thin and is covered by the epicuticle. The epicuticle itself exhibits at least two layers, an outer fuzzy coat and an inner very electron-dense bipartite layer which is presumably the cuticulin layer (Fig. 3 a, 5 b). Both the exocuticle and the epicuticle extend over the porous plates (Fig. 3 a and 5 a). Therefore, the porous plate itself is thought to represent a differentiation of the endocuticle only. This view is supported by the fact, that the exocuticle and epicuticle overlying the porous plate are perforated and the porous plate extends directly to the surface at these places (Fig. 3 a and 5 a). These perforations can also be seen in whole-mount exuviae with both the scanning (Fig. 7 c) and the transmission electron microscope (Fig. 8 a). They are roughly circular and range between 0.1 and 0.25 µm in diameter.

In specimens treated with colloidal lanthanum hydroxide either before, during or after fixation, the tracer is able to penetrate the porous plate and the infoldings of the apical plasma membrane (Fig. 6). Within the porous plate, the lanthanum produces

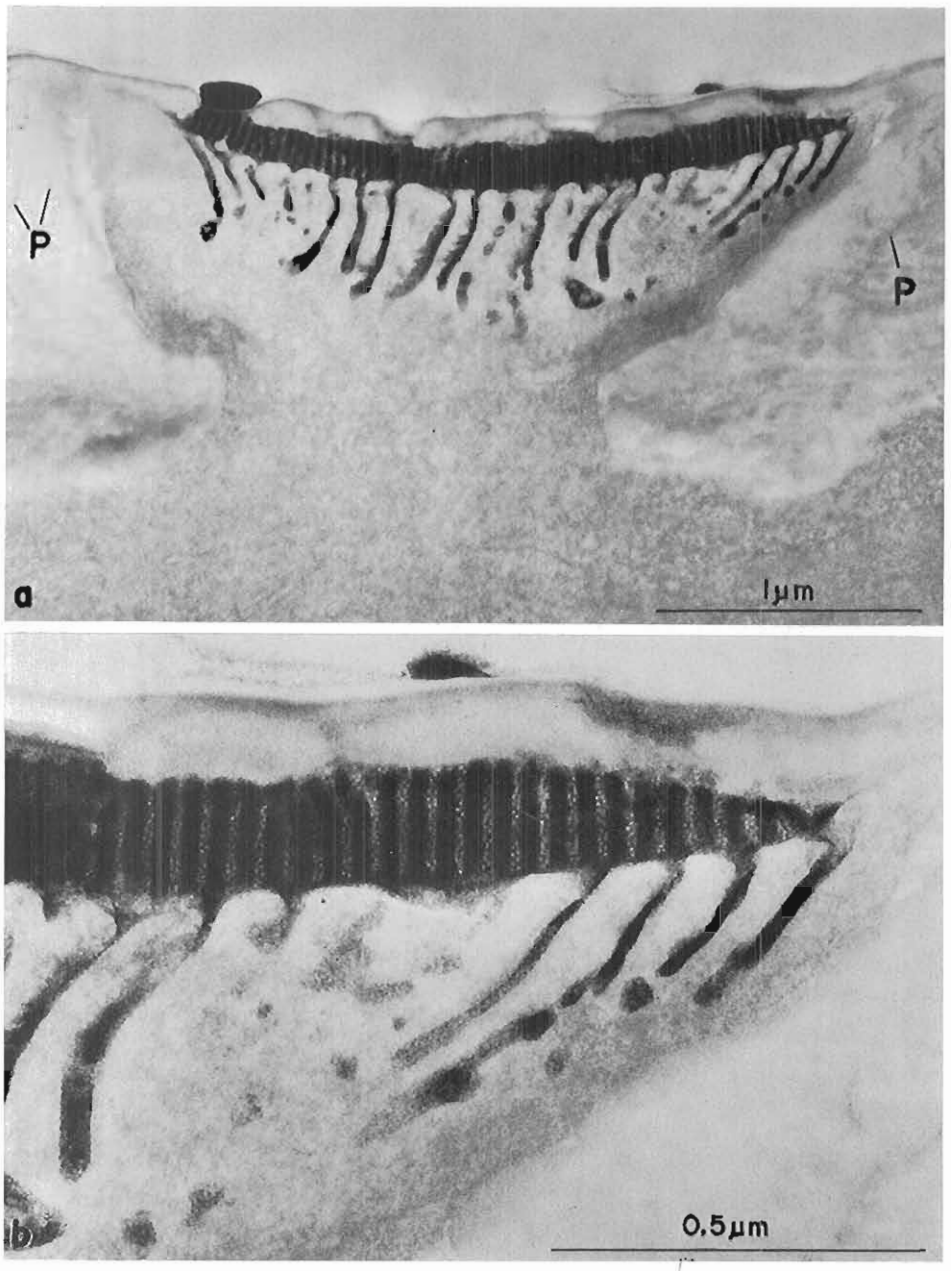


Fig. 6. Porous plate and central cell apex of a chloride cell complex in the tracheal gill epithelium of *Callibaetis* treated with lanthanum hydroxide. – P pore canals within the normal cuticle. – **a.** 35 000 ×; **b.** 100 000 ×.

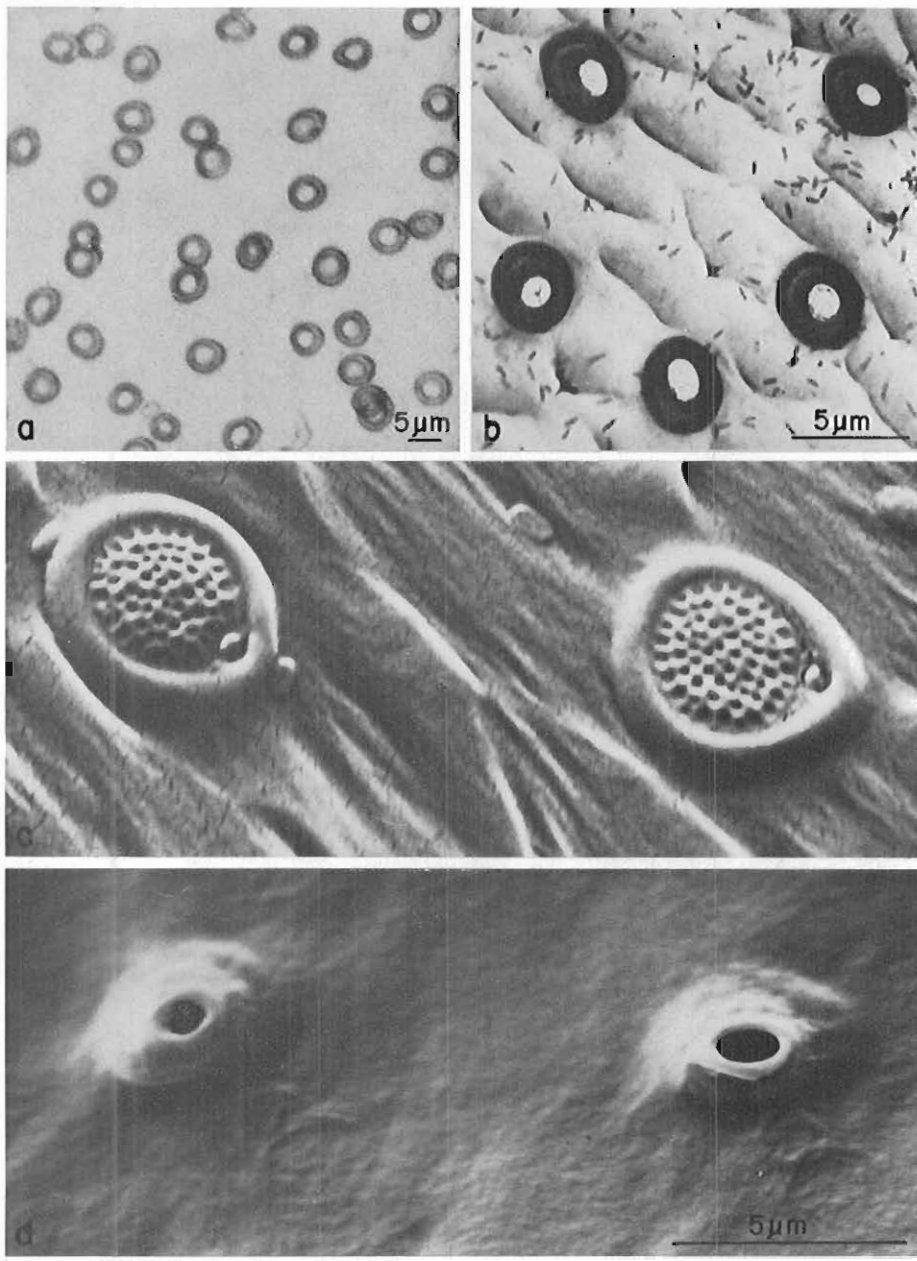


Fig. 7 a. Light microscopical picture of the shed cuticle of a tracheal gill of *Callibaetis*. Since the shed gill cuticle is a very flat sac, some of the rings indicating the porous plates seem to be superimposed, but actually are located at different sides of the cuticular gill envelope. 800 ×. – **b.** Transmission electron micrograph of a shed abdominal tergite of *Callibaetis* showing the outer and inner thickenings around the porous plates, a scale-like surface relief, and attached bacteria. 3000 ×. – **c.** and **d.** Scanning electron micrographs of an abdominal cuticle of *Callibaetis* with two porous plates in each. **c** was taken of the outer surface showing the perforations of the exocuticle overlying the porous plates; **d** was taken of the inner surface showing the mouth of the funnel-shaped recess, into which the central cell apex is extended. 6200 ×.

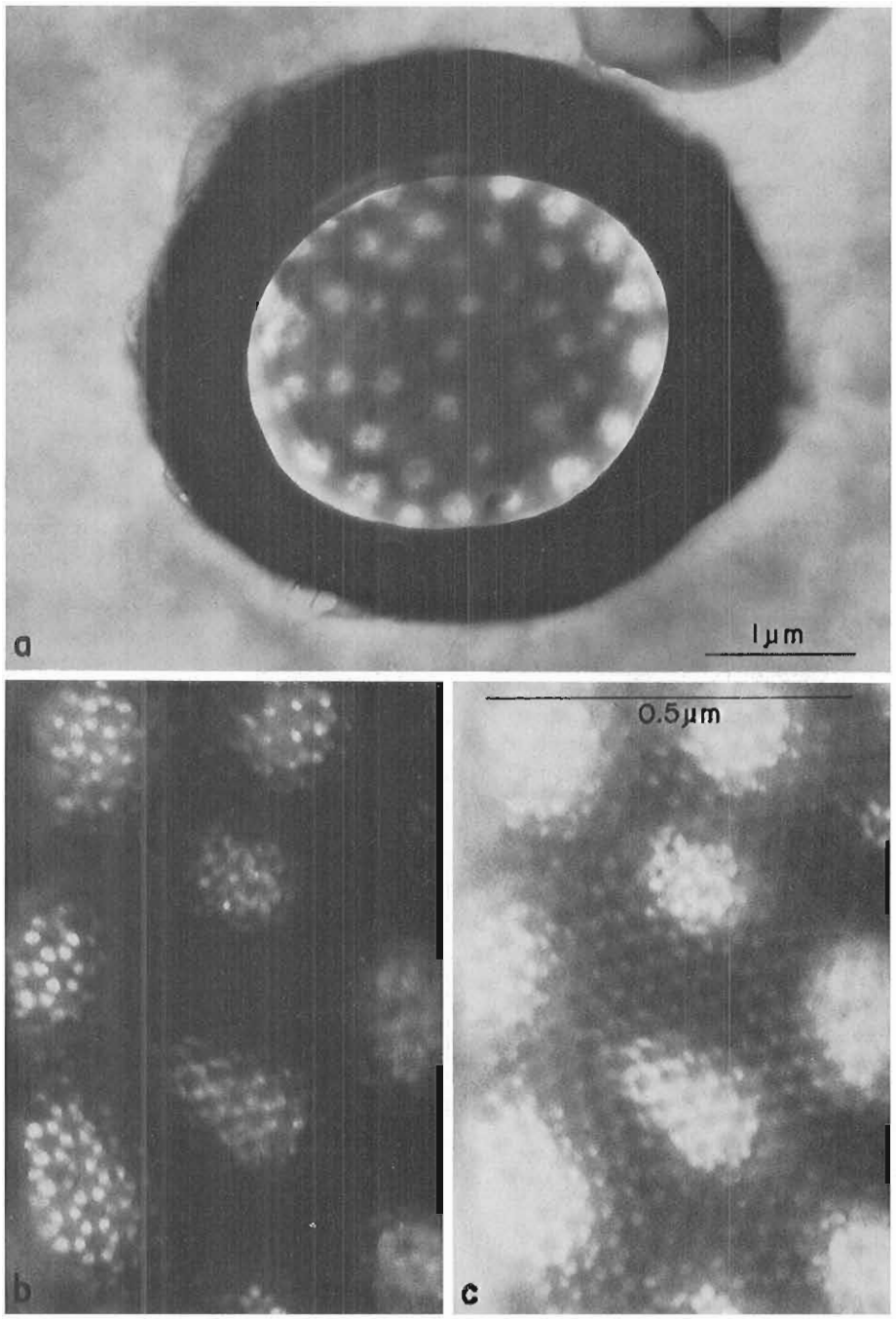


Fig. 8 a. Transmission electron micrograph of a porous plate in the shed cuticle of a tracheal gill of *Callibaetis*. The cuticular envelope was split in this preparation to produce a single layer. 20 000 ×. – **b.** and **c.** Transmission electron micrographs taken with different exposure times at the periphery of a porous plate in an abdominal sternite of *Callibaetis*. The margin of the porous plate is at the left side in both pictures. 100 000 ×.

a striation pattern that is similar to the one observed in untreated specimens (Fig. 3 a, 5 a). These striations are approximately 300 Å wide and are separated by a 200 Å wide interspace. Lanthanum is also observed to form small cross connections between any two adjacent 300 Å channels. This might indicate that tiny cross communicating channels are present. The presence of such channels is also faintly indicated in sections of untreated larvae (Fig. 5 a). Since no lanthanum was observed within the pore canals of the normal cuticle, the porous plates apparently are locally specialized areas of increased cuticular permeability [12].

The normal cuticle which borders the porous plates forms small ridges at the outer and inner surfaces (Fig. 2). In surface views of whole-mount exuviae these thicker borders appear as rings (Fig. 7 a, b) surrounding the porous plates (Fig. 7 c, 8 a). In unfixed and unstained cuticles, the pores can be visualized with the transmission electron microscope using high magnification. At the periphery, where the porous plates are the thinnest (Fig. 3 a), two sets of dots are detected. They appear most clearly at those sites, where the overlying exocuticle is perforated (Fig. 8 b), and more faintly, where the porous plate is covered by the exocuticle (Fig. 8 c). One set consists of electron-translucent dots, which are approximately 200 Å in diameter, the second set is made up by dark dots measuring about 300 Å across and connected by cross bridges (Fig. 8 b). The dots are very regularly arranged, each set forms a hexagonal pattern. Since in thin sections of specimens treated with colloidal lanthanum hydroxide the tracer stains 300 Å wide columns within the porous plates (Fig. 6 b), it is concluded that the dark dots represent the pores. Furthermore, the existence of communications between the pores is also indicated in the lanthanum stained preparations (Fig. 6 b). In unstained layers of equal thickness, contrast is produced by differences in matter density. The pores, if they are actually represented by the dark dots, therefore must contain some material, which is more electron-dense than the cuticular substance. This is also indicated in Fig. 5 a, where one set of the striations of the porous plate has approximately the same electron density as the material contained within the unfoldings of the apical plasma membrane. As judged from previous results of histochemical precipitation of sodium and chloride [12] this material seems also to possess ion accumulating properties similar to that within the central cell apex [4], because the precipitates are often arranged in lines reflecting the striation pattern of the porous plate (see [12] Fig. 6 a and [4] Fig. 8 c and 9 c).

Acknowledgements: We are grateful to Mr. J. A. STANFORD, Department of Zoology, Colorado State University, for identifying the genus, to Miss H. PETERSDORFF, Department of Zoology, University of Illinois, for taking the scanning electron micrographs, and to Miss I. BAAS and Mrs. M. CULOT for technical assistance.

Zusammenfassung

Callibaetis-Larven (*Ephemeroptera, Baetidae*) besitzen Chloridzellen auf beiden Seiten der Tracheenkiemen, an den Seiten der Tergite und Sternite der ersten neun Abdominalsegmente, in den Thorakalsterniten, in den ersten vier Beinsegmenten und in der dorsalen Kopfkapsel. In der feinstrukturellen Organisation entsprechen sie weitgehend den Chloridzellkomplexen von *Cloeon dipterum* [12].

Die Porenplatten werden von der Exo- und Epicuticula überzogen, die etwa 0,1 bis 0,25 µm weite Perforationen aufweisen. Ferner besitzen sie ca. 300 Å weite Poren, die etwa 200 Å voneinander entfernt und in einem hexagonalen Muster angeordnet sind. Die

Poren sind permeabel für colloidales Lanthanhydroxid und scheinen ein elektronendichteres Material zu enthalten, das vermutlich Natrium und Chlorid zu akkumulieren vermag.

References

[1] Diamond, J. M., and W. H. Bossert: Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. J. gen. Physiol. **50**, 2061–2083 (1967).

[2] Diamond, J. M., and W. H. Bossert: Functional consequences of ultrastructural geometry in "backwards" fluid transporting epithelia. J. Cell Biol. **37**, 694–702 (1968).

[3] Komnick, H., und M. Biertiier: Zur histochemischen Ionenlokalisation mit Hilfe der Elektronenmikroskopie unter besonderer Berücksichtigung der Chloridreaktion. Histochemie **18**, 337–362 (1969).

[4] Komnick, H., R. W. Rhees, and J. H. Abel: The function of ephemerid chloride cells. Histochemical, autoradiographic and physiological studies with radioactive chloride on *Callibaetis*. Cytobiologie (in press).

[5] Kushida, H.: A styrene-methocrylate resin embedding method for ultrathin sectioning. J. Electromicr. **10**, 16–19 (1961).

[6] Luft, J.: Improvement in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **4**, 409–414 (1961).

[7] Noble-Nesbitt, J.: Aspects of the structure, formation and function of some insect cuticles. In: J. W. L. Beament and J. E. Theherne (editors): Insects and Physiology. p. 3–16. American Elsevier, New York 1968.

[8] Noirot, C., et C. Noirot-Timothée: Revêrement de la membrane cytoplasmic et absorption des ions dans les papilles rectales d'un termite (Insecta, Isoptera). C. R. Acad. Sci. (Paris) **263**, 1099–1102 (1966).

[9] Revel, J. P., and M. J. Karnovsky: Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. **33**, C 7 (1967).

[10] Stockem, W., und H. Komnick: Erfahrungen mit der Styrol-Methacrylat-Einbettung als Routinemethode für die Licht- und Elektronenmikroskopie. Mikroskopie (Wien) **26**, 199–203 (1970).

[11] Venable, J. H., and R. Coggeshall: A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. **25**, 407–408 (1965).

[12] Wichard, W., and H. Komnick: Electron microscopical and histochemical evidence of chloride cells in tracheal gills of mayfly larvae. Cytobiologie **3**, 215–228 (1971).