Cell Tiss. Res. 160, 263—271 (1975)

The Renal Chloride Cell of the Fresh-water Catfish, *Parasilurus asotus*, with Special Reference to the Tubular Membrane System

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Received January 27, 1975

Summary. The kidney of the fresh-water catfish, *Parasilurus asotus*, was examined by electron microscopy. A special type of cell, very similar in appearance to the chloride cell of the teleostean gill filaments, is found in the kidney and is referred to as a renal chloride cell. This cell is characterized by an extensive tubular membrane system with a rather constant diameter of approximately 600 Å. A number of mitochondria are closely associated with this system. Application of ruthenium red as an extra-cellular space marker revealed that the tubular system is a highly organized derivative of the cell membrane, infolded from the basal and lateral surfaces of the cell. The fine structural resemblance to other types of cells known to possess active transport of electrolytes suggests that these cells are involved in intrarenal osmoregulation.

Key words: Renal chloride cell — Tubular membrane system — Fresh-water catfish, *Parasilurus asotus*.

Introduction

Fine structural studies have indicated that the chloride cells of gill filaments in various species of teleosts contain a peculiar membrane system which communicates directly with the surface cell membrane and is closely associated with abundant mitochondria (Philpott and Copeland, 1963; Threadgold and Houston, 1964; Philpott, 1967; Conte, 1969; Shirai and Utida, 1970; see also Lennep and Lanzing, 1967).

In fresh-water teleosts, the ionic composition of the blood is regulated by these chloride cells. Their kidney functions mainly as a water excretory device (Hickman and Trump, 1969). One of the authors (Yamamoto, 1966), however, previously described a special type of cell in the renal tubules of fresh-water catfish, which is quite similar in fine structure to the chloride cell of the gill filaments, and therefore, suggested that the ionic composition of the blood could be regulated by the kidneys as well as by the gill filaments. In order to confirm this idea, the present paper describes in greater detail a fine structural study of this type of cell with special reference to its membrane system. Ruthenium red (Luft, 1966) was also applied as an extracellular space marker to determine whether the membrane system is continuous with the cell membrane.

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Material and Methods

Fresh-water catfish (*Parasilurus asotus*) were used in the present study. The kidney tissue was removed from the unanesthetized animal and fixed in an ice-cold fixative consisting of 4% glutaraldehyde buffered with 0.2 M cacodylate solution at pH 7.4 (Sabatini *et al.*, 1963) for 2 hrs., followed by post-fixation in 2% OsO_4 for 3 hrs. In some cases 500 ppM ruthenium red (Luft, 1966) was added to the fixatives as an extracellular space marker.

After dehydration through a series of graded ethanols, the specimens were embedded in Epon epoxy resin (Luft, 1961). Thin sections were cut on a Porter-Blum microtome, stained with lead tartrate (Millonig, 1961), and examined with a Hitachi Hs-7 or Hu-12 electron microscope.

Observations

According to the classification of Ogawa (1961) for the teleostean kidney, the kidney of the fresh-water catfish may belong to Type III, where the bilateral kidneys are fused at their posterior ends, taking on a V-form with a thickened bottom. A small number of renal corpuscles are distributed among the loose hematopoietic tissue. Renal tubules are lined with various kinds of cells, such as ciliated columnar cells, columnar cells with prominent brush border and mucous cells, although the type and proportion of the cells varies along different segments as has been described in various species of teleosts (Hickman and Trump, 1969).

In the distal segment and collecting tubules, a special type of cell that has not been reported heretofore occurs intermingled with mucous cells (Figs. 1, 2). When observed under the light microscope, they are columnar or cuboidal in shape and are characterized by having pale cytoplasm and basal granulations, which represent an accumulation of mitochondria. Since, as described below, these cells bear some fine-structural resemblances to the chloride cells of the gill filaments, they are referred to here as "renal chloride cells". In sections, these cells are usually separated from each other by intervening mucous sells that often have an expanded mushroom-like apical cytoplasmic process. Their free surfaces are, therefore, often reduced or even almost completely shielded by the mucous cell processes (Fig. 2). The apical part of the renal chloride cell frequently contains a number of deep invaginations and vacuoles. Vesicles with a diameter of approximately 900 Å are also encountered (Fig. 3). The lumina of urinary tubules contain flocculent materials of moderate density, apparently secreted by the mucous cells. Some of the renal chloride cells, however, contain fewer apical invaginations, vacuoles and vesicles but have several microvilli instead (Fig. 1). The morphological variation of apical parts could represent functional states or maturing phases of these cells.

The most striking feature of the renal chloride cell is the content of a tubular membrane system occupying nearly the whole cytoplasm (Figs. 1, 2). These tubules are rather constant in diameter, ~600 Å; their limiting membrane is approximately 90 Å in overall thickness. They contain a moderately electron-dense core ~200 Å in diameter in their luminal center (Fig. 4). Although repeating subunits apparently integrated in the extracellular aspect of the unit membrane of the tubular structures have been reported in the chloride cell of *Fundulus* (Ritch and Philpott, 1969), comparable structures have not been observed in this study.

The tubules take a more or less angular course and appear to branch and anastomose with each other. Occasionally, they also show a honeycomb profile

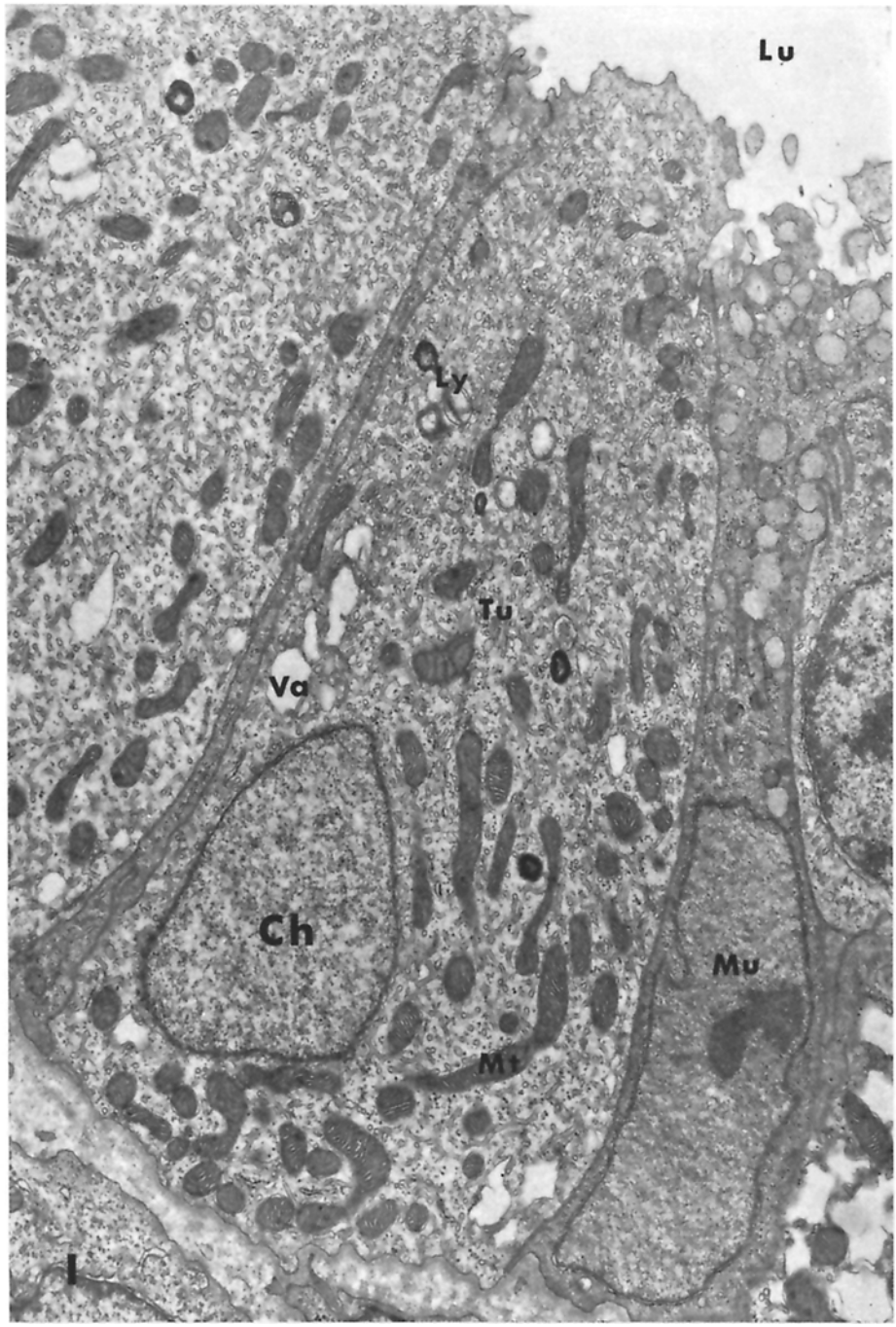

Fig. 1. An electron micrograph showing a renal chloride cell (*Ch*). Almost the entire cytoplasm is filled with an extensive tubular membrane system (*Tu*) and a number of mitochondria (*Mt*). Free surface with a few microvilli protrudes into the lumen (*Lu*). Vacuoles (*Va*) and lysosomal dense bodies (*Ly*) are also seen. *Mu*; mucous cell. ×11000

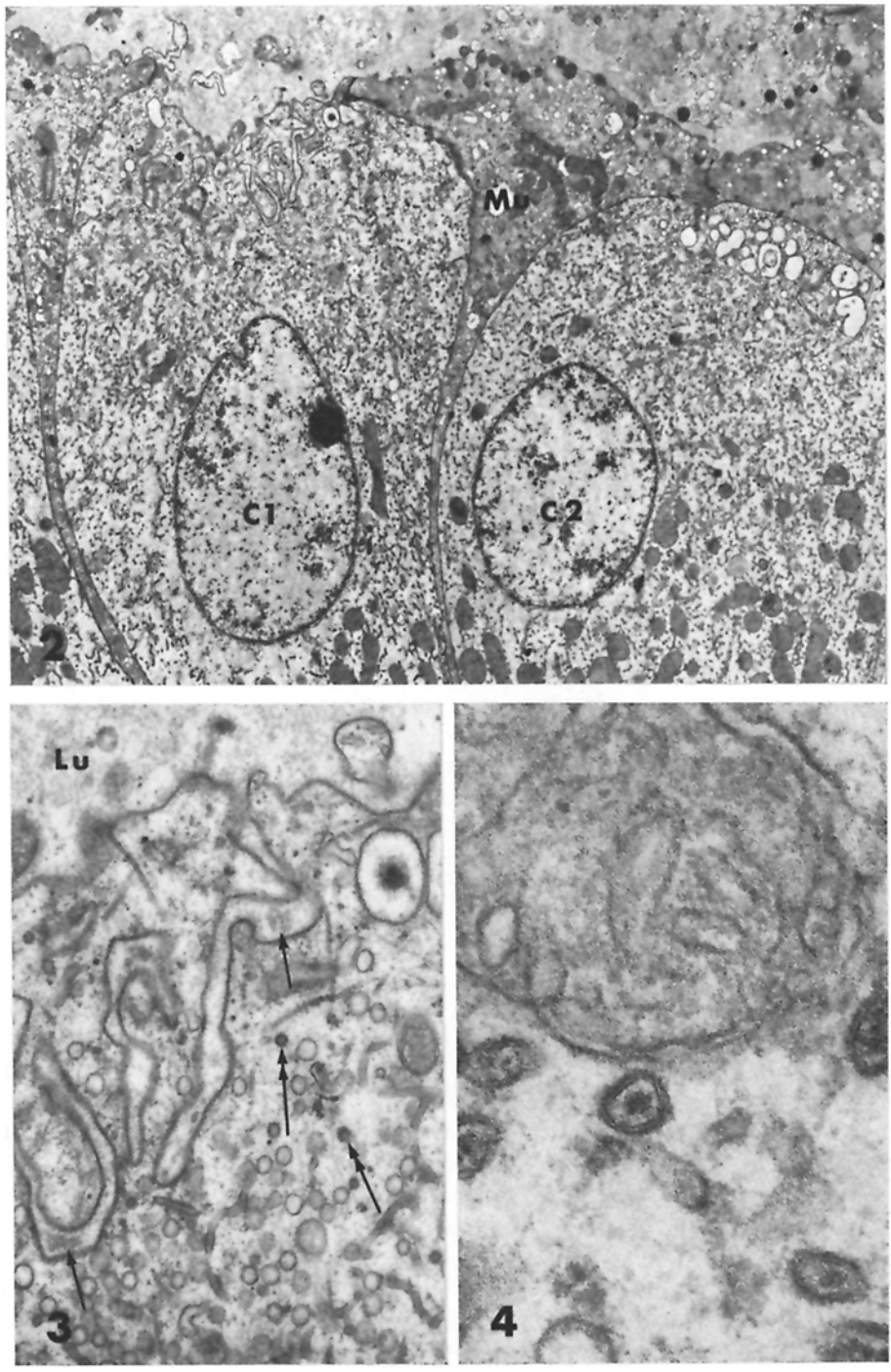

Fig. 2. An electron micrograph showing an apical differentiation of renal chloride cells. Apical invaginations, many vacuoles and vesicles can be seen both in cell 1 (*C1*) and cell (*C2*). Mucous cells (*Mu*) cover a rather large area of the luminal surface of the renal chloride cells. ×5000

which seems to represent a basic structural unit. These profiles seen in sections seem to constitute a three dimensional network with a hexagonal basic unit (Fig. 5). By their constant diameter, their thicker membrane and unique arrangement, the tubular membrane system could be clearly distinguished from the endoplasmic reticulum.

Another prominent feature of the renal chloride cells is their content of abundant mitochondria, closely associated with the tubular membrane system. These mitochondria are oval or elongated, contain a number of cristae and are particularly abundant in the basal half of the cell (Figs. 1, 2, 5). Outer membranes of the mitochondria and the tubules often appear to be contiguous, leaving only a narrow interspace as close as 100 Å (Fig. 6). Both granular and agranular endoplasmic reticulum are poorly developed in these chloride cells, and a typical Golgi apparatus is rarely encountered. These features would indicate that the renal chloride cells are not actively engaged in synthetic function. Lysosomal dense bodies and large cytoplasmic vacuoles are occasionally seen (Fig. 1).

When ruthenium red is applied as an extracellular space marker, every tubule contains electron dense deposit, accumulated as a dense layer of about 50–70 Å in thickness lining the luminal aspect of the tubular membrane (Figs. 5, 6). This would be a strong indication that the tubules communicate with the extracellular space through either the lateral or basal surfaces of the cells, although the direct continuity is difficult to visualize in untreated material. As shown in Fig. 6, where the tubular membrane system and endoplasmic reticulum are superimposed on each other, the two systems are still more clearly identifiable by the presence of the electron-dense deposits in the lumen of the one system and the absence in the other.

Discussion

Cells characterized by the vast amplification of surface cell membrane associated with abundant mitochondria have been demonstrated in various systems; salt secreting cells of the nasal gland of the marine birds (Doyle, 1960) and reptiles (Philpott and Templeton, 1964), epithelial cells of rectal or anal papillae of insects (Copeland, 1964; Gupta and Berridge, 1966a, b; Berridge and Gupta, 1967), the abdominal chloride epithelia in caddisfly (Wichard and Komnick, 1973), the gastric acid secreting cells of various animals (Ito, 1961; Sedar, 1962, 1969; Nomura, 1966; Lillibridge, 1968), and the chloride cells in the gill filaments of teleosts (Philpott and Copeland, 1963; Threadgold and Houston, 1964; Conte,

Fig. 3. Deep invaginations contain flocculent material (*arrows*) similar to that in the lumen (*Lu*). A vacuole in the upper right corner contains condensed material. Small coated vesicles (*double headed arrows*) can be seen among the plain vesicles. ×20000

Fig. 4. An electron micrograph at high magnification showing a cross section of the tubules. Dense deposits can be seen in the encircled unit membrane walls. Overall thickness of the tubular membrane appears to be thicker than the mitochondrial membrane. ×100000

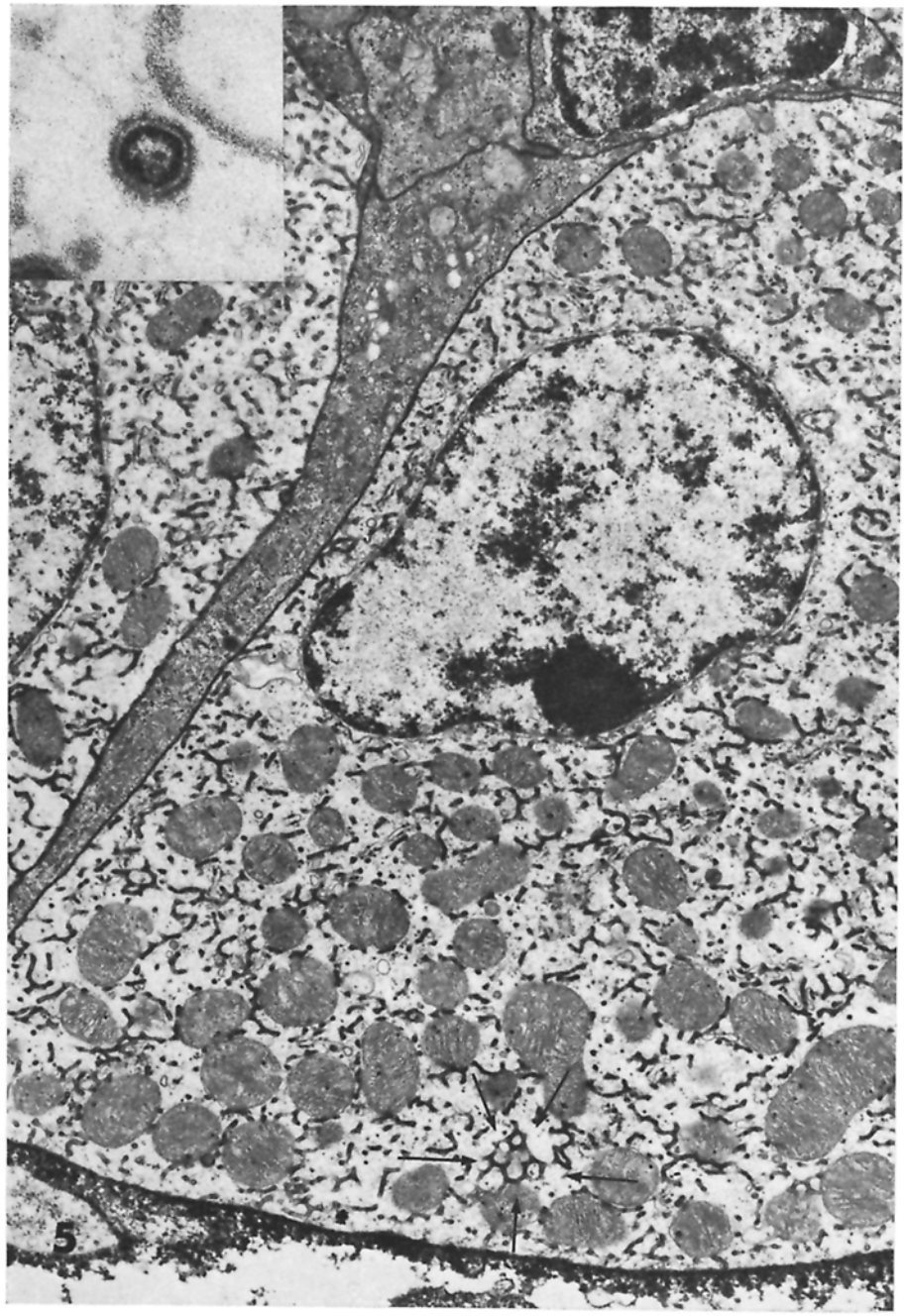

Fig. 5. An electron micrograph showing an extensive tubular membrane system stained with ruthenium red. It is clear that the tubules branch and anastomose in angular or tortuous form. Honeycomb array can be seen in the basal part (*arrows*). ×12000. Insert: A high magnified electron micrograph of a cross section of a tubule stained with ruthenium red. The luminal surface of the unit membrane is lined with a layer of dense deposit, with an average thickness of 60 Å. ×200000

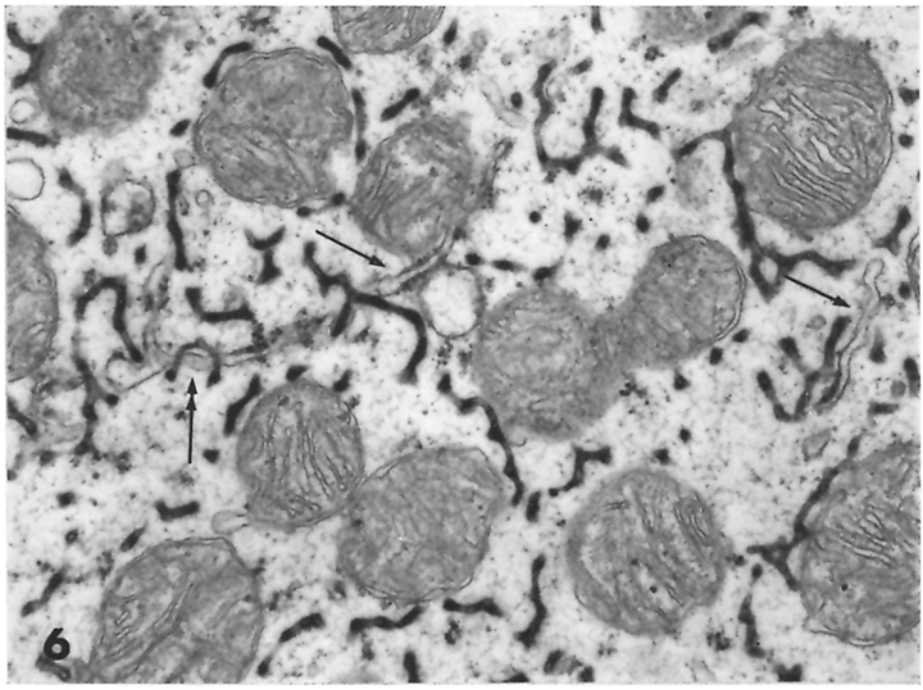

Fig. 6. A juxtanuclear region of a renal chloride cell treated with ruthenium red. A striking contrast can be observed in the presence or the absence of the deposit in tubules and in cisternae of the endoplasmic reticulum, even when they are superimposed within the thickness of a section (*double headed arrow*). ×35000

1969; Shirai and Utida, 1970). These cells are generally suggested to participate actively in the transport of ions, in which the associated mitochondria can favorably provide energy required for an active process, presumably carried out on an amplified membrane system. Lillibridge (1968) demonstrated that a comparable membrane system in the gastric oxyntic cells of the bull-frog had a thicker membrane and could be distinguished from the endoplasmic reticulum. Continuity of these membrane systems to the surface cell membrane was proven in the developing human gastric parietal cell (Nomura, 1966), and was also confirmed by extra-cellular space markers such as peroxidase in the bull-frog gastric oxyntic cell (Sedar, 1969) and lanthanum compound in the *Fundulus* chloride cell (Philpott, 1967).

The present study revealed that the special type of cell referred to as "the renal chloride cell" in the fresh-water catfish contained an homologous membrane system to those reported in various cell types mentioned above, which are actively involved in ion transport.

The dynamic aspect of the transport of electrolytes through gill filaments has been discussed with respect to the environmental ionic concentration. In the sea-water fishes, the chloride cells secrete excess ions from the blood (Threadgold and Houston, 1964; Conte, 1969), whereby electrolytes are suggested to be absorbed somewhere in the basal area of the cytoplasm and then concentrated during

transport to the apical cavities where release to the environment occurs (Philpott and Copeland, 1963). In fresh-water fishes, these cells absorb ions to keep an adequate concentration of electrolytes (Krogh, 1937). In the kidney of those fishes exposed to a diluted ionic environment, some mechanism of ionic regulation would be expected to take place in order to prevent a loss of electrolytes. Therefore, it is likely that the renal chloride cell in the fresh-water catfish could be involved in an absorption of ions rather than secretion. The evidence that Cl^- is almost completely reabsorbed from the glomerular filtrate in fresh-water fishes (Hickman and Trump, 1969) would support this idea.

Accordingly, in the renal chloride cell, ions might be actively incorporated into the apical invaginations and vacuoles, and succesively transferred to the tubular membrane system, possibly by interposition of vesicles, and finally released into the tissue space through openings in the tubules.

Electron-dense amorphous material found in the invaginations and vacuoles of the renal chloride cells are similar in appearance to those which have been reported in the gill filament chloride cells of *Fundulus*. As has been postulated by Philpott and Copeland (1967), this material could play an important role in the ionic movement, possibly functioning as a trap and/or a vehicle for electrolytes.

Acknowledgement. The authors thank Dr. Y. Uehara, Department of Anatomy, Ehime University for helpful discussion and for his critical reading of the manuscript.

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