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High-energy adhering junctional complexes or with mitochondrial coupling

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Summary. A variety of *adhering junction* is found in the ependyma of the domestic cat with a coupling of mitochondria. These are symmetrically situated (in mirror form) at both sides of the intercellular cleft, which always maintain the same separating distance, thereby leaving a limiting cellular space of a constant amplitude. The *hypothesis* is put forward that the energy (ATP), provided by the mitochondria over adhering junctional complexes, would produce separate fields of force which would position in a lengthwise direction the molecules which give rise to the anchoring filaments. The *mitochondrial energy* provided and the electrostatic forces generated would produce an adhering, intercellular junction which is functionally very strong and which could be called: *high-energy adhering junctional complexes or with mitochondrial coupling.*

Key words: Junctional complexes - Adhering junctions - Mitochondria - Ependymocytes - Ependymal epithelium - Domestic cat

Introduction

The intercellular cohesion which provides the group of cells of the tissues of multicellular organisms with specificity, develops specialized ultrastructures of certain points of contact between the cell membranes of two adjacent cells which are known as *intercellular junctions*. The varied ultrastructure of these junctions means that they may be classified into *four types*, which began to be made known in 1963 by Farquhar and Palade and by Roberston. They had previously been examined by Sjostrand et al. (1958), Fawcett (1958, 1961) and Karrer (1960). Research was later carried out on these by Dewey and Barr (1964), Kelly (1966), Revel and Karnovsky (1967), Overton (1968), McNutt and Fawcett (1969), Rayns et al. (1969), Staehellin et al. (1969), Douglas et al. (1970), Goodnough and Revel (1970), McNutt et al. (1970), Skerrow and Matoltsy (1974), Staehlin (1974), Staehlin and Hull (1978), Singh et al. (1980), Merchan and Gil-Loyzaga (1980), Seitz et al. (1981), Saunders et al. (1982) among others.

Four basic types of *intercellular junctional complexes* are distinguished as follows: 1.- *Occluding junction.* 2.- *Adhering junction.* 3.- *Desmosomic junction.* 4.- *Communicating junction (Nexus).* Some other types have subsequently been added, which may be considered to be variations or subtypes of the above-mentioned four basic types. Some of them only correspond to variations in name or terminology.

These four basic types of junctional complexes are characterized by their complex ultrastructure, although each one of them is designed to fulfil one or more of the following functions: to obliterate the intercellular space; to block the circulation through the intercellular space; to prevent the free dissemination of molecules; to constitute a barrier to the dissemination of ions; to provide hydroionic impermeability; to act as a mechanical, compact and stable intercellular junction (Brighman and Reese, 1969); to act as points for the transmission of active forces intercellularly generated and transmitted by the cytoskeleton to support the interstitial or endocellular, cell movement; to act as support points in the muscle contraction; to act as points for the agglutination of cellular clones for blastomeres and continue with the development of embryonic tissue (morphogenetic movements) (Sheffield, 1970: Sheffield and Moscona, 1970); to act as connecting points of the cytoskeleton of neighbouring cells; to act as distribution points of the mechanical forces of the tensions (stresses and pressures) to which a tissue is subjected; to act as a basis for the cellular interaction through intercellular interchanging of molecules, be it by electrical or chemical coupling; to act as a transmitting point of cardiac contractions or nervous stimulation impulses (synapsis); to act as the point of dissemination of the changes in potential between

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adjacent cells; to act as points allowing for the unidirectional transfer of impulses; to act as points of low ionic resistance for the electrical coupling between myocardial cells; to act as points of ionic interchanging and small molecules; to provide the microchannels transporting ions; to act as points through which the induction between embryonic cells may take place; to act as points through which the morphofunctional harmony of the cellular clones which make up all tissues may take place.

The essential, ultrastructural characteristics of each of the *four types* of *intercellular junctions* already mentioned above, may be summed up as follows: 1.- *Occluding junctions* fuse the external laminae of adjoining cell membranes. 2.- *Adhering junctions* possess a cleft of 15-25 nm between the cell membranes, full of a material with a low electrodensity and the internal lamina of both cell membranes is reinforced by a plate with a high electrodensity where 7 nm filaments are anchored. 3.- *Desmosomic junctions* leave a separating cleft of 22-35 nm full of an electrodense material which forms a dense, central plate of the intracellular space, and the internal lamina of both cell membranes is reinforced by a very dense, thick plate from which fork-shaped filaments of 10 nm emerge. 4.- *Communicating junctions* leave a separating cleft of 2-3 nm which is crossed by numerous microchannels forming geometrical, hexagonal units perforated at the centre by a micropore of 1-2 nm which connects the two cells.

Among the great variety of *subtypes* of intercellular junctions, classified within each of the four types mentioned, assigning them their corresponding functions and their complete and complex ultrastructure, we found no reference in the bibliography consulted to any *junctional complex* as presented in this paper, and which we classify as a variation or *subtype of adhering junction.*

Materials and methods

Normal, adult domestic cats were perfused with glutaraldehyde. Following careful dissection, the diencephalon was removed and subsequently cut into pieces at the level of the third ventricle, submerged in buffer-sucrose and microdissected through a binocular magnifying glass. Small 1 mm^3 cubes of nerve tissue were obtained, one side of which was the ependymal epithelium of the third ventricle. These cubes were postfixed in 3% osmium tetroxide for 2 hours, embedded in araldite, and oriented so that the ependymal epithelium could be cut perpendicularly.

In some cases, to obtain a perfectly perpendicular cut, several successive reinclusions had to be made (González Santander, 1969). Ultrafine sections were made on a Reichert Om-U2-ultramicrotome, stained via Reynold's method (1963) and observed under the transmission electron microscope.

Results

At the intercellular junctions of the ependymocytes Fig. 1) and in the area closest to the ependymal space, there is a group of structures associated with the *intercellular junctional complexes.* These are notable by virtue of the apposition of separate groups of mitochondria, forming mitochondrial plates on both sides of the intercellular junctions. They are always at the same distance, leaving a limiting cellular space with a constant amplitude.

These are lateral and apical intercellular junctions between the ependymocytes, which have several *adhering junctions* (seven in Fig. 2) and which are evenly distributed, alternating with slightly dilated, intercellular spaces. Separate strings of mitochondria (which vary in number) are situated on both sides but they tend to mate at the level of each of the adhering junctions. These mitochondria are usually rounded although occasionally some may be elongated. This string-like layout of the mitochondria, situated as mirror images on both sides of the intercellular space, creates *a cellular space* in the form of separate corridors (Fig. 3c). In the outermost part of both corridors there are some intracytoplasmic *free condensations* (Fig. 3 j,f,c) situated in front of the mitochondria and almost joined to these (*juxtamitochondrial, free condensations*).

Description of the structures included in these junction complexes with mitochondrial reinforcement. The group is in fact a *complex* due to the fact that there are several *junctions* as well as several mitochondria and *juxtamitochondrial free condensations*, which provide the group with a fairly complex *structural unity* (Fig. 3).

The central plane of the complex is marked by the *intercellular space* which is nearly always arrenged perpendiculary to the ependymal space. This central plane has an amplitude (measured perpendicularly to the ependymal space) of approximately 2 μm. On both sides of this central plane, which passes through the intercellular space, are situated all other structures, approaching (as far as their layout is concerned) a *bilateral symmetry.* Along the axis perpendicular to the ependymal space which passes through the central plane are the *adhering junctions* which vary in number from five to ten (7-8 is the most frequent number), separated by small, intercellular spaces of a greater amplitude (Fig.3).

1.— *The cell membrane* of the intercellular space is similar to the rest of the cell membrane of the ependymocyte (7.5-8 nm) (Fig. 3).

2.— *Anchoring plates* in apposition are two highly osmiophilic condensations, in the form of reinforcement plates, which adhere to the internal lamina of the cell membrane. There are two (bilateral) plates, arranged in parallel at the sides of the intercellular space, separated by a *cleft* of about 15 nm which delimit the external laminae of the cell membranes of the two ependymocytes in contact. Each plate forms a single unit with the cell membrane, has a constant thickness (of 0.15 μm) and a varying, bilateral, coupling amplitude (between 0.07 and 0.20 μm). These plates have two surfaces: a smoother *external or free surface* which faces the intercellular cleft,

Fig. 1. Electron micrograph of the ependymal epithelium of the third ventricle of the domestic cat. Note the more apical section of part of two ependymocytes joined by a junctional complex. In the ependymal space (L), cilia (ci) and microvellosities (mv) can be seen. In the junctional complex there are several adhering junctions (bordered by two arrows) flanked by two paramitochondrial, bilateral corridors (c) and a plate of aligned mitochondria (M). × 5,000

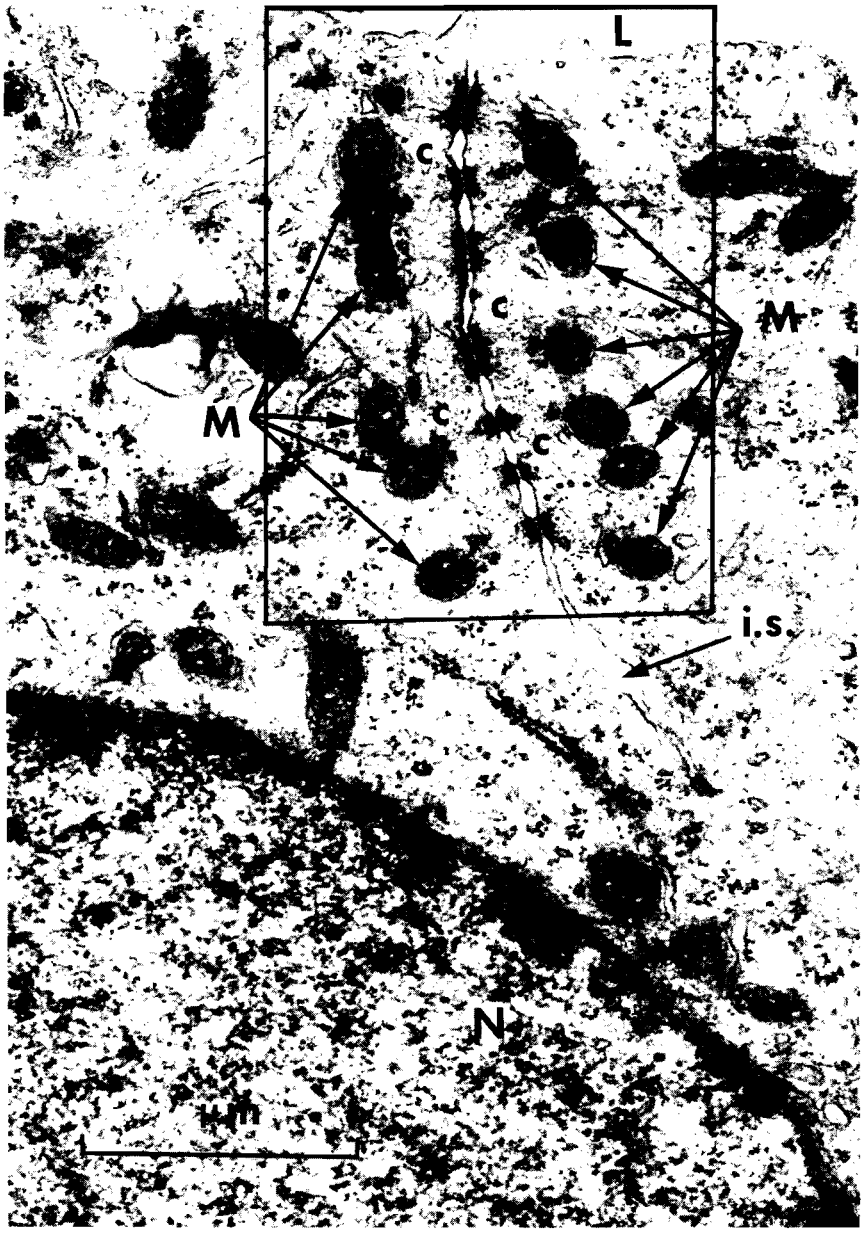


Fig. 2. Electron micrograph of the intercellular limit between two ependymocytes covering the space (L) of the third ventricle of the domestic cat in which the two mitochondria (M) plates can be clearly seen, almost bilaterally symmetrical, at the sides of the intercellular space (i.s.) and the even nature of the amplitude of the paramitochondrial, bilateral corridor (c) separating both structures. The nucleus (N) of one of the two cells can be seen in the bottom left-hand corner. × 5,000

where the interconnecting filaments are fixed; and another rougher *internal or cytoplasmic surface* in contact with the cytoplasm where the lateral filaments are anchored. By virtue of this dual characteristic, we call it *anchoring plate* (Figs 3-6).

3.— *Interconnecting filaments* cross the intercellular cleft perpendicularly, fixing on to the external or free surfaces of the two anchoring plates in apposition, through the cell membranes covering them. They are about 15 nm long and 5-7 nm thick. The separating space between the filaments ranges between 10 and 30 nm (Fig. 3).

4.— *Intercellular, cohesion material* is a homogeneous material of low density which appears in the cleft, where interconnecting filaments cannot be seen or between these when they are widely separated. These are light condensations similar to the *glycocalyx*. This material is observed only at the level of the *adhering junctions*, i.e. between the anchoring plates which delimit the clefts (Fig. 3).

5.— *Bilateral mitochondria.* Mitochondrial plates. The mitochondria are generally rounded, with a diameter of 0.25 µm., although they may sometimes be lenghtened (0.25 µm in diameter by 0.70 µm in length) with their long axis situated parallel to the central plane of the *junction complex*. The mitochondria have very numerous crests and a highly dense mitochondrial matrix, giving them a compact appearance. Their situation is characteristic as they form a group of 4 to 8 (6 being the most frequent number) bilaterally aligned mitochondria thereby forming as a whole two *mitochondrial plates* which are parallel to each other and flank the

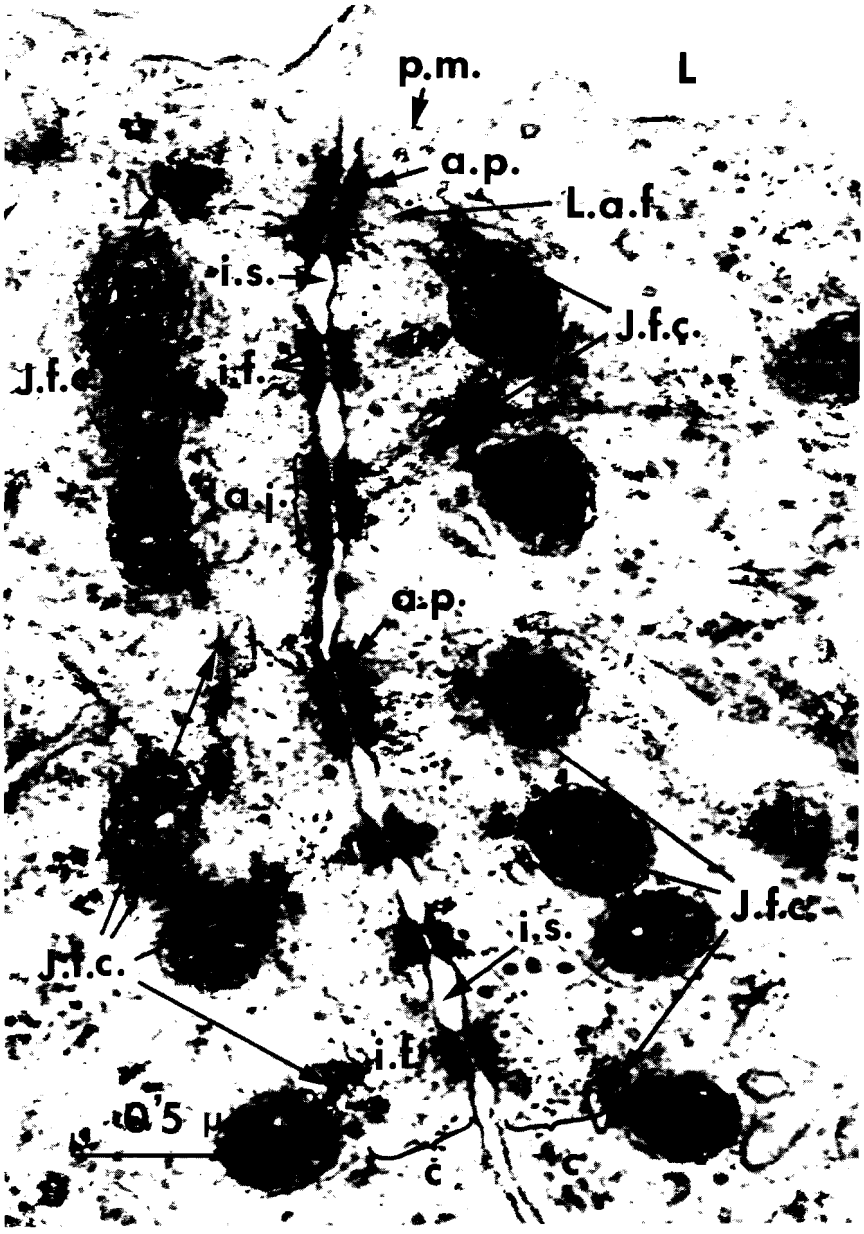


Fig. 3. Magnification of the above figure, showing the inset and in which the following are visible: the ependymal space (L), the plasmatic membrane (p.m.) of the two ependymocites, the intercellular space (i.s) which is the central plane of the junctional complex, seven adhering junctions (a.j.) with several interconnecting filaments (i.f.), anchoring plates (a.p.) lateral, anchoring filamentes (l.a.f.), amplitude of the paramitochondrial, bilateral corridor (c), two parallel plates of mitochondria (M) and two parallel groups of juxtamitochondrial, free condensations (j.f.c.) × 9,200

junction complex on both sides leaving a distance between them of approximately 0.25-0.30 μm. Each of the mitochondria and of bilateral pairs, tend to be linked (and more or less on the same level) to the *anchoring plates*. Sometimes a round mitochondrium may appear to be at the level of the space separating two *anchoring plates*. On these occasions an oblong mitochondrium may cover the large level of two anchoring plates or more. (Fig.3).

6.— *Paramitochondrial, bilateral corridor.* This is an oblong, cellular space, 0.23 μm wide and 2 μm long, which covers the entire length of the junction complex, on both sides of its central plane so that these are two (which are bilateral) delimited by the group of mitochondria (*mitochondrial plates*) on one side and by the group of *anchoring plates* on the other side. The most characteristic feature of the corridor is its constant amplitude. The cytoplasm which includes this corridor contains very variable structures in arrangement, morphology, number and density: juxtamitochondrial, free condensations, homogeneous granular material and lateral, anchoring filaments. (Figs 3,5, 6 c).

7.— *Juxtamitochondrial, free condensations.* These are free, cytoplasmic condensations, situated in the paramitochondrial corridor, precisely beside each of the mitochondria (with some exceptions), in front of these. They are highly osmiophilic, electrondense, irregularly-shaped condensations with uneven edges but which tend to have a slightly oblong shape, parallel to the central plane of the *junctional complex*. They vary in thickness between 30 and 45 nm. Their lengthvaries in relation to the size of the mitochondria, beside which they are situated

Figs. 4-6 Electron micrographs of the ependymal epithelium of the third ventricle of the domestic cat, showing three junctional complexes with mitochondrial reinforcement, where separate, clear, limiting spaces are obvious as paramitochondrial, bilateral corridors with a constant amplitude (c) and the large number of juxtamitochondrial, free condensations (j.f.c.) which form two, almost continous, parallel lines, *juxtamitochondrial condensation lines* (j.c.l.) (indicated by thick arrows). Beyond these very large mitochondria (M) are partially visible. Large anchoring plates (a.p.), interconnecting filaments (i.f.) and the ependymal space (L) can also be seen. — Fig. 4 × 9,200 Figs. 5 & 6 × 5,000

(juxtamitochondrial). When they are situated beside a round mitochondrium, the corresponding condensation tends to be rounded or slightly oval-shaped; and when they are situated beside oblong-shaped mitochondria, the condensation is oblong-shaped and its longitudinal axis may reach 0.20 - 0.40 µm. These oblong-shaped, juxtamitochondrial, free condensations may sometimes be seen to approach each other tending to form a *beaded or continuous, linear condensation*, like a *juxtamitochondrial, condensation line*. In these cases two parallel, bilateral lines are formed, marking the lateral or external edge of each of the *paramitochondrial bilateral corridors*. (Figs. 3-5, 6 j,f,c)

8. — *Homogeneous, granular material.* This is a light, granular material which is only found in some places of the paramitochondrial, bilateral corridor, which is mainly situated beside the internal surface of the *anchoring plates* and to a lesser extent around the *juxtamitochondrial, free condensations* and to an even lesser extent in the space of the *corridor* which joins the two previous structure crosswise (Fig. 3).

9. — *Lateral, anchoring filaments* (transversal). These are a series of fine filaments, between 4 and 5 nm in thickness, which are anchored to the internal surface of the *anchoring plates*, transversally crossing the *corridor* in the direction of the *juxtamitochondrial, free condensation* but without reaching it. At the anchoring level, they are found among the *homogeneous granular material* and by their form of insertion they are *terminal* filaments. They are usually interrupted at various points in their course and therefore they are usually discontinuous (Fig. 3, 1, a, f).

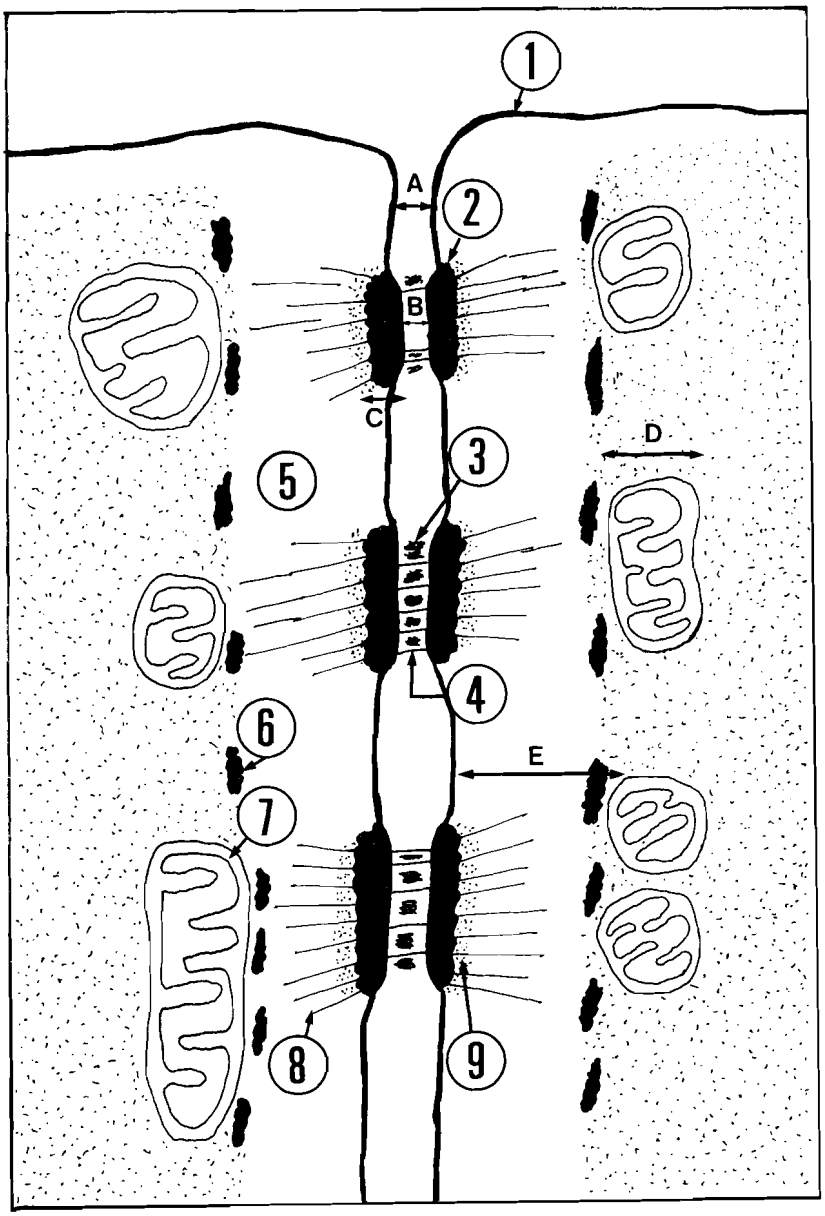
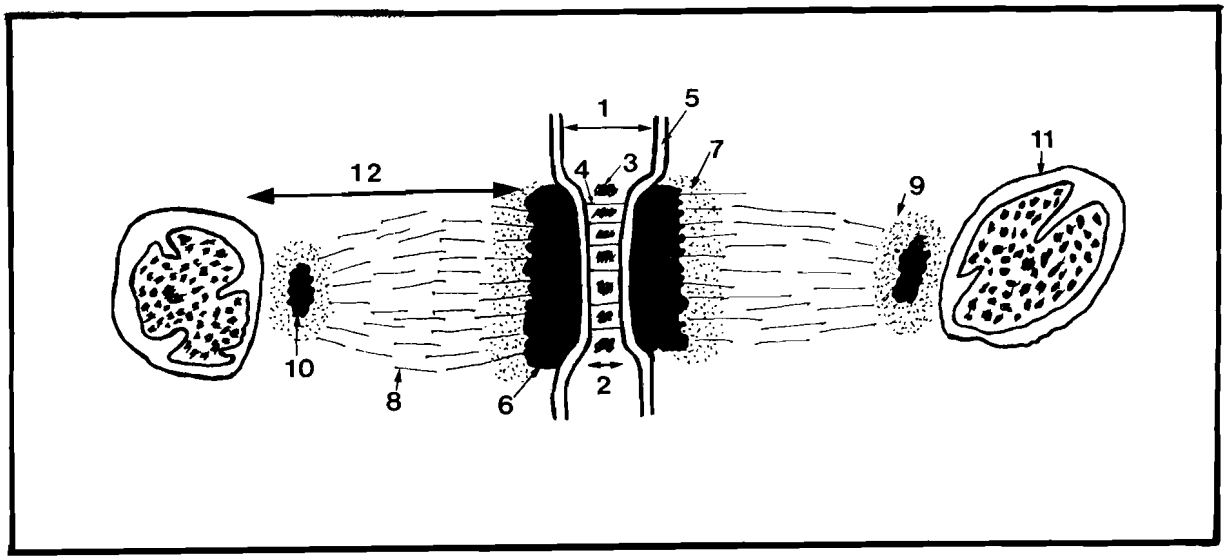


Fig.7. Diagrammatic representation of the adhering junctional complexes with mitochondrial reinforcement. 1.— Cell membrane. 2.— Anchoring plate. 3.— Intercellular cohesion material. 4.— Interconnecting filaments. 5.— Paramitochondrial, bilateral corridor. 6.— Juxtamitochondrial, free condensations. 7.— Bilateral mitochondria. 8.— Lateral, anchoring filaments. 9.— Homogeneous, granular material. A: Intercellular space, (45-60 nm), B: Cleft in the adhering junction, (15 nm). C: Thickness of the anchoring plate (0.15 μm). D: Thickness of the mitochondria (0.25 μm). E: Amplitude of the paramitochondrial, bilateral corridor (0.23 μm).

Fig. 8. Diagrammatic representation of the *functional unit* of the adhering junctional complex, indicating its structure and dimenstions: 1.— Intercellular space (45-60 nm). 2.— Adhering cleft (15 nm). 3.— Intercellular, cohesion material. 4.— Interconnecting filaments (15 nm by 5-7 nm). 5.— Plasmatic membrane (7.5-8 nm) (bilateral). 6.— Anchoring plate (0.15 μm by 0.07-0.20 μm) (bilateral). 7.— Homogeneous, granular material (bilateral). 8.— Lateral, anchoring filaments (4-5nm) (bilateral). 9.— Homogeneous, granular material (bilateral). 10.— Juxtamitochondrial, free condensation (bilateral). 11.— Mitochondrium (0.25 μm) (bilateral). 12.— Functional space or field of attracting forces in dynamic balance (0.23 μm) (bilateral).



Discussion

The intercellular junctions which are described in this paper are of the *adhering junctional* type and are *complex* structures because they include a variety of ultrastructures and are connected in a complex way, by virtue of their number (there are several units, usually seven) and also their layout. They are not occluding junctions because there is no fusion of the cell membranes and there appears an intracellular cleft. They are not desmosomic junctions because they have no dense central plate in the cleft nor fork-shaped filaments in the lateral plates.

These complexes have several units of adhering junctions on both sides of which there are strings of mitochondria (Fig. 7, «7») which unite at the level of each of the adhering junctions. The mitochondrial strings, symmetrically situated on both sides of the intercellular cleft, leave a paramitochondrial, bilateral corridor with a constant amplitude of 0.23 μm (Fig. 7, «E»). In the outermost part of both corridors there are some *juxtamitochondrial, free condensations* (Fig. 7, «6»).

Fig. 7 contains a diagram of these *adhering junctional complexes with mitochondrial reinforcements* which shows the following new structures of these complexes: interconnecting filaments (4), paramitochondrial, bilateral corridor (5), with its amplitude of 0,23 μm («E») the juxtamitochondrial, free condensations (6), bilateral mitochondria (7) with a thickness of 0.25 μm («D») and the homogeneous, granular material (9). It also shows the known structures in the adhering junctions the cell membrane (1), anchoring plates (2), intercellular, cohesion material (3), lateral, anchoring filaments (8), the intercellular space of 45-60 nm (A), the adhering junction cleft of 15 nm («B») and the thickness of the anchoring plate of 0.15 μm («C»).

The functional interpretation of these *adhering junctional complexes with mitochondrial reinforcement* suggest to us a *hypothesis* for interpreting such a complex ultrastructure, which would explain the bilateral symmetry of the mitochondrial arrangement and of the juxtamitochondrial, free condensations with their surrounding, homogeneous material and, in particular, would explain the constant amplitude of the paramitochondrial, bilateral corridor. Could we consider the possibility of intercellular junctions maintained by the energy provided by the mitochondria in a balance of forces where the molecular order produced by the mitochondrial energy would be responsible for the visualization of the interconnecting filaments and the lateral, anchoring filaments?

The explanation of this hypothesis would involve a coupling of mitochondrial energy which would define them as energetic, adhering junctions with coupling by dynamic balance. The molecular, ultrastructural order by static or electrostatic forces generated in the mitochondria might suggest the name of *high-energy, adhering junctional complexes* (or with mitochondrial coupling). That is to say, all the energy is required which in bioenergetics is necessary to maintain the connection of the cells of the ependymal epithelium in the area closest to the ventricular space and provide an effective barrier to the cephalorachydian liquid.

To support this hypothesis and the name of *high-energy, adhering junctional complexes* we could mention the following facts already accepted by most authors: the distribution of the mitochondria in the cytoplasm must be considered in relation to its energy-providing function; the mitochondria are constantly located near the region where the energy (ATP) is supposedly most necessary, as shown by the presence of succino-dehydrogenase activity (Chason and Pearse, 1961); in the ependymal epithelium the mitochondria accumulate near the apical portion, where the intercellular junctions are laterally situated; the number of intercellular junctions is related to their functional capacity. On the other hand, it was already known that the intercellular junctions transmit active forces generated within the cells (by means of the cytoskeleton) as an active response to certain functional situations, i.e. that they transmit the intrinsic mechanical energy generated by the actual cells and that these are places were extrinsic, mechanical forces of traction and pressure are transmitted.

As shown in Figure 7, each *adhering junctional complex* may be considered to be formed by several units (3 in the case of Figure 7), which may be called *functional units* of the high-energy, adhering junctional complex. To explain this, the diagram in Figure 8 shows a *functional unit* with all the elements of which it is composed and their respective dimensions: the adhering cleft of 15 nm (2), with the *intercellular cohesion material* (3) between interconnecting filaments of 5-7 nm diameter (4); the cell membrane of 7.5-8 nm (5) the internal lamina of which is fused to the *anchroing plate* (6) of 0.07-0.20 μm thickness; the *homogeneous, granular material* (7) at both ends of the functional space or field of attracting forces in dynamic balance, with a constant amplitude of 0.23 μm (12); the *lateral, anchoring filaments* of 4-5 nm thickness (8); the *juxtamitochondrial, free condensations (10);* and the *mitochondria* with their dense matrix (11).

The *functional unit of the high energy, adhering junctional complex* is formed by the coupling of two, equidistant mitochondria on either side of the intercellular cleft, which provide the energy (ATP) necessary to produce separate fields of force which longitudinally guide the molecules giving rise to discontinous, *anchoring filaments.* When they are still disorientated, these molecules concentrate around the *juxtamitochondrial, free condensation* and the *anchoring plate,* giving rise to the *homogeneous, granular material. The anchoring filaments* (by virtue of their arrangement and thickness) could be similar to the F-actin ones shown for other cells of the column epithelium (Ishikawa et al., 1969; Tilney and Mooseker, 1971). According to Pollard and Korn, (1971), the F-actin filaments combine with heavy meromyosin in the presence of ATP in cytoplasmic motility. Likewise, the high density of the mitochondrial matrix may be assumed to be due to a higher concentration

of ATP, calcium and other cations within the mitochondrium.

The fact that the mitochondria concentrate in the apical part of the ependymocytes (Brightman and Palay, 1963) means that when a more effective barrier to the cephalorachydian liquid is required, they may be coupled to the adhering junctions to provide the high energy required for the greater sealing, i.e. these high-energy, adhering junctions would be formed to meet special, functional requirements, would be temporary rather than permanent, according to the general facts demonstrated by the microcinematography which shows that the cells do not establish permanent links but merely temporary connections.

To sum up: *the high-energy, adhering junctional complexes* or with *mitochondrial coupling*, may be a subtype of the adhering junctions, where the bioenergetics generate electrostatic forces to maintain the intercellular cohesion more effectively and more powerfully, even though this may be of a temporary nature in order to meet, special, functional requirements.

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