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The Salt Absorbing Cells in the Gills of the Blue Crab (*Callinectes sapidus* Rathbun) with Notes on Modified Mitochondria*

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Summary. A modified, glandular epithelium lining the respiratory platelets of gills in crabs known to be absorbing salt is described. The cell surface adjacent to the surface cuticle is thrown into folds whose spaces are filled with flocculent material that is subject to pinocytosis. The lateral surface areas of neighboring cells are thrown into narrow, flat sheets that deeply interdigitate with one another on a one to one alternating basis. There is considerable spacing between the interdigitating plasma membranes that communicates with the haemolymph surface of the epithelium. Tremendously flattened mitochondria with modified cristae are seen. Both flattened mitochondria and more normally shaped mitochondria are seen to form mitochondrial pumps.

Introduction

This report describes the fine structure of a highly developed epithelial layer of cells located in the respiratory (lamellar) platelets of the crab gill. These cells are presumed to provide the mechanism for salt absorption by active transport when the animal is adapted to living in low salinities.

Considerable knowledge has been gained of the physiology of extra-renal mechanisms for salt excretion and salt absorption in various animals (for summaries see Krogh, 1939; Prosser and Brown, 1961; Potts and Parry, 1964; and Potts, 1968). Of the crustaceans, the crabs and crayfish have undergone extensive investigations. The earlier literature on these animals is reviewed by Robertson (1960) and Lockwood (1962). More recent investigations have been made by Potts and Parry (1964); Croghan, Curra and Lockwood (1965); Dehnel (1966); Gross et al. (1966); King (1966); Quinn and Lane (1966); Rudy (1966, 1967); Burton (1967); Kerley and Pritchard (1967) and Smith (1967). In this body of literature there is general agreement that the main route of salt absorption, when the forms are in hypo-osmotic environmental conditions, is through the gills. There is far less certainty of the route(s) of salt excretion under hyper-osmotic conditions (Dall, 1967). In this connection it is interesting to note that Mantel (1967) has found changes in the membrane potentials of isolated *Callinectes* gills which indicate that at salinities above normal seawater concentration the gill may reverse its function and excrete salt.

The normal histology of the gills of various crabs has been described by Bernecker (1909), Chen (1933), Webb (1940), and Flemister (1959). To the best of our knowledge, no one has described the fine structure of the cells in the respiratory platelets of the gills of either crabs or crayfish except for preliminary reports on the present study (Copeland, 1963, 1964a).

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Materials and Methods

Blue Crabs were studied in two different localities, Massachusetts and Louisiana. At the Marine Biological Laboratory, in Woods Hole, Massachusetts, crabs were adapted to decreasing salinities by mixing seawater with fresh water from Long Pond, the water supply source for Woods Hole (see PHILPOTT and COPELAND, 1963, for a water analysis). Animals were adapted to concentrations of 2 parts per thousand($^0/_{00}$) total salinity, or less, for at least a week before

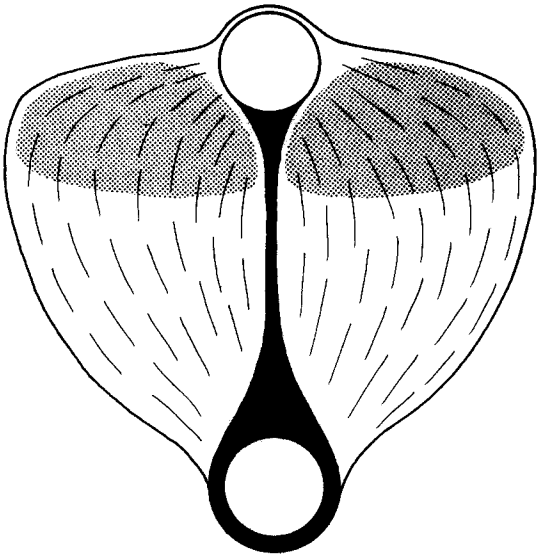

Fig. 1. Diagram of a cross section of a gill stem (black) showing a respiratory platelet attached on each side. Afferent vessel is at top, efferent vessel at bottom. The darkened areas in the platelets, near the afferent vessel, represent the patches of cells presumed to absorb salt. The curved lines represent the distribution of pilaster cells and the resultant path of blood flow through the respiratory exchange areas

sacrifice. At Tulane University in New Orleans, Louisiana, the crabs studied were fixed immediately upon collection from Lake Penchant, a low salinity body of water in the Louisiana marshes. The lake is reached by traveling some twenty miles of interconnecting bayous by boat from the nearest road. It is also connected to the Gulf of Mexico by another series of bayous and small lakes totaling about thirty miles in distance. During the several years of study, salinity measurements were made at monthly intervals at thirteen stations in and near the lake. Tides along the shallow Louisiana coast are small, normally not exceeding two feet. However, a strong prevailing south wind may double the tidal rise forcing salt water into the marshes up to the point that normal run-off balances the incursion. Although the water level of Lake Penchant might occasionally fluctuate, the lake itself was beyond the point of any significant salt water influx, yet was in free communication with the salinity gradient of the more coastal marshes. Lake Penchant did not exceed $0.5^0/_{00}$ total salinity and was usually about $0.2^0/_{00}$. In contrast, Carrion Crow Bayou, Bayou DeCade and Lost Lake, which lie a few miles closer to the Gulf, would read as high as $5.0^0/_{00}$.

During the summer months, Lake Penchant is invaded by a large population of Blue Crabs which disappear again during the winter months. This agrees with the ecological observations of ODUM (1953) and others. There is the very good possibility that these crabs represent a process of genetic selection and consequently are capable of more complete adaptation to fresh water than is the average Blue Crab.

In a preliminary survey of the gill tissues, a cellular patch (Fig. 1) was located on the respiratory lamellae by use of 0.01 M $AgNO_3$ following the method of KOCH (1934). Once the patch was identified it could just as easily be demonstrated after osmic fixation.

Gills of adapted animals were exposed by removal of the overlying carapace after removal of the legs by autotomy. The usual protocol involved placing the crab on chipped ice in the bottom of a deep polystyrofoam ice chest and dripping cold fixative on the gills for about five minutes. The second and third gills (counting from the posterior) were then excised and the outermost vessel (afferent) gently perfused with cold fixative by minimum pressures through a number 22 hypodermic needle. The needle was modified by grinding off the point and casting an epon plastic shoulder immediately in back of the new, rounded tip. The epon plastic was turned down on a drill press to a taper fine enough to readily seat the needle into the vessel of the gill. Perfusion was done only long enough to be certain that fixative had penetrated the entire gill and usually took less than a minute to accomplish. The gills were then dropped into vials of chilled fixative.

Fixation was for a total of $1^1/_2$ to 3 hours in 5% glutaraldehyde (SABATINI et al., 1963) buffered to pH 7.4 with either phosphate buffer (MILLONIG, 1961) or cacodylate buffer (TICE and BARRNETT, 1962). The phosphate buffer afforded more "brilliant" results but this may be due to loss of background material preserved by the cacodylate buffer. (For example, see KIHARA et al., 1961). The osmotic tonicity of the fixative was adjusted to about 800 milliosmoles by the addition of sucrose (CAULFIELD, 1957). The excess glutaraldehyde was then removed by buffer rinse over a period of 3 hours or, if more convenient, over night. Post fixation was done in 2% osmium tetroxide for 1 hour using the same buffer. The osmotic values of the buffer rinse and the post fixative were adjusted to that of the initial fixative by the addition of sucrose. Dehydration was done rapidly, starting with 50% ethanol and reaching 100% in about 1 hour with constant agitation.

All treatment fluids up to and including the first change of 100% ethanol were chilled on ice. Once in 100% ethanol, the gills were allowed to warm to room temperature for the final trimming. Embedment was Epon 812 according to the procedure of LUFT (1961). Thin sections were stained in uranyl acetate (WATSON, 1958) followed by lead citrate (REYNOLDS, 1963).

Results

It was quickly discovered that the size of the area of the lamellar patch of cells, in the respiratory platelets (Fig. 1) varied with the position of the gill in the animal, the posterior gills having the larger patches and anterior gills having small patches or none at all. It was also noted that the size of the cell patch varied within any one gill, dependent on the salinity of adaptation. Animals taken from seawater of about 30% salinity had relatively small patches of cells. Animals adapted to decreasing salinities showed a marked hypertrophy of the area of the patch. Animals adapted at Woods Hole showed a doubling and almost tripling of the area in a period of one to three weeks. A faint shadow would indicate the boundary of the baseline (seawater) adaption. Crabs captured in Lake Penchant showed maximal development, approximately a quadrupling of the cellular area over that of the marine forms. The nature of the cells, as seen at the electron microscope level, was essentially the same in both the old and newly established areas. A description of the gross morphology of the gill and the changes associated with salinity adaptation is being prepared for a separate publication.

The lamellar cell patch is composed of a single layer of cells lining the cuticle on each side of the lamella (respiratory leaflet) with periodic connections (pilaster cells) forming pillars across the haemolymph space. The positioning of these pillars undoubtedly determines the pattern of haemolymph flow through the lamella (diagrammed in Fig. 1). The gross histology follows closely that described by BERNECKER (1909) for *Pagurus bernhardus*, CHEN (1933) for *Grapsus grapsus*, WEBB (1940) for *Carcinus maenas*, and FLEMISTER (1959) for *Ocypode albicans*.

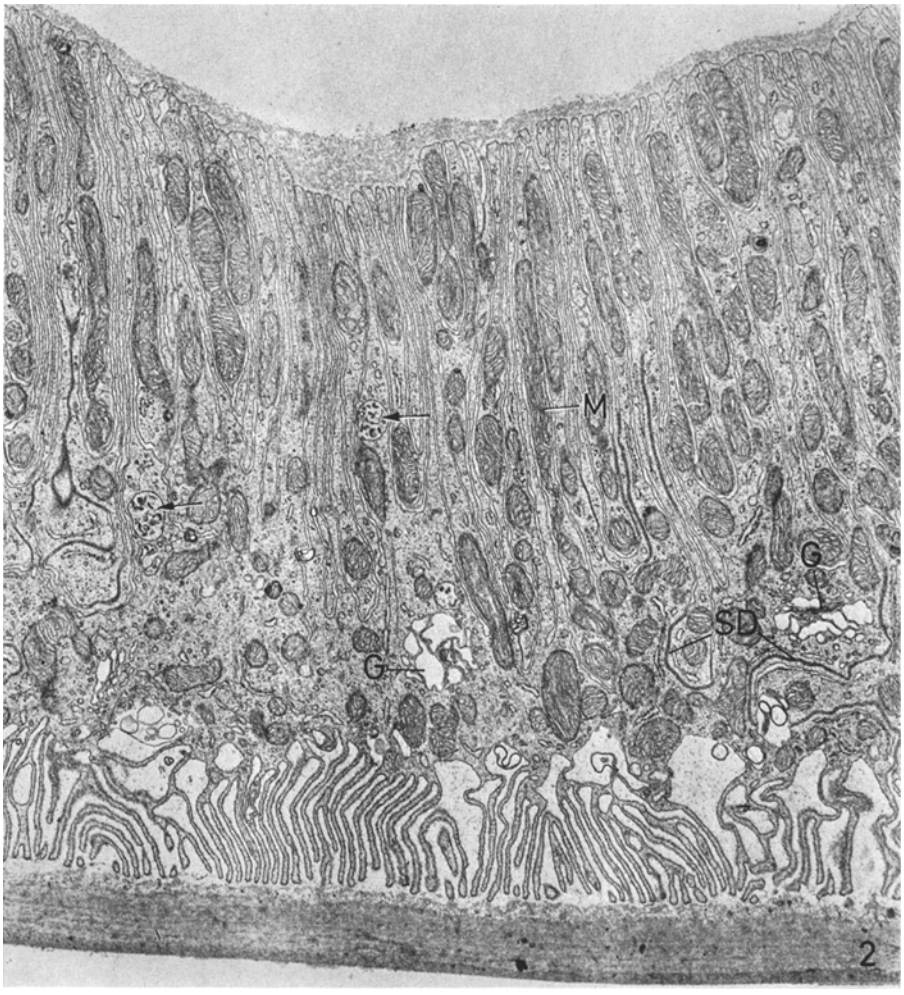

Fig. 2. Cross section of the salt absorbing epithelium lining the respiratory platelets of the gill. Cuticular surface below, haemolymph surface with granular basement membrane at top. Arrows indicate two multivesiculate bodies. A mitochondrion (*M*) with longitudinal cristae is also seen. Golgi-apparatus (*G*) and septate desmosomes (*SD*). See text for other points. 11,500 ×

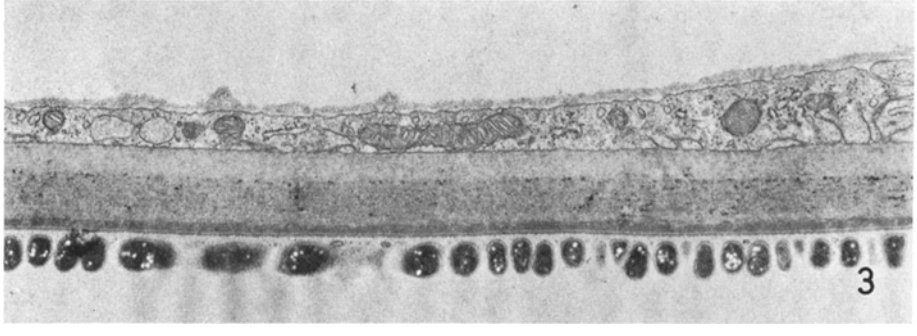

Fig. 3. Cross section of gill respiratory platelet in the respiratory exchange area, showing a very thin epithelium. The dark bodies on the outer surface (bottom) of the cuticle are symbiotic alga-like forms. 11,500 ×

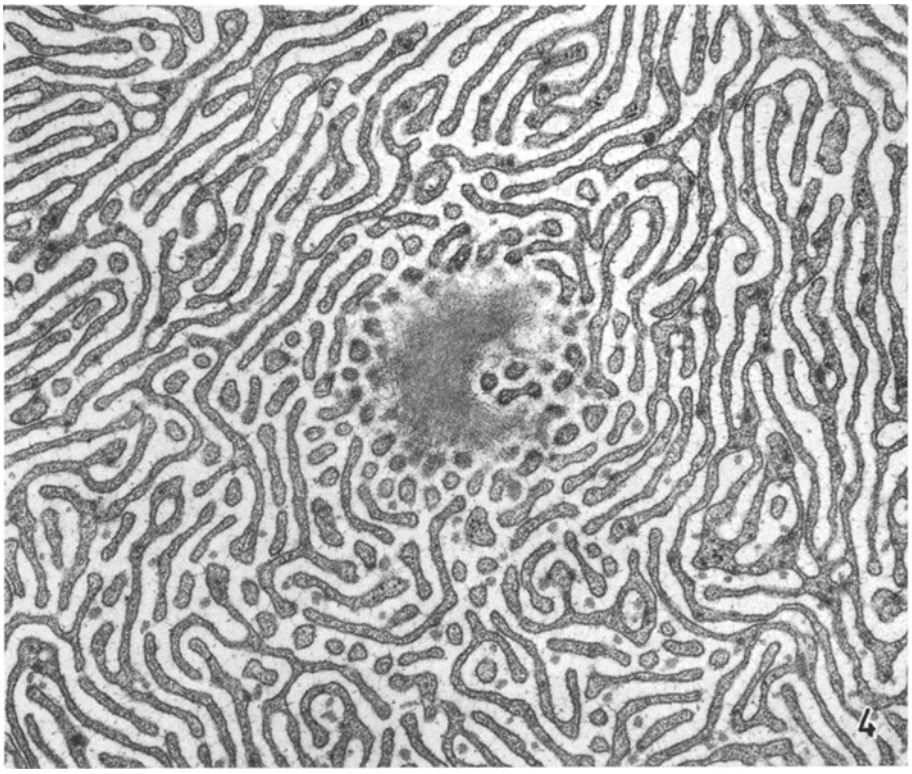

Fig. 4. Basal folds of the absorptive epithelium sectioned parallel and close to the cuticle. The dark area in the center is cuticle grazed by the section. Immediately about the cuticular area are the round profiles of the small, button-like projections found on the free edges of the folds. 16,500 ×

The salt absorbing cells are relatively thick, measuring about 10 microns as compared to about 0.5 micron for the endothelial lining of the respiratory areas of the remainder of the lamella (Fig. 2 as compared to Fig. 3).

Three areas (Fig. 2) can be distinguished within the cells: 1. a layer of folds adjacent to the cuticle, 2. a relatively homogenous cytoplasmic layer superimposed on the folds and 3. the rest of the cell (the major share) which is thrown into narrow leaflets that interdigitate with neighbouring cells.

The individual cells are separated in their basal regions by plasma membranes in the form of septate desmosomes (Fig. 2). However, in the region of the cellular interdigitations, the septate nature of the adjoining plasma membranes disappears.

The surface of the basal parts of the cells, adjacent to the respiratory cuticle, is thrown into folds. In cross section (Figs. 2, 5, 6) the basal folds look like widely spaced, somewhat irregular microvilli. However, a frontal section (i.e. one parallel to the cuticular surface) reveals the projections to be indeed folds (Fig. 4). The basal folds are arranged in random whorls but with a relatively constant spacing between folds and the folds in turn are of a relatively constant thickness. The free ends of the folds have small, button-like projections. These, again, are best seen in frontal sections (Fig. 4).

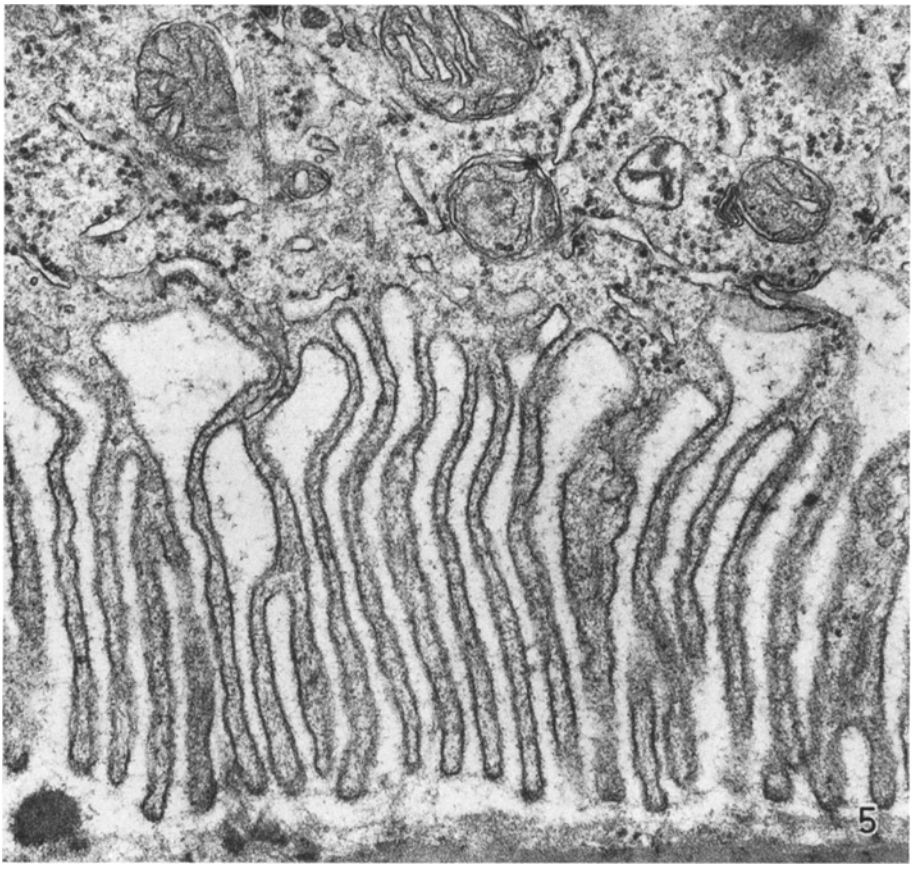

Fig. 5. Higher power of the basal folds. Minimal or no pinocytosis in this photo. Rough endoplasmic reticulum is closely associated with the bases of the folds. 38,000 ×

The spaces between the basal folds frequently contain a fuzzy, fibrous material. The open spaces are larger at the bases of the folds because two or three folds may share a common base (Fig. 5). Pinocytosis frequently occurs from these enlarged spaces (Fig. 6). In the process, the flocculent or fibrous material between the folds is engulfed and progressively condensed into formed bodies. The fate of the formed bodies could not be determined. A degree of pinocytosis is usually seen; Figs. 5 and 6 represent the extremes of no pinocytosis and very active pinocytosis. The basal folds, in contrast to the distal folds described below, do not interdigitate between cells.

The cytoplasmic layer immediately above the basal folds is well populated with organelles. In addition to formed bodies and granules, there are mitochondria, Golgi complexes, rough endoplasmic reticulum and microtubules. Smooth endoplasmic reticulum is minimal in amount or absent. The mitochondria, in this part of the cell, have the form of spheres or short rods. The cisternae of the Golgi complexes are distended and appear empty (Fig. 2).

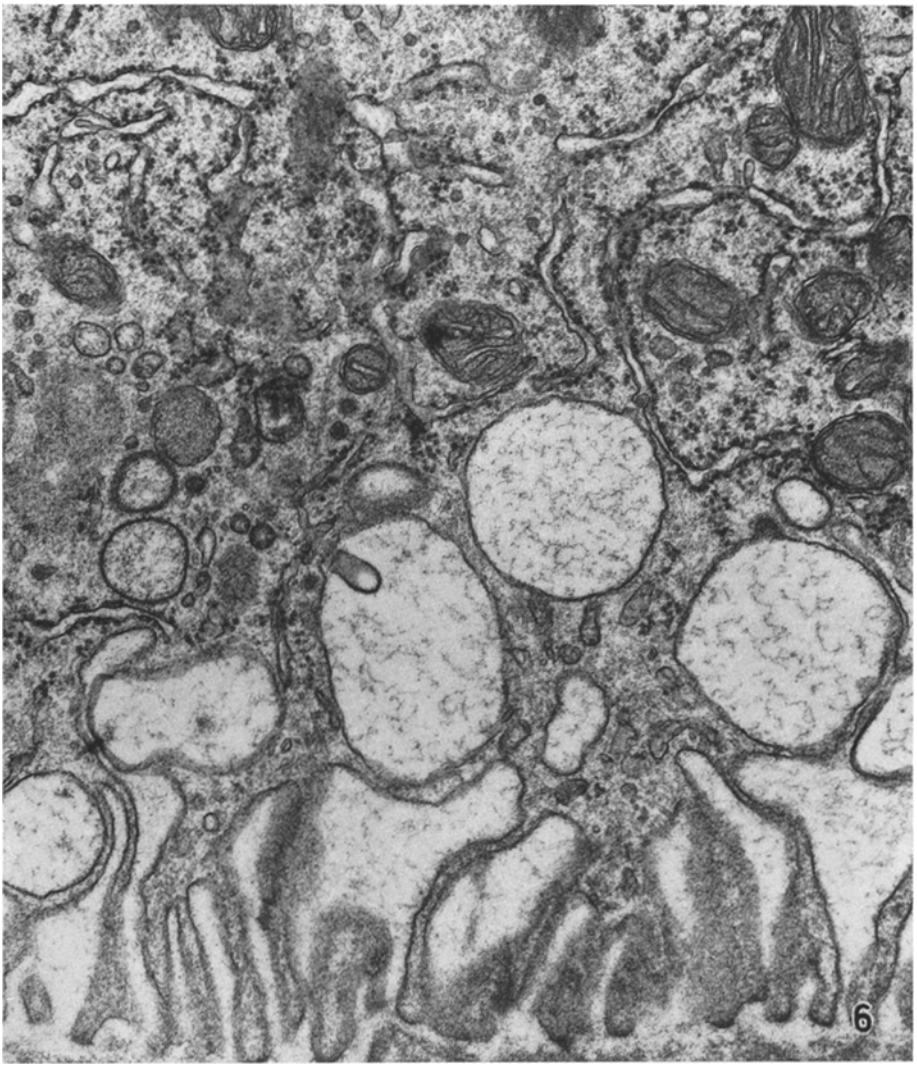

Fig. 6. Higher power of basal folds. High degree of pinocytosis depicted in this section. Note flocculent material in the inter-fold spaces and in the vesicles. 26,000 ×

The major share of the cell is cast in the form of leaflets or very narrow folds that interdigitate with those of neighbouring cells. The plane of the folds is perpendicular to the haemolymph surface of the cells and, in general, radiates from a small cytoplasmic area about the nucleus (Figs. 7, 8). The folds extend from the haemolymph surface to within a short distance of the cuticular folds (Fig. 2).

The interdigitating folds, though not of even dimensions, show a reasonably constant thickness compared to their great length (Fig. 7). Equally noteworthy, the folds of one cell interdigitate with the folds of an adjacent cell on a one to one basis, thus producing evenly spaced, alternating layers of cytoplasm. Usually, the cytoplasm of the salt absorbing epithelium is of an even density. The

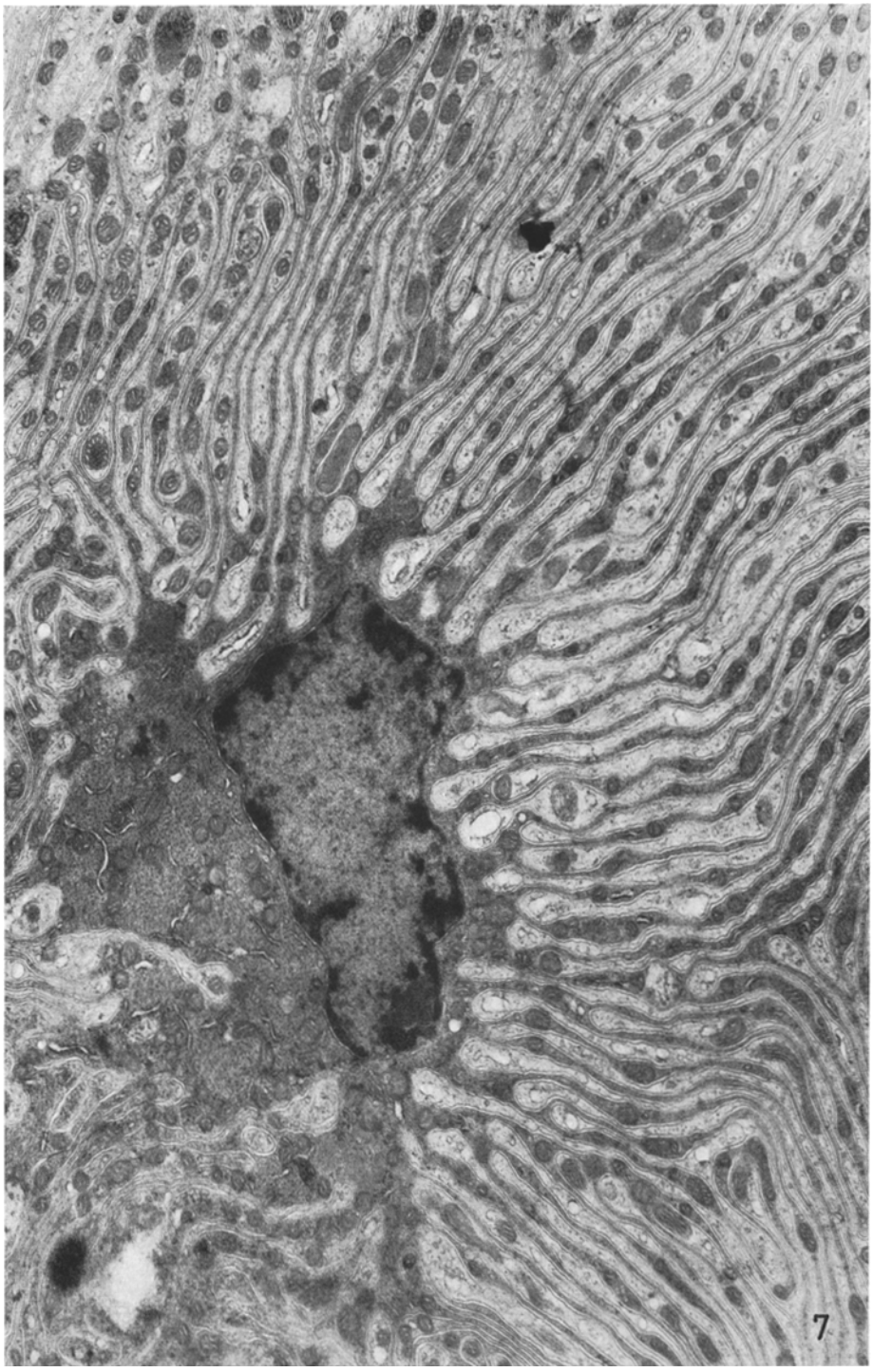

Fig. 7. Moribund cell (dark) cut in cross section to its longitudinal axis (i. e. plane of section is parallel to the cuticle). This illustrates the extensive one to one interdigitation of the neighboring cells. 6,000 ×

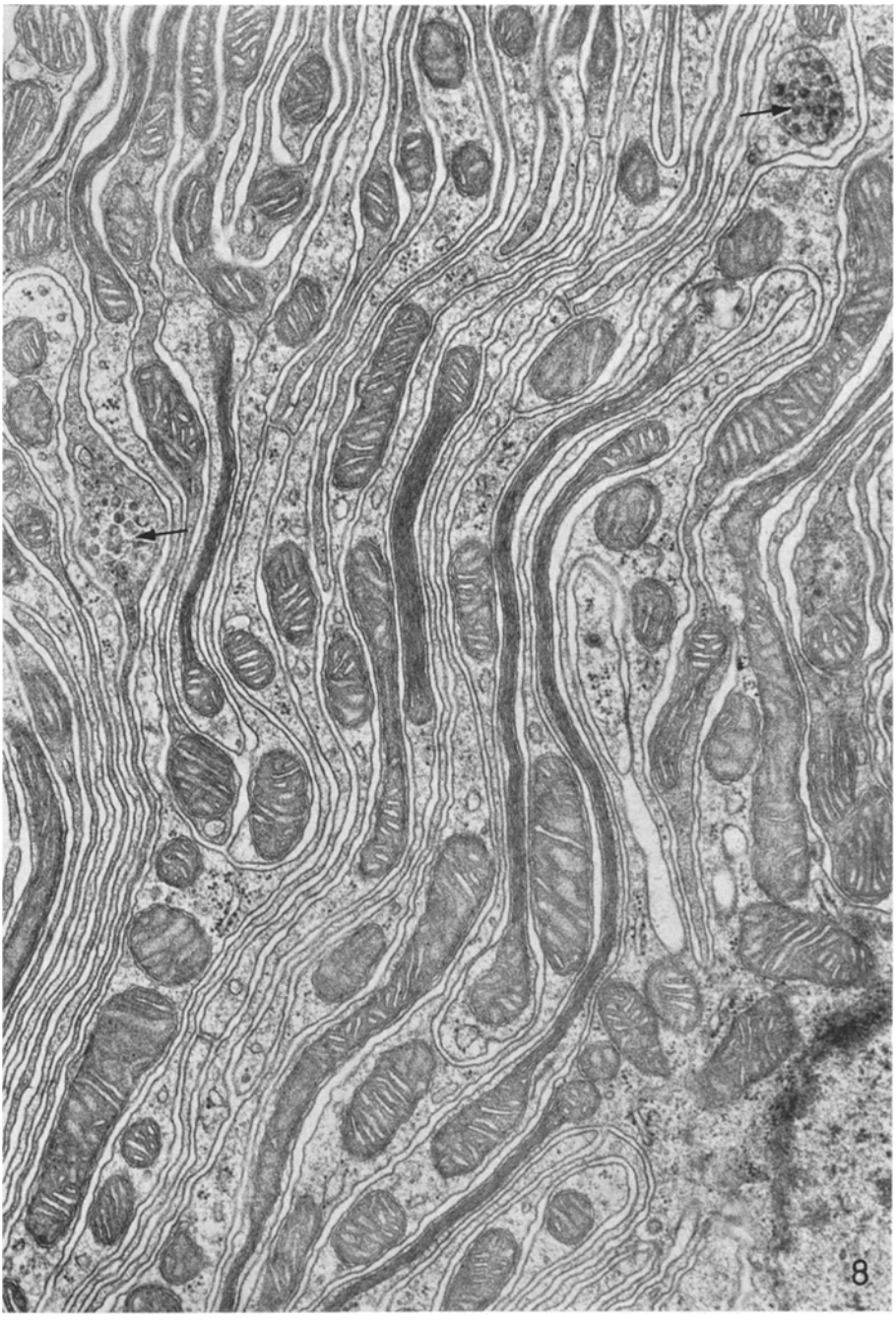

Fig. 8. Detail of cellular interdigitations in a section parallel to the cuticle and at the level of the nucleus (on the lower right). Note small volume of the cytoplasmic body about the nucleus. Note flattened, attenuated mitochondria. Two multivesiculate bodies are indicated by arrows. 15,000×

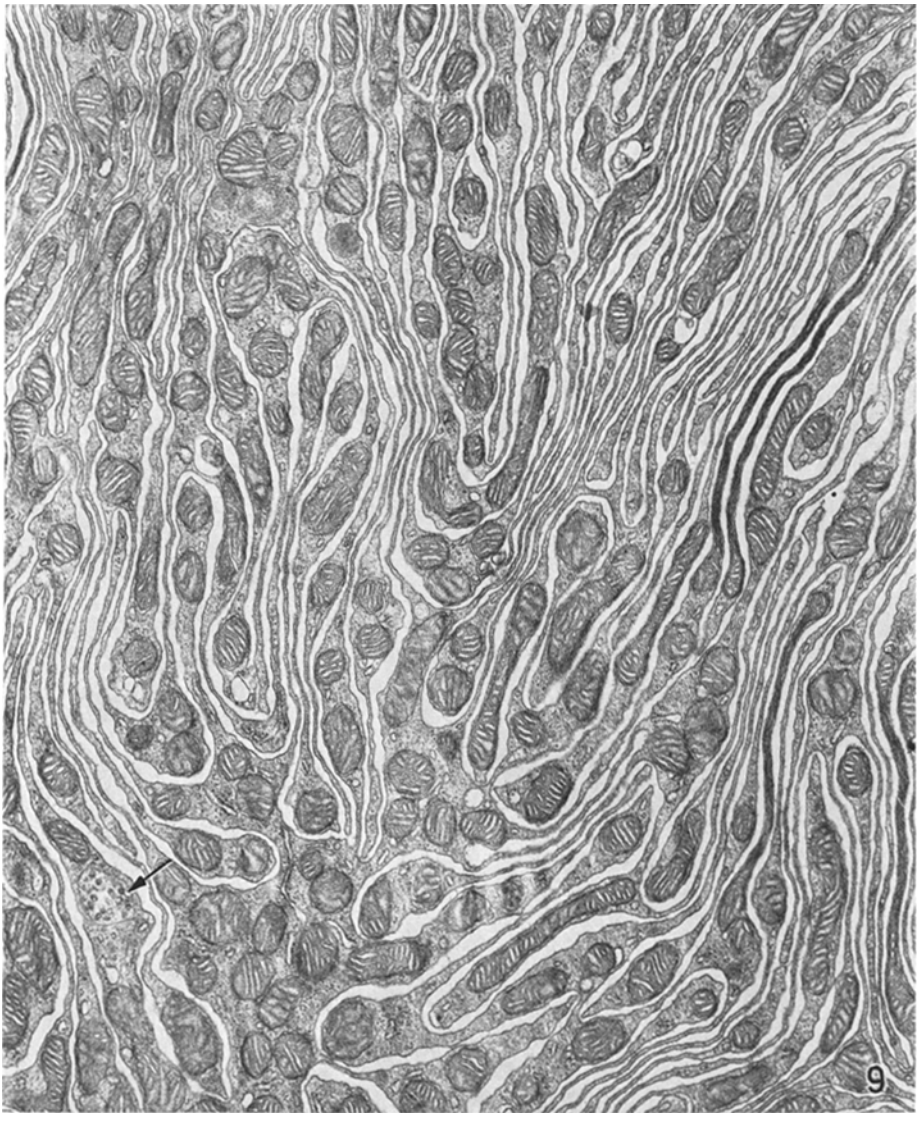

Fig. 9. Detail of cellular interdigitations in a section parallel to and close to the haemolymph surface. Note the dimensional regularity of both the folds and the spacing between the folds. Multivesiculate body indicated by arrow. 9,000 ×

"dark-light" cells in Fig. 7 are an exceptional condition; it is shown only to illustrate the extensiveness and the regularity of the interdigitations.

Most noteworthy is the fact that the interdigitating folds are separated by a space much larger than that usually found between the plasma membranes of neighboring epithelial cells (Figs. 8, 9, 12). When first observed, I suspected the spaces to be caused by fixation distortions. However, the spaces are seen under

almost all conditions of fixation and, in particular, when the rest of the tissue seems adequately fixed. They are indeed spaces of considerable dimension, averaging about 600—800 Å in diameter. The spaces may narrow slightly at the point of communication with the haemolymph space (Figs. 12, 14). The extremely numerous and extensive intercellular spaces produced by the distal interdigitating folds communicate freely with the haemolymph through the granular membrane which lines the entire haemolymph space. There are no desmosomes between the folds. Occasionally, adjoining plasma membranes may be close enough to resemble a tight junction but these are not seen with enough regularity to justify being labelled as such and are probably fixation artifacts.

The majority of the mitochondria within the cytoplasm of the interdigitating folds are long and sausage shaped. The tubular cristae are at right angles to the long axis or at a slight angle to it (Fig. 2). However, mitochondria are quite frequently observed in a flattened, pancake-like condition with a complete rearrangement of the cristae. As the mitochondrion flattens, the cristae shift to a length-wise position and become evenly parallel to each other (Fig. 11). As the mitochondrion continues to lengthen and flatten, the number of cristae diminishes to two or three slot-like spaces that run in continuous fashion from one edge of the plate-like mitochondrion to the other end (Fig. 14). On rare occasions, an extremely attenuated mitochondrion with no cristae at all can be observed (Fig. 10).

The flattened nature of these mitochondria can be checked by two circumstances. First, it is unlikely that tubular mitochondria of such thin diameter

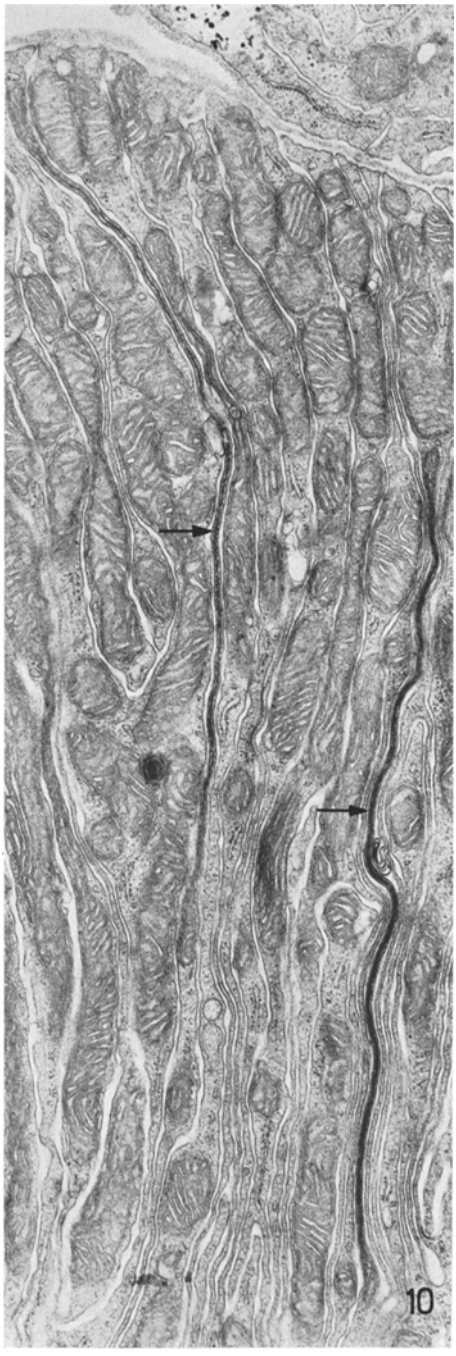

Fig. 10. Two instances of extremely flattened mitochondria (arrows) with no cristae. 10,500 ×

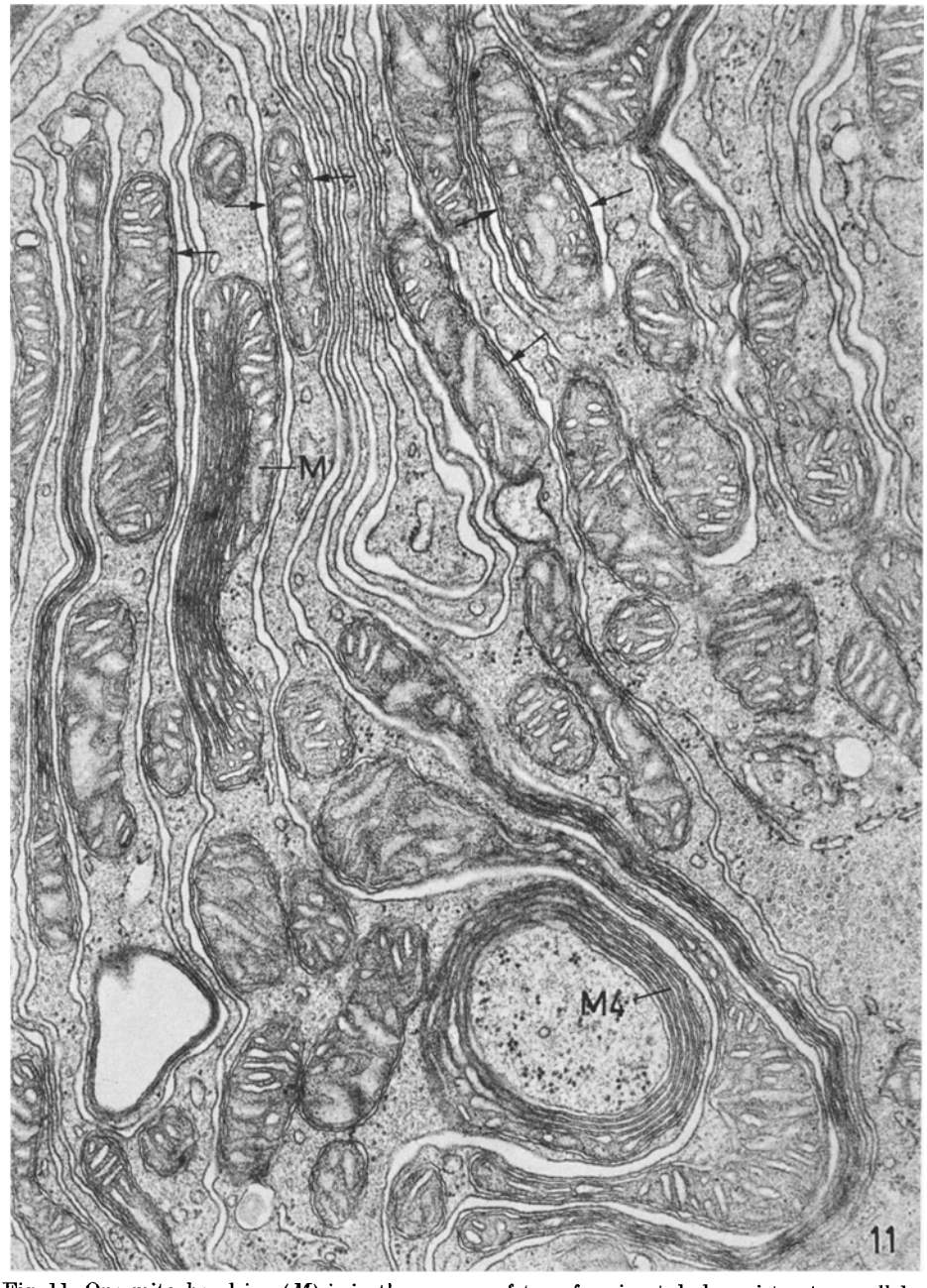

Fig. 11. One mitochondrion (*M*) is in the process of transforming tubular cristae to parallel, plate-like cristae. The circular mitochondrion (*M 4*) shows four flattened cristae. Mitochondria with tubular cristae also form mitochondrial pumps (arrows). 31,500 ×

would remain consistently in the plane of section (Figs. 10, 15). Secondly, the flattened mitochondria occasionally are found in nesting, cup-like folds (Fig. 14),

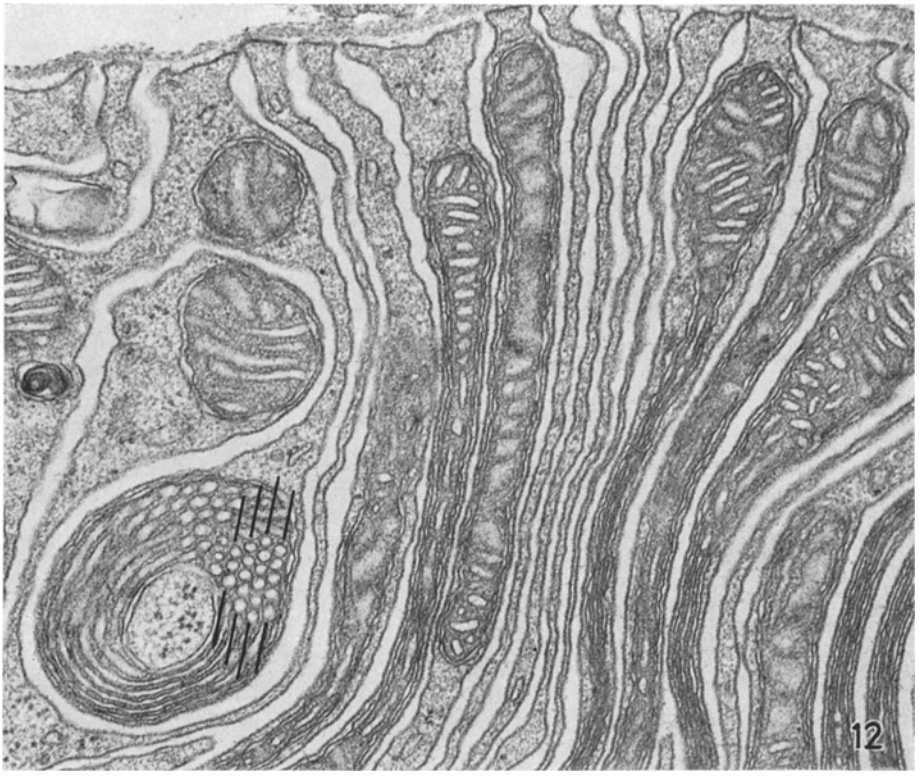

Fig. 12. Transformation of cristae. Note that the tubules are in parallel rows. Each will presumably fuse to form a plate-like or slot-like cristae. 22,500 ×

in which case cross sections of the nesting cups reveal circular mitochondrial profiles (Figs. 11, 13).

Fig. 16 is a diagram of the configuration of a flattened, single crista type mitochondrion. Vestiges of the tubular cristae system are usually seen in the bulging rim of the flattened mitochondria. A typical transitional condition, i.e. multiple cristae becoming parallel to the long axis of the mitochondrion, can be seen in Fig. 11. At first the length-wise cristae are tubular as the mitochondrion begins to flatten. Approximately twenty-one such cristae can be seen in the circular profile (flattened mitochondrion) in Fig. 12. Note that the cristae are oriented in four rows (possibly five). It is probable that the cristae fuse laterally in any one row to form the flattened slot-like cristae seen so well in Figs. 13—15.

The flattened mitochondria usually have only one crista. Two cristae are fairly commonly observed. Three flattened cristae are occasionally observed and four cristae rarely. Only in rare happenstance are attenuated, darkly staining mitochondria seen with no cristae at all (Fig. 10).

Another characteristic of the flattened mitochondria is that their external membranes become closely approximated to the interdigitating plasma membranes (the alpha cytomembranes of SJÖSTRAND). This is noticeable even at low magnification (Fig. 9). Usually the surfaces of any one mitochondrion are adherent

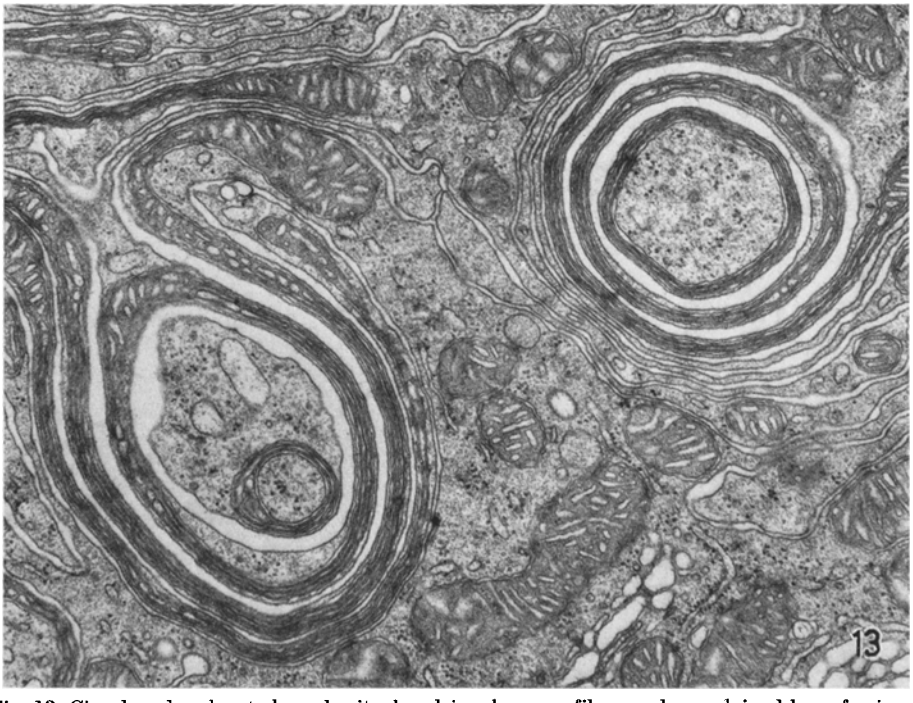

Fig. 13. Circular, doughnut-shaped mitochondria whose profiles can be explained by referring to Figure 15. 17,300 ×

to the plasma membrane on both surfaces of the fold (Figs. 12, 13). If the fold is of any great thickness, then the mitochondrion adheres to at least one of the surfaces.

The distance between the envelope membrane of the flattened mitochondria and the plasma membrane is approximately 110—140 Å (Fig. 15). This happens to be about the same dimension as the distance of the envelope membrane to the inner membrane of the mitochondrion and also for the inner membrane to the first crista membrane and, in turn, for the crista space itself. This makes it difficult, at first glance, to determine the number of cristae involved in any one instance.

The distance of 110—140 Å between the two membranes (i.e. plasma membrane and mitochondrial surface membrane) is a constant feature in respect to the flattened mitochondria. It is also to be found in regard to the more normal appearing mitochondria still in posession of tubular cristae (Fig. 11). This close and regular association of membranes is referred to as "mitochondrial pump" in the Discussion.

Characteristic multivesiculate type formed bodies are seen usually in the basal areas of the interdigitating folds and, occasionally, in other areas. They are membrane bounded with a light, granular matrix in which are embedded small vesicles. The vesicles or particles are shaped much like mammalian erythrocytes, except that they are not completely symmetrical (Figs. 2, 8, 9).

Discussion

Ever since the classical work by HOMER SMITH (1930) on the extra-renal secretion of salt by marine teleosts it has been normal procedure to test the gills of other aquatic forms as a possible locus of salt secretion and/or salt absorption.

The gills of crabs are no exception. NAGEL (1934) was the first investigator to show that the crab does indeed possess an ability to actively absorb salt from the medium. His approach was quite simple and consisted of reducing the concentration of salt in the haemolymph of *Carcinus maenas* by washing the animals in water of low salinity. The salinity of the external medium was then increased, but still kept below the tonicity of the haemolymph. Nevertheless, the crabs proceeded to increase the tonicity of the haemolymph by absorbing salt from the hypotonic external medium, indicating active uptake. KOCH, in the same year (1934), treated the gills with silver nitrate and suggested that the blackened areas indicated the sites of active salt absorption (see Fig. 1).

KROGH (1938), using techniques similar to those of NAGEL (1934), studied in more exhaustive detail both uptake and loss of a variety of ions, including NaCl, in *Eriocheir sinensis*. He noted that, when the crabs were first put into the distilled water rinse medium, there was a rapid initial loss of salt through the gill region and an appreciable lag time before salt absorption would occur. He found that crabs given "training" at intermediate salinity responded best to his final experiments. This undoubtedly allowed time for hypertrophy of the cellular apparatus reported here. A similar line of evidence is found in the report by ANDERSON and PROSSER (1953) wherein *Callinectes* collected in the areas of low salinity of the upper reaches of the Pettaquamscutt River in Rhode Island could adapt to much lower final salinities than those crabs collected at the mouth of the river in more saline areas.

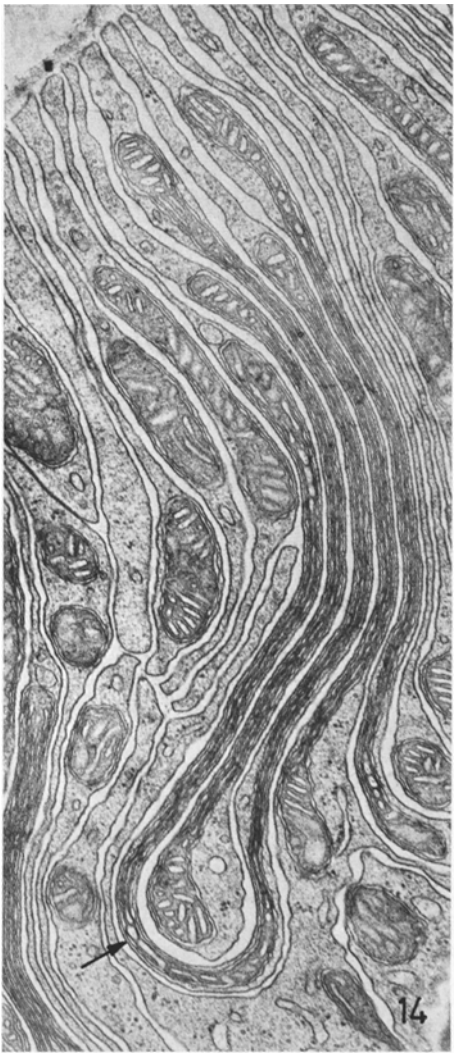

Fig. 14. Flattened, cup-shaped mitochondrion (arrow) which if cut at right angle would present a circular profile. See Fig. 16 for a three dimensional interpretation of the cup-shape. Each of the four associated mitochondria have a single longitudinal cristae. 17,300 ×

Conclusive proof that the gills of crabs do indeed absorb salt from dilute media was not provided, however, until KOCH, EVANS and SCHICKS (1954) perfused isolated gills of *Eriocheir sinensis* which were bathed in solutions of radio-isotopes of salts. Transfer occurred and it was also found that various metabolic inhibitors would block the absorption of salts, indicating that the transport was dependent on metabolic energy (KOCH, 1954).

Callinectes must be considered as a marine form (i. e., reproduction occurs only in seawater), but it readily invades brackish water. Seawater adapted crabs ($35^0/_{00}$) can be abruptly moved to water of about $7^0/_{00}$. If exposed to weak or more intermediate concentrations, they can be adapted to about $0.5^0/_{00}$. If allowed to invade fresh waters at a rate determined by their own

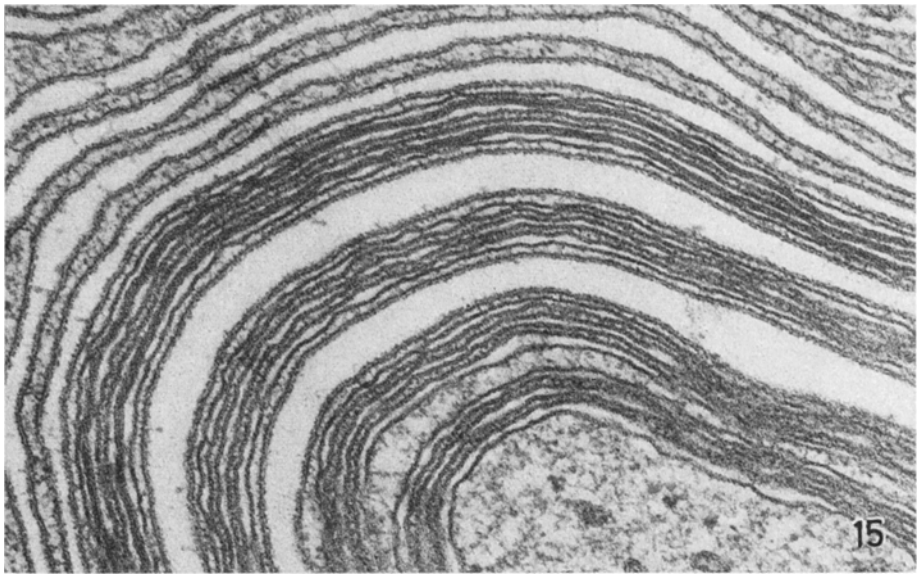

Fig. 15. Detail of the cristae in the flattened mitochondria and detail of the plasma membrane-mitochondrial membrane association to form mitochondrial pumps. The uppermost mitochondrion has two cristae, the other three have one cristae each. 64,500 ×

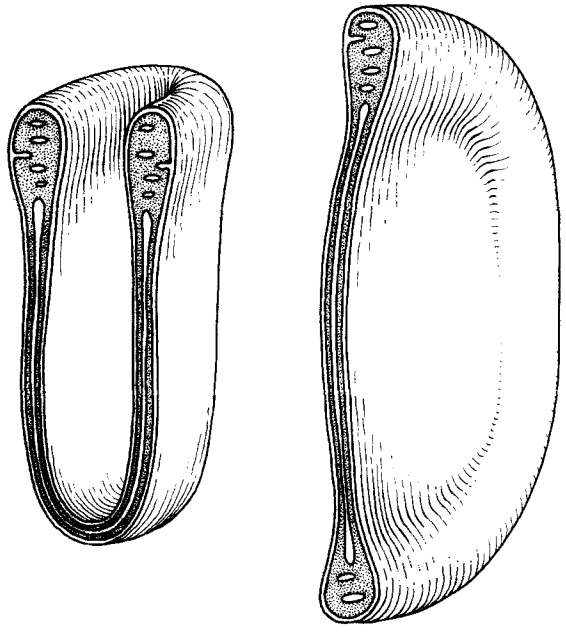

Fig. 16. Diagrammatic drawings depicting two flattened mitochondria cut in half. The one on the right is the more commonly seen. The one on the left is cup-shaped (seen in various orientations in Figures 13 and 14)

volution, adaptation to $0.1^0/_{00}$ is not unusual (our observations on Lake Penchant in Louisiana). The lowest recorded concentration for adaptation is $0.01^0/_{00}$ for crabs invading the rivers and marshes of Florida (ODUM, 1953). In the latter two cases, genetic selection could be a factor, i. e. those crabs best able to invade fesh water may have propagated their own kind.

Although not of direct concern to this report, it is interesting to note that *Callinectes* can adapt to salinities above that of ordinary seawater. We have adapted them to seawater supplemented with salt to the level of $52^0/_{00}$. GIFFORD (1962) reports that in the Laguna Madre on the Texas Gulf Coast, where evaporation concentrates natural seawater, *Callinectes* can adapt to $72^0/_{00}$. The latter salinity is almost double that of the average for seawater.

Starting with the classical work of LUNDEGARDH and BURSTROM (1933) on salt absorption in the root of wheat seedlings, a tremendous literature has evolved concerning the act of moving salt across living membranes against osmotic pressure. A sampling of recent investigations and reviews is as follows: CZAKY (1965), OPIT and CHARNOCK (1965), POST and SEN (1965), RAPAPORT (1965), SKOU (1965), VAIDHYANATHAN (1965), HOSHIKO and LINDLEY (1966), MARTIN and DIAMOND (1966), SNELL and CHOWDHURY (1966), HAAS (1967) and HEINZ (1967).

Unfortunate to our present consideration, most of these investigations explore the chemistry and physics of the phenomenon. Almost no attention is given to possible cellular architecture, except to the plasma membrane itself and then usually in terms of possible molecular arrangement in the form of model systems.

Correlations between the physiology of active transport and the structural mechanism (s) of active transport are still largely speculative. Some generalizations, however, regarding the cytoarchitecture of salt transport can be drawn from the comparative studies of the "salt glands" found in diverse animals but the intimate processes by which these structures operate still awaits elucidation.

Common to all the salt transporting tissues is an extensive elaboration of the plasma membranes in the form of tubules, crypts, infoldings and/or interdigitations. Invariably, mitochondria are to be found in some association with the membranous systems. Representative types of tissues are as follows: salt secreting cells in fish gills (PHILPOTT and COPELAND, 1963); salt gland of herring gull (KOMNICK, 1963); rectal gland of shark (BULGER, 1963; KOMNICK and WOHLFARTH-BOTTERMANN, 1966); anal papillae of mosquito larvae (COPELAND, 1964b; SOHAL and COPELAND, 1966); salt glands of marine turtles (ABEL and ELLIS, 1966); potassium transport in *Cecropia* midgut (ANDERSON and HARVEY, 1966); rectal papillae of the blowfly (GUPTA and BERRIDGE, 1966; BERRIDGE and GUPTA, 1967); rectal papillae of termites (NOIROT et al., 1966, 1967), salt secreting cells of the brine-shrimp *Artemia* (COPELAND, 1967).

Comparison of the salt transport tissues just mentioned to that in the *Callinectes* gill gives no ready answer to the problem of functional morphology. Perhaps the most unique and distinctive coincidence is the presence of "mitochondrial pumps" in the tissues of 1. a mosquito larva (COPELAND, 1964) which can absorb salt from concentrations as low as $0.009^0/_{00}$, 2. a brine-shrimp (COPELAND, 1967) which can secrete salt against crystalizing brine of about $500^0/_{00}$ and 3. the presently described *Callinectes* which can readily adapt from seawater to fresh water.

It is probable that the membranous association which constitutes the mitochondrial pump[1] may exist in other tissues, namely the tubules of the kidney. The cells of the proximal tubules are highly interdigitated and the mitochondria are frequently closely associated with the infolded plasma membranes. For example, see Fig. 26 on page 519 of RHODIN (1958). The tempting assumption is that the mitochondria furnish the metabolic backing for the adjacent plasma membranes to carry on salt transportation.

[1] The mitochondrial pump is defined as follows: "The mitochondrial pump is a metabolically linked ion pump located in the cell plasma membranes intimately associated with the mitochondrial membranes at a distance of several hundred Ångströms or less". (COPELAND, 1967, p. 380).

The other noteworthy membranous arrangement in the *Callinectes* are the folds adjacent to the cuticle. A degree of pinocytosis is almost always seen at the base of the spaces between the folds. It could be interpreted that salt (and water) may be taken into the gill by pinocytosis in the sense of GRIM (1963). Another interpretation that would not require transporting energy on the part of a plasma membrane is the possibility that the flocculent amorphous material in the spaces between the folds would bind the salt. The bound salt would then be ingested by the pinocytotic vesicle. In the latter circumstance, it then might be assumed that the bound salt is then released in the cytoplasmic vicinity of the mitochondrial pumps and subsequently transported to the haemolymph connected spaces by the metabolically active plasma membranes.

Work by physiologists dealing with gill transport mechanisms does not give many clues as to the responsible morphology. KOCH (1954) in his report on the perfusing of isolated gills of *Eriocheir* comes to the conclusion that the salt absorbing mechanism is located on the outer surface of the gill. SHAW (1960) in work on the gills of *Astacus* does not attempt to cytologically locate the function but does believe that Na^+ is the primary ion to be moved and that Cl^- may lag behind but still be moved by ion exchange mechanisms. On the other hand CROGHAN et al. (1965) working on isolated gills of *Austropotambius* concludes that the act of salt absorption is accomplished at two sites: Cl^- is pumped by the outer membranes of the cell of the gills and Na^+ by the inner (haemolymph) membranes of the cells.

The multivesiculate bodies, although ubiquitous in the crab gill, need not be directly related to salt transport. It is easier to interpret them as being lysosomal in nature and merely symptomatic of a metabolically active tissue. It is interesting to note, however, that very similar bodies are described in the salt excreting cells of the plant, *Tamarix aphylla* (THOMSON and LIU, 1967, Fig. 18, page 213).

If attention is turned to the possible significance of the relatively narrow but long spacings of the interdigitating membranes of the cells, one can apply Diamond's theory of transport by a standing osmotic gradient (DIAMOND and TORMEY 1966; TORMEY and DIAMOND, 1967; DIAMOND and BOSSERT, 1967). In this instance the infolded, interdigitating plasma membranes, with associated mitochondria, would actively transport the salt and the long channels leading to the haemolymph surface would provide the dimensions for an osmotic gradient.

Another theory of water and salt movement (CURRAN and MCINTOSH, 1962) might also be applied if the infolded plasma membranes with the associated mitochondria are considered to be the "active" membranes of their theory and the granular membrane (basement membrane) which lines the haemolymph space and covers the long channels would constitute the physiological equivalent of the "inactive" or porous membrane of their theory.

The observations on the salt absorbing cells in the blue crab gills are in essential agreement with the picture seen in the land crab *Gecarcinus* obtained from Bermuda (COPELAND, 1968). The interdigitating folds in the land crab are somewhat thicker and more irregular. The mitochondria are not as completely flattened. However, the spaces separating the interdigitating folds and the dimensions of the mitochondrial pump systems are similar. The land crab is unique in that

its gills are air breathing. The surface of the gills is kept moist by ground water attracted from damp sand by a complicated irrigation system (BLISS, 1963). The physiology of osmoregulation in this animal is further complicated by the possible participation of the pericardial sac in water balance (BLISS, 1966). A tentative conclusion (COPELAND, 1968) is that the pericardial sac does not absorb water and/or salt by metabolic transport.

We have also made some preliminary observations on the gills of the fresh water crab *Pseudothelphusa* from Costa Rica. Fixation has been inadequate but we can report that the membrane folds along the cuticular border are much deeper, more narrow and show more pinocytosis than those in the blue crab.

The observations on the blue crab gill reported here constitute an interesting contribution to the fine morphology of salt transport organs. However, they do not as yet pinpoint the exact location of the living membrane(s) responsible for the transport. The answer will probably come with the histochemical demonstration of suitable enzymes at the electron microscope level. In this regard, it is unfortunate that there is as yet no universally acceptable technique for the localization of Na und K activated ATPase.

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