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Paracrystalline Arrays in Mitochondria

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I. *Introduction*

Electron micrographs of four very striking instances of mitochondrial paracrystalline array patterns are shown in Figs. 1–4. The "Star of David" pattern has been found in the *in situ* mitochondria of the jumping muscle of the migratory locust[1] (Fig. 1); patterns which appear to be "helical structures" have been found in the *in situ* mitochondria of both the root tip cells of the mung bean *Phaseolus vulgaris*[2], as well as in the *in situ* mitochondria of guinea pig liver[3] (Figs. 2 and 3, respectively); and a pattern of triangles surrounded by "structured areas" has been found in the *in situ* mitochondria from the astrocyte of the Syrian hamster[4] (Fig. 4). When considered as examples of mitochondrial paracrystalline array patterns, such patterns are seemingly so far removed from the "normal" morphology seen in mitochondria, that they make it quite understandable why no general picture for describing mitochondrial paracrystalline array patterns has as yet been forthcoming.

At least one hundred papers have been published in the past five years which contain descriptions of unusual patterns within the mitochondrial interior. The designation "paracrystalline arrays" has been useful as an umbrella term to cover the full gamut of these ultrastructural patterns. The rather extensive literature concerning these paracrystalline arrays has been partially reviewed by Suzuki and Mastafi[5] and Mugaini,[6] as well as treated in a general way by Lehninger.[7] Explanations of the patterns have generally been sought in terms of non-mitochondrial entities such as inclusion bodies,[8] DNA,[9] ribosomes,[10] and microtubules,[11] which in some way form separate domains within the mitochondrion after invading the mitochondrion in some unexplained fashion. André[12] has been among the observers who have not excluded the possibility that these paracrystalline array patterns arise from the mitochondrial membranes. In

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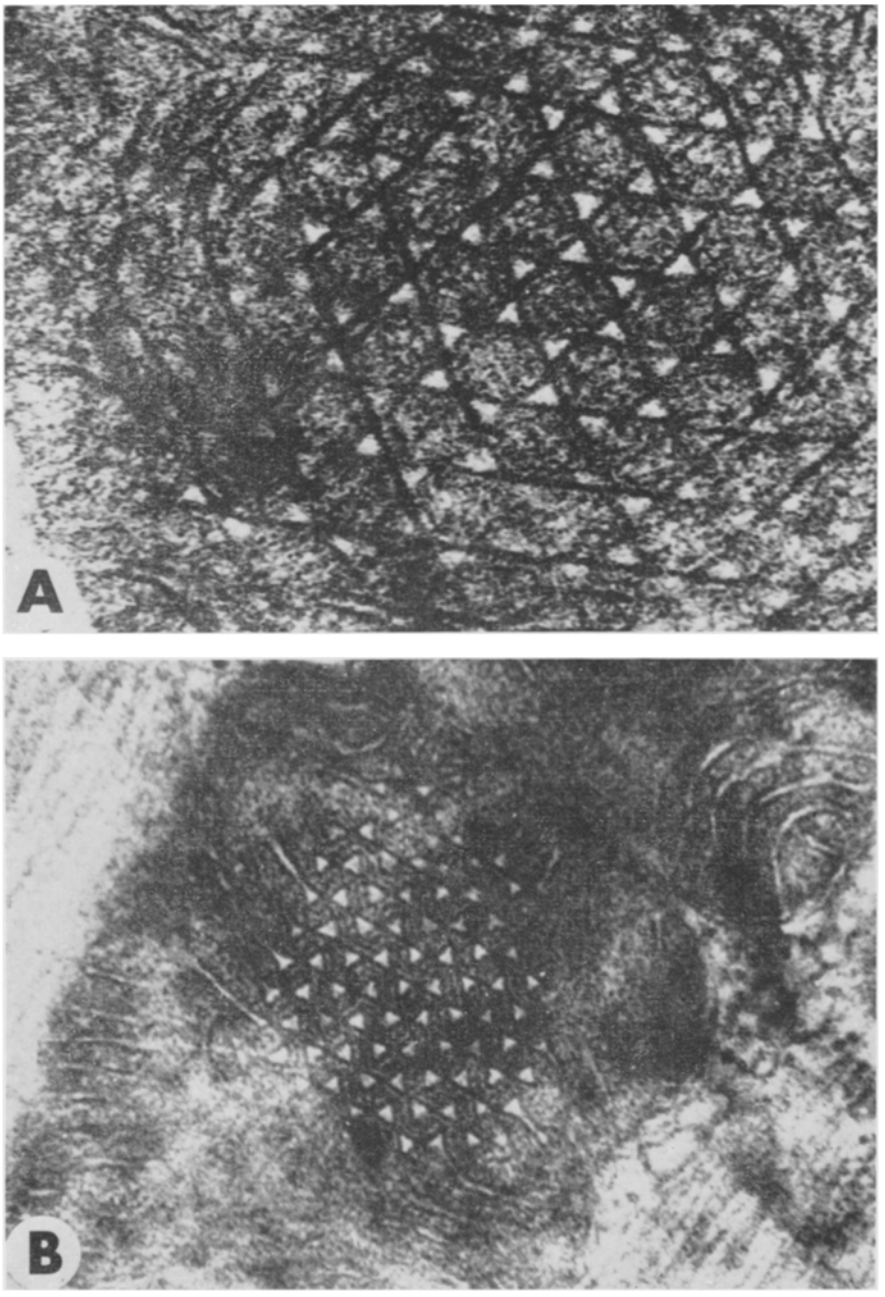

Figure 1. Electron micrographs of regions of a sectioned mitochondrion from the jumping muscle of the migratory locust *in situ* (1). (A) The paracrystalline array patterns in the form of "Stars of David". (B) The paracrystalline array patterns in the form of "alternating triangles".

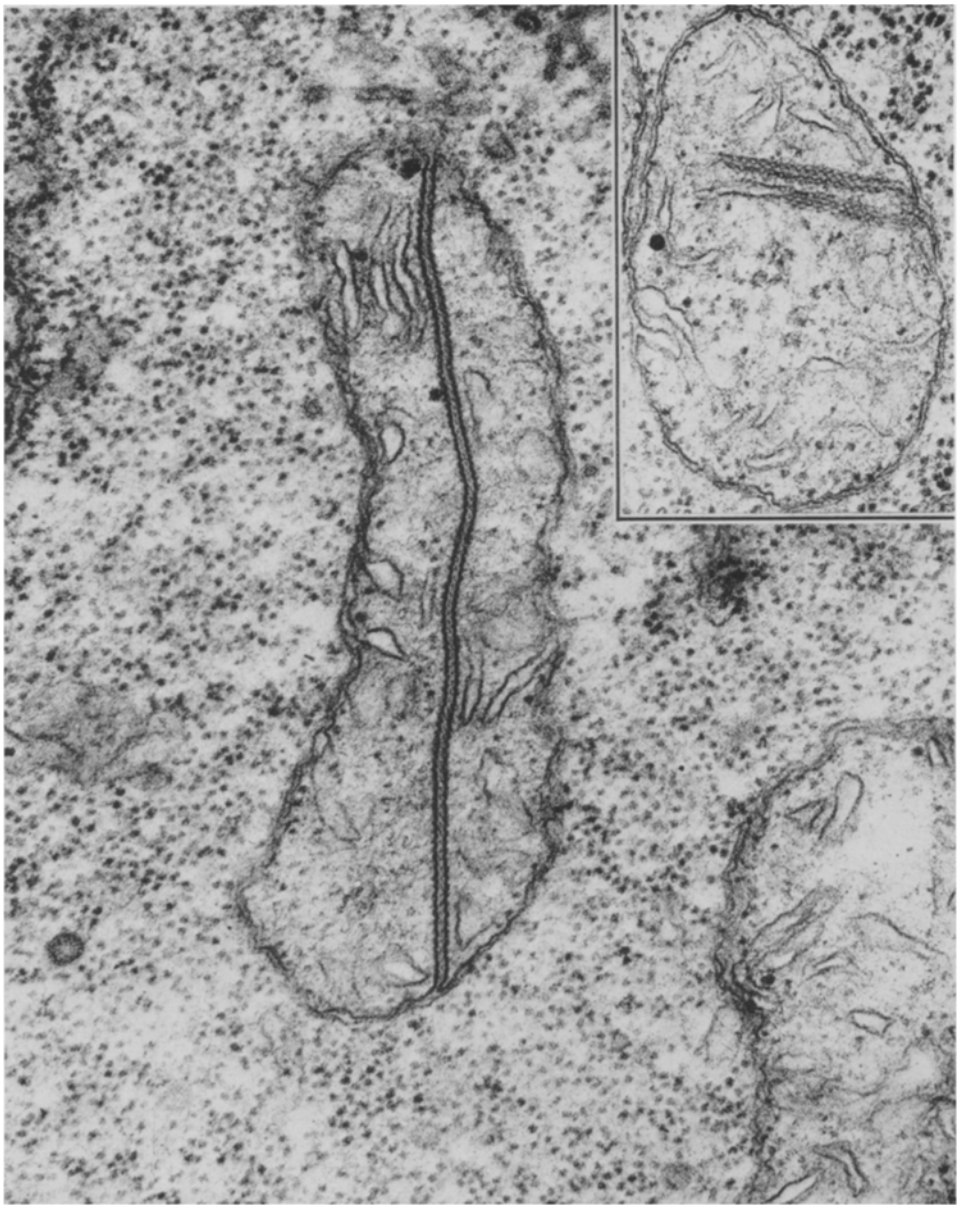

Figure 2. An electron micrograph of a sectioned mitochondrion from the root tip of the mung bean *Phaseolus vulgaris in situ.*[7] The paracrystalline array pattern is in the form of a long "helical structure". Insert: a mitochondrion with several "cross-hatched" paracrystalline array patterns. ×100,000.

addition, Hackenbrock[13] has discussed evidence for ordered structures in the mitochondrial matrix space.

Figures 1–4 have purposely been chosen from the vast number of examples of mitochondrial paracrystalline array patterns because they are not only bizzare and puzzling, but because they graphically illustrate the underlying principles of a general picture

which we have deduced applies to certain mitochondrial paracrystalline array patterns, and which we will develop in this communication. The impetus for the development of that picture came from the fact that in mitochondria from a wide variety of tissue sources, all normal morphologies can be rationalized as distortions of the mitochondrial inner membrane during the energy cycle.[14] These distortions occur without disruption of the mitochondrial inner membrane as a surface continuum, i.e., without tearing or shredding the membrane or puncturing holes through it. The success in explaining normal mito-

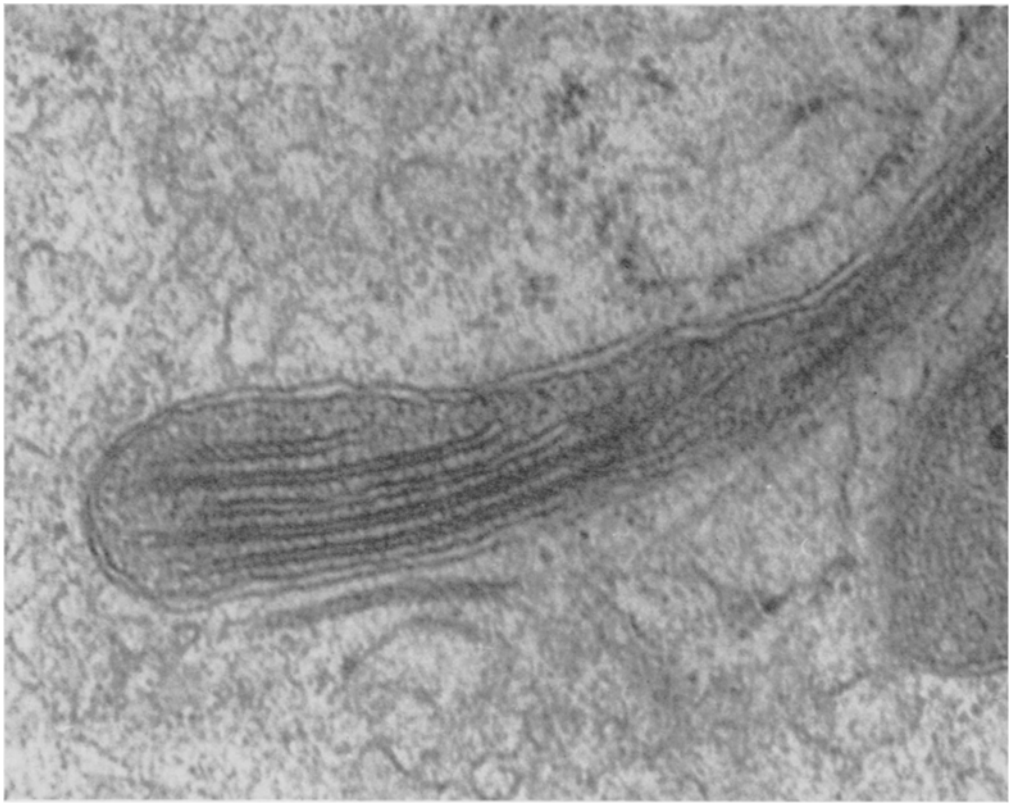

Figure 3. An electron micrograph of a sectioned mitochondrion from guinea pig liver *in situ*. The paracrystalline array pattern is in the form of long "helical structures". ×180,000.

chondrial morphology in these terms led us to consider the possibility that at least some of the mitochondrial paracrystalline array patterns could be similarly rationalized. By this we mean that every possible explanation involving inherent mitochondrial structures and morphologies should be carefully examined before invoking non-mitochondrial entities to explain the paracrystalline array patterns. This tactic can, of course, be more fruitfully pursued now than in the past, primarily because it has been possible since only very recently to rationalize the normal mitochondrial morphologies in understandable terms. With the new insights available to us now, we can attempt to rationalize mitochondrial paracrystalline array patterns in this way. For example, the Star of David

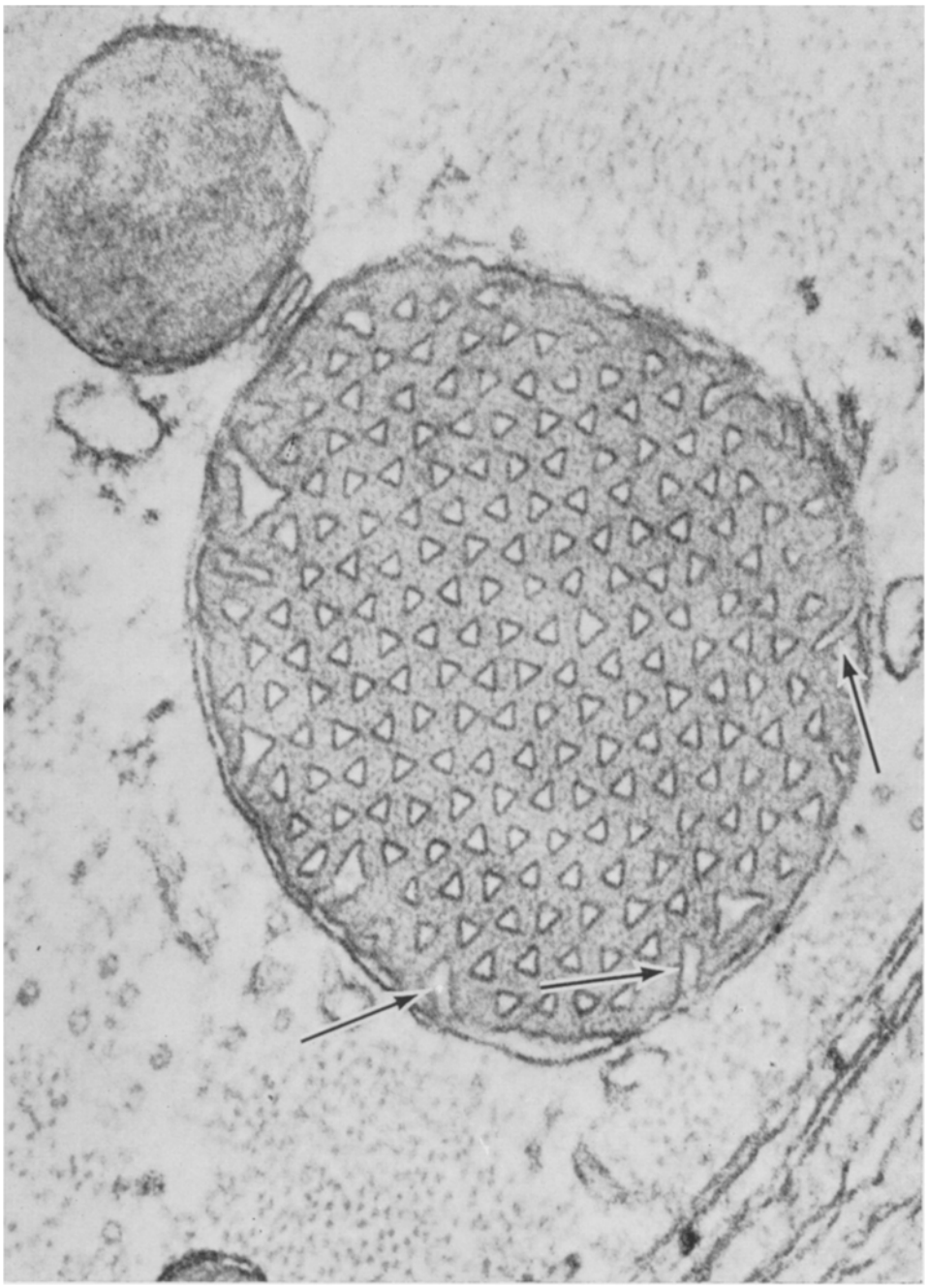

Figure 4. An electron micrograph of a sectioned mitochondrion from a Syrian hamster astrocyte cell *in situ*.[4] Enclosed within an outer and inner membrane, both of normal appearance, are tubular cristae (see arrows), and triangular, dark-walled structures surrounded by "structural material", which gives rise to the paracrystalline array patterns of dark spots. ×120,000.

pattern [Fig. 1(A)] can be fully explained in terms of the mitochondrial inner membrane *per se* in one of its characteristic configurational states.

The mitochondrial inner membrane *per se*, however, explains only some of the mitochondrial paracrystalline array patterns. We therefore looked to other structures within the mitochondrion for their involvement in these patterns. The outer membrane was initially dismissed as a participant because the patterns usually occur within what look like unperturbed outer membranes. We later learned that the outer membrane is a participant in certain paracrystalline array patterns, but its participation is indirect. The only other possible locale of the structures within the mitochondrion which could give rise to the pattern is the intracristal space or the matrix space. These spaces have been thought of in the past not so much as structures, but rather as regions or compartments within the mitochondrion. The possibility that these spaces could contain structures has not been overlooked, however. We now have evidence from heavy beef heart mitochondria, that in certain normal morphologies the intracristal space contains "structured materials" which give rise to paracrystalline array patterns (see Section IV, 2). We will attempt to show by deduction that the paracrystalline array patterns seen in the mitochondria of *Phaseolus vulgaris* and of guinea pig liver arise in an almost identical way.

Since certain paracrystalline array patterns occur as the result of structured material in the intracristal space, we expected that other patterns could arise as the result of structured materials in the matrix space as well. It is almost immediately self-evident that such is the case in the astrocyte mitochondrion.

From this brief description, the outlines of our general picture can be easily discerned. Our thesis is that certain paracrystalline array patterns seen within mitochondria can result from the visualizations of structures inherent to the mitochondrion, namely (a) a structure or structures of the mitochondrial inner membrane *per se*, or (b) structured materials within one or both of the mitochondrial compartments. These two categories are not mutually exclusive. That is, the mitochondrial inner membrane, because of its multiplicity of morphological states, cannot only assume forms which are themselves paracrystalline array patterns, but can influence the formation of patterns in the mitochondrial compartments. It can do so by modulating the relative volumes of these compartments, thereby modulating the form or structure of their contents. The outer membrane can take part indirectly by acting as a boundary within which paracrystalline array patterns can form in the intracristal space.

The general picture outlined here, of course, constitutes a lesson in the "geography" of the paracrystalline array patterns. The details of this lesson require prior study of normal mitochondrial morphology to decide even such a simple question as which compartment contains a particular paracrystalline array pattern. We can now make some fairly firm assignments. The more intriguing, but far more difficult to answer, questions about the nature and function of the paracrystalline array patterns can now be entertained.

II. *Topological Parameters*

We shall commence our study of mitochondrial paracrystalline array patterns by first briefly commenting on the mitochondrial inner membrane as a membrane continuum

per se. It has been deduced that the mitochondrial inner membrane can be treated as a topological surface within certain boundary conditions. First of all, all mitochondrial inner membranes can be considered as topologically identical surfaces. Secondly, they can all be considered as members of the topological genus zero, i.e., topologically equivalent to a hollow sphere. Thirdly, all mitochondrial inner membranes can be considered as having constant topology. A complete treatment of this topic has been given by Korman *et al.*[14]

III. *The Energy Cycle and Configurational States*

A correlation between the paracrystalline array patterns and the mitochondrial inner membrane considered as surface continuum *per se* also requires a clear understanding of the various configurations of that membrane as the configurations relate to the energy cycle. The energy cycle is that sequence of events in which the mitochondrial inner membrane is first put into the energized state by the oxidation of substrate in the presence of oxygen or by the hydrolysis of ATP, and is then discharged back to the nonenergized state by a work performance or by an uncoupler. It has been demonstrated in our laboratory that the various configurations of the mitochondrial inner membrane bear a one-to-one relationship to the energy cycle in non-steady state conditions.[15–18] The morphological details of the mitochondrial inner membrane in relation to the energy cycle have been described in connection with the development of a unified model which satisfies the topological boundary conditions outlined above in Section II.[14]

IV. *The Rationalization of the Paracrystalline Array Patterns*

We shall now present our rationalization of the structures of the various paracrystalline array patterns shown in Figs. 1–4. In the process we shall formulate our general picture of these patterns.

1. *The Paracrystalline Array Patterns in Mitochondria of the Locust; The Star of David Pattern*

The Star of David seen in the *in situ* mitochondria of the jumping muscle of the migratory locust can be briefly described as consisting of hexagonal electron dense areas, at the six sides of which are electron-transparent equilateral triangular areas. A whole field of these patterns shows the hexagons touching each other at their vertices, but they never share a common side [see Fig. 1(A)].

Our rationalization of the Star of David pattern derives from the work of Williams *et al.*[19] in our laboratory on rat heart mitochondria *in situ*. In that tissue, under conditions which lead to the energized (twisted) configuration, i.e., under aerobic energizing conditions in the presence of inorgainc phosphate, a Star of David pattern was observed [See Fig. 5(A)]. We deduce that the Star of David pattern arises in rat heart mitochondria as the result of the longitudinal hexagonal parallel packing together of tubular cristae having uniform circular cross-sections. Since the energized (twisted) configuration has previously been shown to be a configuration associated with a tubularization of the mitochondrial inner membrane,[14, 15] such a rationalization is quite reasonable. The Star of David pattern results from the geometric fact that a circle (the cross-section of a right circular cylindrical tube) with a given diameter can be tangent to, at most, six

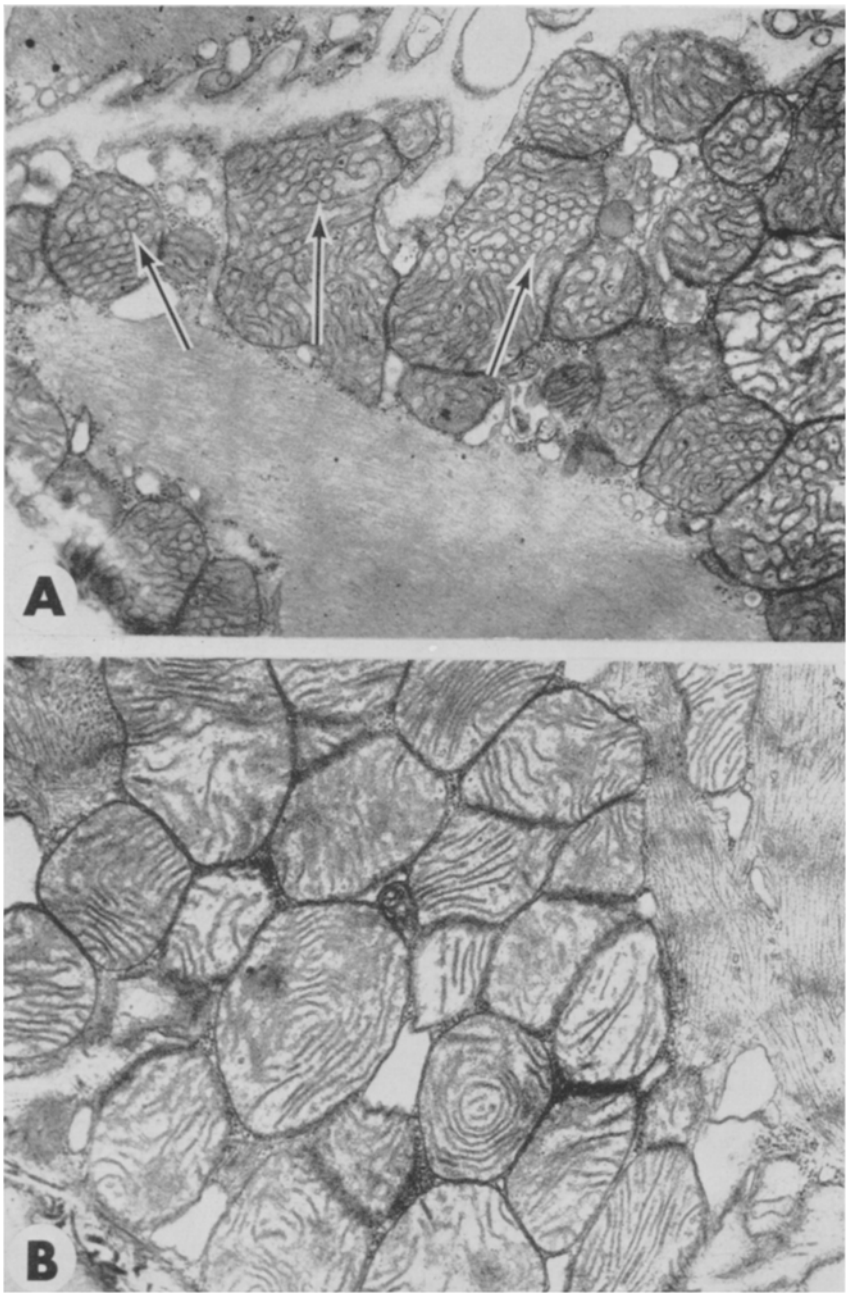

Figure 5. Electron micrographs of rat heart mitochondria *ir situ*.[6] (A) Rat heart mitochondria *in situ* under energizing conditions which lead to the energized (twisted) configuration. The sectioning of large-diameter tubular cristae which are packed hexagonally gives rise to the Star of David paracrystalline array patterns (see arrow). (B) Rat heart mitochondria *in situ* uncoupled with dinitrophenol. All the mitochondria are in the nonenergized (orthodox) configuration. ×30,000.

other circles (cross-sections of other right circular cylindrical tubes) with equivalent diameters. This fact is illustrated in Fig. 6. A cross-section of a set of such tubes cut perpendicular to the longitudinal axis of the tubes looks like a Star of David, except for the difference that the electron-transparent equilateral triangular spaces seen in the mitochondria of the jumping muscle of the migratory locust are slightly modified. In rat heart mitochondria, those spaces are really tricuspid* in shape. These tricuspid-shaped areas are the regions enclosed by the arcs of three circular cross-sections of tubular cristae which are mutually tangent. When the tubes are physically widely separated from one another, one sees only many circular cross-sections of the tubular cristae but no Star of David pattern. When the tubes begin to physically approach each other, a Star of David pattern begins to be discernible in cross-section, and when tubular cristae are actually touching, the Star of David pattern in cross-section is unmistakable.

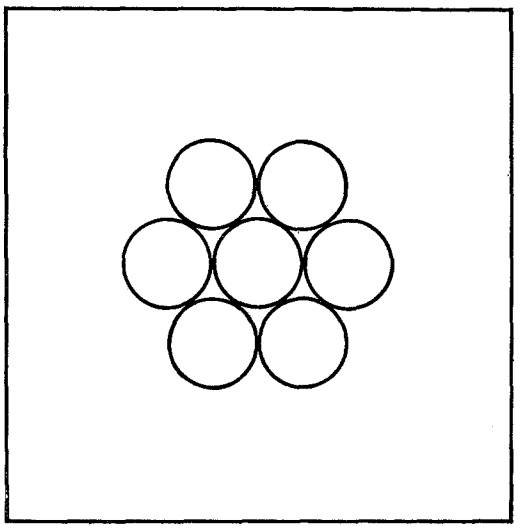

Figure 6. A diagrammatic representation of a cross-section cut perpendicular to the longitudinal axes of a set of right circular cylinders in closest hexagonal packing. Note the Star of David pattern and tricuspid areas.

By analogy with the explanation of the Star of David pattern in rat heart mitochondria, the Star of David pattern in the mitochondria of the jumping muscle of the migratory locust is deduced by us to result from the sectioning of the mitochondrial inner membrane in the energized (twisted) configuration. The cristae are visualized as tubes with hexagonal cross-sections, with headpiece-stalk sectors oriented inward into the lumens of the tubes (the matrix space), and lining the length of the tubes. The lumens of the hexagonal tubes contain not only the headpiece-stalk sectors but matrix protein as well, and are electron-dense in electron microscopy. The electron-transparent triangular spaces are interpreted as the intracristal space.

In addition to proposing that the mitochondrial inner membrane of the *in situ* mitochondria of the jumping muscle of the migratory locust are in the energized (twisted) configuration with tubes having hexagonal cross-sections, we also suggest that the tubes are corkscrew or coil-spring in form. The corkscrew form of the tubes can be thought of as resulting from a hexagonal tube wrapping itself around a right circular cylinder (see Fig. 7). This corkscrew form of the tubes with hexagonal cross-sections packed in parallel hexagonal arrays is offered to allow us not only to explain the Star of David pattern, but simultaneously to explain another pattern which occurs in these same mitochondria [see Fig. 1(B)]. Here we see what look like numerous densely stained twisted tubes, all lying in the same direction and with triangular electron-transparent spaces. Along any

* There are two possible kinds of tricuspid-shaped areas, one shaped like a shamrock, the other shaped like an equilateral triangle. This latter type, with three concave arcs in place of three straight lines for the three sides of the triangle, is the shape to which we refer.

pair of twisted tubes, these triangular spaces are oriented alternately with one of their vertices pointing first up then down, if the longitudinal direction of the tubes is considered horizontal. The twisted tubes appear to be filled with a row of darkly staining dots, which we interpret are the headpieces. The corkscrew nature of the tubes explains the origin of the triangular spaces which point in alternating directions. They arise from viewing a section or cut made parallel to the longitudinal direction of the corkscrew tubes and thick enough to contain a corkscrew. The projection of the electron-transparent intracristal spaces between two adjacent corkscrew tubes on a flat plane results in the visualization of a set of equilateral triangular electron-transparent spaces oriented as described above. In the diagram in Fig. 7(B), the tubes are shown in the position of closest packing. We see several tubular corkscrews in a form which is equivalent to the helical wrapping of each identical hexagonal tube around a right circular cylinder, where the cylinders have identical diameters and where all the corkscrews have identical pitch angles of wrapping. Expressed in an equivalent way, the tubes have identical frequencies and wavelengths of wrapping. From the study of models of corkscrew tubes we have learned that when two corkscrews are in the position of closest packing, they do not touch at points all along their length, but touch only at two points per half wavelength (see Fig. 8). A flat projection of what is seen when two such corkscrews touch can give rise to the features seen in Fig. 1(B). A result of such a structure is that the intracristal space is completely continuous, that is, it is not subdivided into long triangular tubular volumes as would be the case if the cristae were hexagonal tubes which were straight rather than helical, and were touching at their vertices all along their lengths. This latter structure, shown diagrammatically in Fig. 9, is probably incorrect, since sectioning such a structure, although it can give rise to the Star of David pattern when sectioned perpendicular to the longitudinal axis of the tubes, could in no way give rise to the pattern seen in Fig. 1(B).

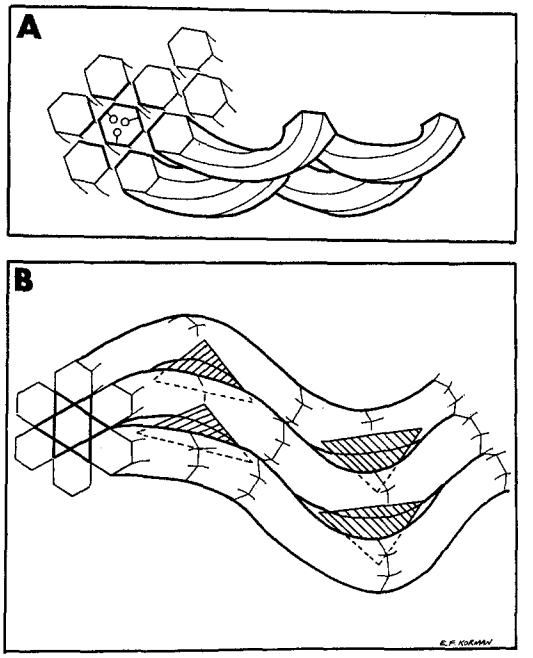

Figure 7. A diagrammatic representation of the interpretation of the Star of David and alternating triangular paracrystalline array patterns. (A) Tubular "corkscrew" cristae with hexagonal cross-sections in close hexagonal packing, vertex to vertex. The heavy lines indicate the edges of cristal membrane giving rise to the Star of David pattern which results when a set of such cristae are sectioned perpendicular to the longitudinal axis of the tubular cristae. The headpiece-stalks indicate the sidedness of the membranes. The interiors of the tubes are the matrix space; the space around the tubes is the intracristal space. (B) The touching of corkscrew cristae at their half wavelengths generates triangular regions in the intracristal space (shaded triangles).

The equating of the Star of David pattern with the energized (twisted) configuration was made on the basis of the pattern seen in rat heart mitochondria *in situ* under conditions which lead to the energized (twisted) configuration. The Star of David pattern

should, therefore, be eliminated by discharging the energized (twisted) configuration. As predicted, there is a total loss of the Star of David pattern when rat heart mitochondria *in situ* in the energized (twisted) configuration are subsequently treated with uncouplers such as dinitrophenol. The configuration is converted to the nonenergized (orthodox) configuration under these conditions [see Fig. 5(B)].

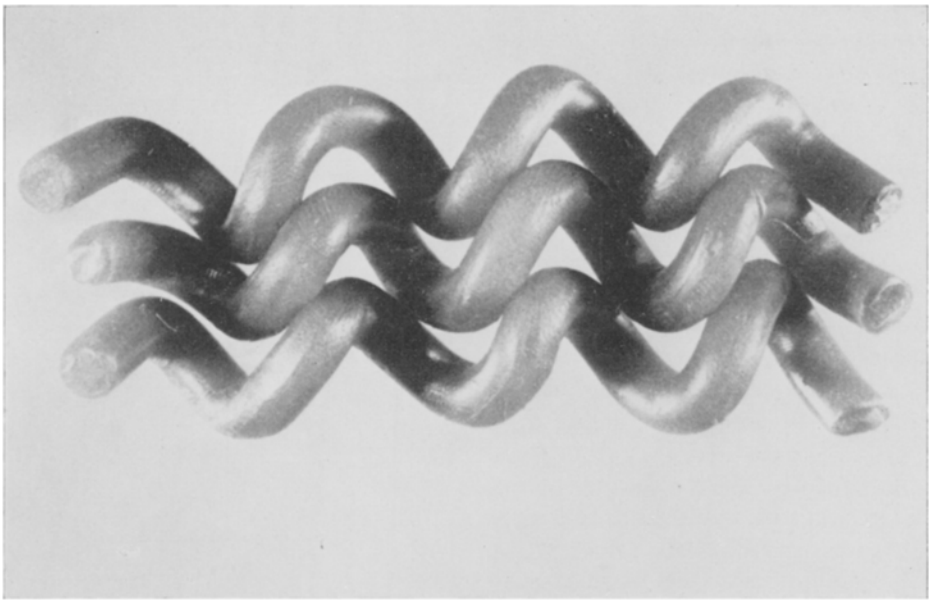

Figure 8. A photograph of a model of corkscrews of identical cross-section, identical wavelength, and identical pitch. The corkscrews touch at half-wavelength points along their length. The view generates triangular regions (projected on the plane of the photograph) which alternately have vertices pointing up, then down.

2. *The Paracrystalline Array Patterns in Phaseolus vulgaris and Guinea Pig Liver Mitochondria*

A rather rare (approximately one mitochondrion out of a thousand) but puzzling paracrystalline array pattern different from the Star of David pattern occurs in the mitochondria of the mung bean root tip and in guinea pig liver (see Figs. 2 and 3, respectively). In both of these mitochondria, we see a very thin long pattern, often stretching from one end of the mitochondrion to the other. The mitochondrion is itself often elongated. Usually what is seen is a pattern of two very long, quite dark lines between which is an "ordered" pattern. The detailed pattern is sometimes not clear when a "cross-hatching" pattern is seen, as shown in the insert in Fig. 2. This latter pattern may be due to either a surface cut of these structures, as was suggested by Newcomb,[2] or possibly an oblique section of the structure.

The interpretation of this pattern proved to be quite a bit more difficult than the rather direct interpretation of the Star of David pattern. Unlike what one might suppose, the structure is not a long tube in a helical form, as we ourselves originally concluded from superficial appearances. Newcomb[2] has deduced that in sections from one and the same mitochondrion possessing these patterns, the structures are probably sheet-like,

and not tubular. Valdivia[3] has deduced a similar conclusion with sections of the mitochondrion from guinea pig liver. This conclusion makes untenable the possibility that this pattern could be the expression of a tubularized energized (twisted) configuration which we originally tried to invoke.

A simple examination of the other regions of the mitochondrion within which these extraordinarily long paracrystalline array patterns are found indicates that they are in the orthodox configuration. In addition, it was found that in guinea pig liver mitochondria the number of such paracrystalline array patterns could be vastly increased by treatment of the whole animal with dinitrophenol. This treatment is known to discharge the energized or energized (twisted) configuration of the mitochondrial inner membrane of isolated mitochondria to a nonenergized configuration, of which the orthodox configuration is one form.[19] These facts were difficult to reconcile with the paracrystalline array pattern, because we could find no simple way to rationalize what we know of the orthodox configuration and its relation to the energy cycle with the paracrystalline array pattern.

The clue for the explanation of these paracrystalline array patterns came from an unexpected direction. A careful examination made by T. Wakabayashi in our laboratory of electron micrographs of heavy beef heart mitochondria in the energized configuration revealed a paracrystalline array pattern unusually similar to the patterns seen in the paracrystalline arrays in question[20] (see Figs. 10 and 11). It is clear that the paracrystalline array patterns seen in the orthodox configurations of the inner membranes of *Phaseolus vulgaris* and guinea pig liver mitochondria have their counterpart in the beef heart mitochondrial patterns, except that in the beef heart case the mitochondrial inner membrane is not in the orthodox configuration but rather in the energized configuration. This fact was very disconcerting, until it was realized that these patterns, despite the differences in the energy state of the mitochondrial inner membrane, are an expression

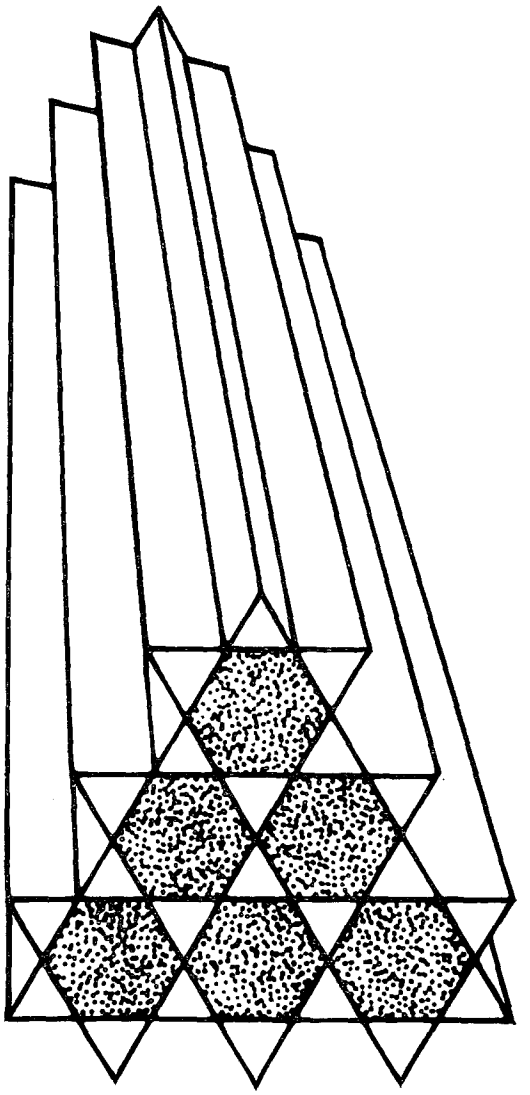

Figure 9. A diagrammatic representation of the origin of the Stars of David.[1] The tubes touch at their vertives all along their lengths. A section through such a structure at any random angle cannot generate the "alternating triangular" paracrystalline array pattern.

of material in the *intracristal space*, and that the feature common to them all is the close apposition of membranes basepiece-to-basepiece. The energized configuration in beef

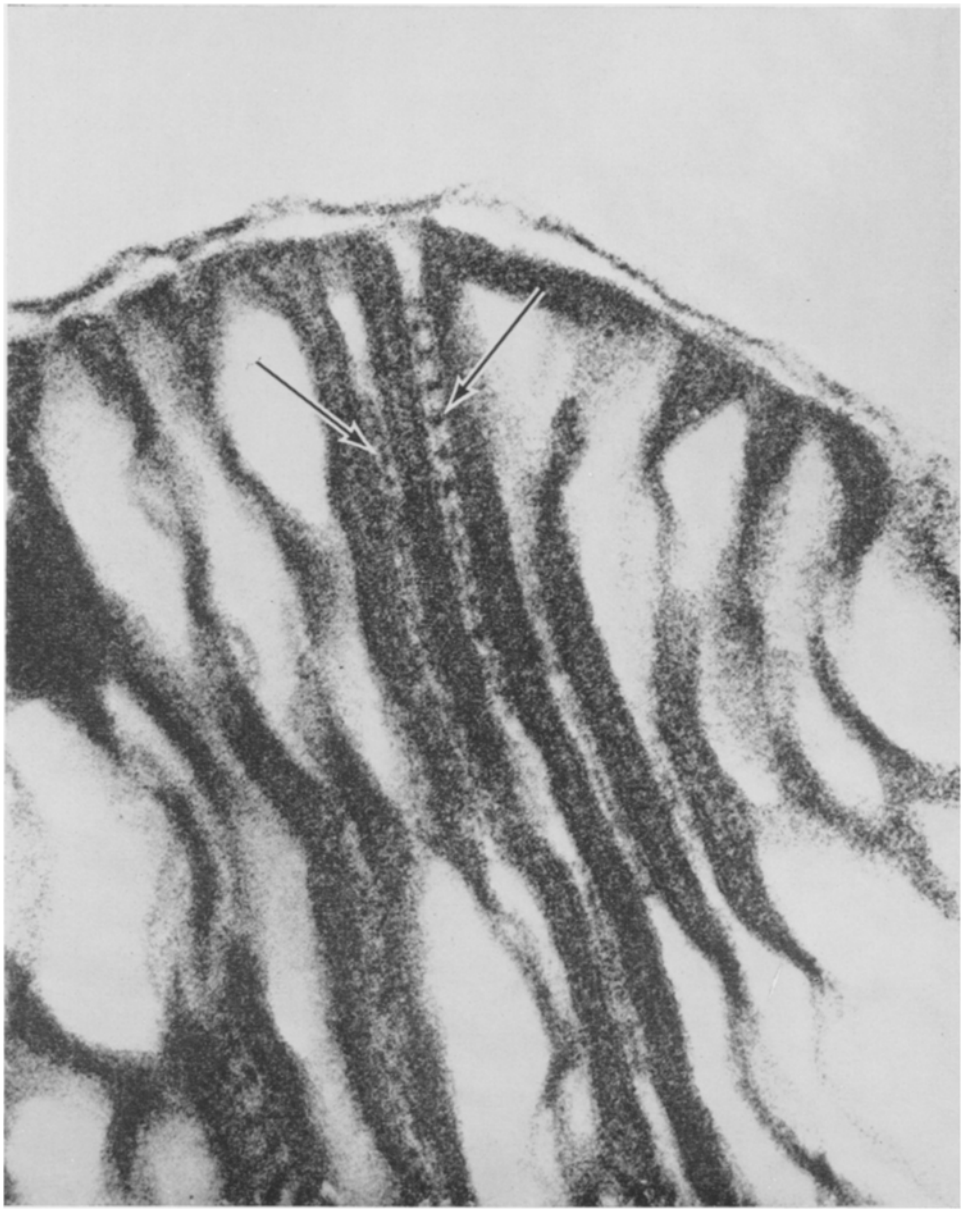

Figure 10. An electron micrograph of a region of a sectioned heavy beef heart mitochondrion in the energized configuration. The intracristal space contains "structured material" pointed to by the arrows. ×300,000.

heart mitochondria is ordinarily characterized by a distension or ballooning-out of the intracristal space[14, 15] but it is clear from the electron micrographs that there are regions which, although truly energized (as evidenced by the apposition of headpieces of one

cristal membrane very close to headpieces from a second cristal membrane), nevertheless at the same time retain close basepiece-to-basepiece apposition of cristal membranes (see Fig. 10). In addition, very similar patterns are seen between the outer membrane and the inner boundary membrane in energized beef heart mitochondria (see Fig. 11).

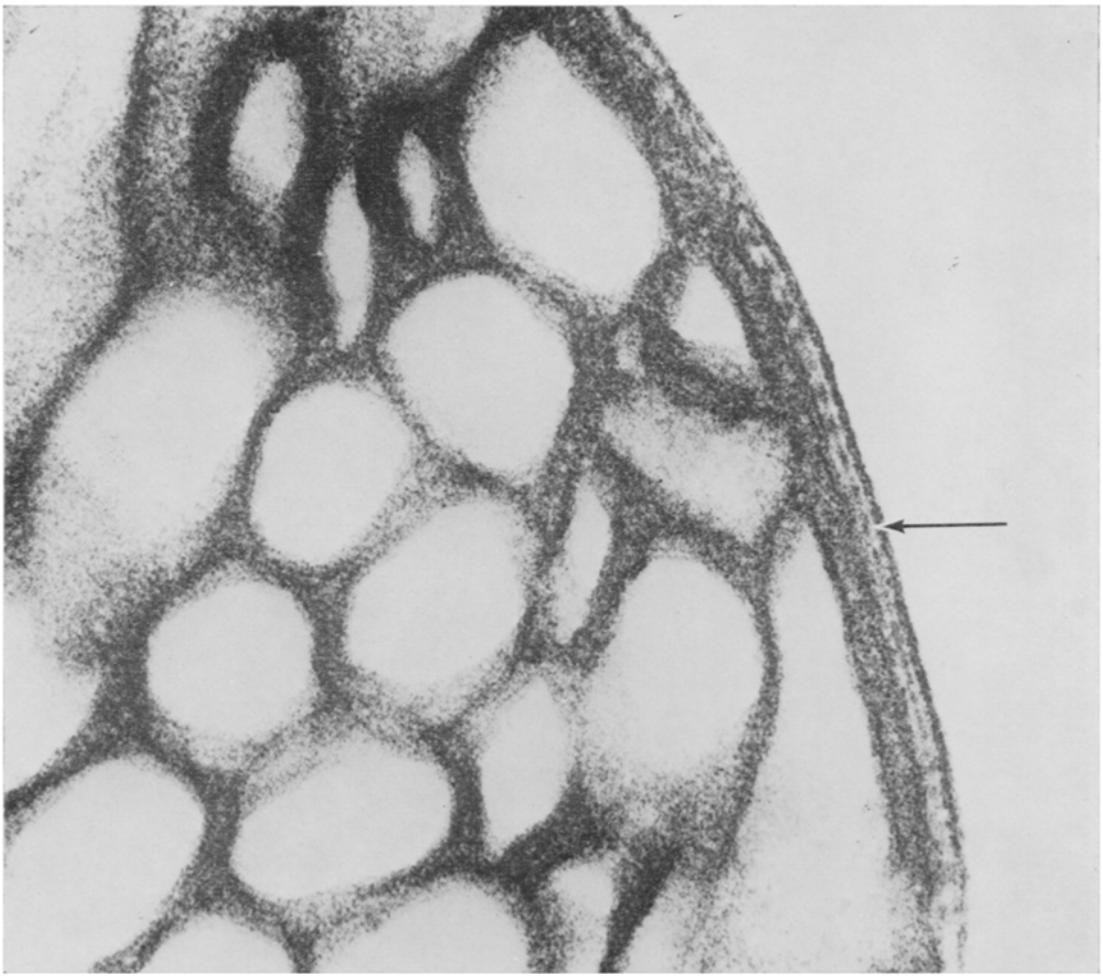

Figure 11. An electron micrograph of a region of a sectioned heavy beef heart mitochondrion in the energized configuration. The region of the intracristal space between the outer membrane and the inner boundary membrane is filled with "structured material". See arrow. ×300,000.

The paracrystalline array patterns in beef heart mitochondria are seen only where there is a rather extensive apposition of two membranes. In this regard, extensive apposition is increased if the mitochondrion is itself elongated, which causes the diameter of the mitochondrion to be reduced and forces the cristae into closer apposition. Elongation of guinea pig liver mitochondria can rather easily be achieved either by cooling down the whole animal or subjecting the whole animal to hypobaric conditions, i.e., low oxygen tension, for extended periods[3] prior to examination of the mitochondria *in situ*. The

number of elongated mitochondria, as well as the number of paracrystalline array patterns, is very greatly increased by these procedures.

The explanation of the paracrystalline array patterns seen in *Phaseolus vulgaris* and in guinea pig liver mitochondria is now at hand. The patterns are obviously associated with the orthodox configuration where there is maximal opportunity for close and extensive basepiece-to-basepiece apposition. We interpret the patterns as arising from the staining of ordered intracristal materials. Thus, although we have observed an essentially identical paracrystalline array pattern in two different energy states and their associated configurations, there is a common denominator for their appearance, namely the close apposition of two cristal membranes, basepiece-to-basepiece.

This finding has been exciting not only because it opens a new way to think about paracrystalline array patterns in very divergent kinds of mitochondria and in different energy states, but it has allowed a new view of the organization of materials in the mitochondrion, with the possibility of localizing some of the enzymatic activities of the mitochondrion whose place in the mitochondrion is as yet uncertain.

3. *The Paracrystalline Array Patterns in Astrocyte Mitochondria*

The interpretation given above for the paracrystalline array patterns in *Phaseolus vulgaris* mitochondria, guinea pig liver mitochondria, and heavy beef heart mitochondria, i.e., the visualization of structured material in the intracristal space, immediately suggested the possibility that some paracrystalline array patterns could be rationalized by structured material in the matrix space. The paracrystalline array pattern seen in the *in situ* mitochondrion of the astrocyte of the Syrian hamster is quite clearly an example of such a system (see Fig. 4). We see a mitochondrion within which there are triangles, each triangle of which has very dark walls and an electron-transparent interior. The triangles are surrounded by a paracrystalline array pattern in the form of dark spots. The triangles are interpreted as tubular cristae with triangular cross-sections which are arranged parallel to one another and which hve been sectioned perpendicular to their longitudinal axes. The triangles of this paracrystalline array pattern are, therefore, attributed to a particular form of the mitochondrial inner membrane *per se*, just as the pattern of Stars of David was ascribed to the energized (twisted) configuration of the mitochondrial inner membrane *per se*. We deduce that the cristae are tubes with a triangular cross-section, since certain of them, especially those we see near the inner boundary membrane, have been sectioned along part of their lengths, and thus give rise to elongated structures. The interiors of the triangles are the intracristal space, as evidenced by the fact that there are quite clear continuities linking the electron-transparent space between the outer and inner boundary membranes with the electron-transparent space within the interiors of the tubular cristae seen near the inner boundary membrane. The space surrounding the cristae and containing the paracrystalline array pattern of dark spots is the matrix space. The dark spots are long structures which lie parallel to the longitudinal axes of most of the cristae. This is known because sections made of mitochondria *in situ* of the astrocytes of the Syrian hamster at random angles in a pattern, not of dark spots, but rather in a pattern of dark lines in the matrix space. The exact nature of the structured material in the matrix space is as yet unknown.

With an example of a mitochondrial paracrystalline array pattern involving the matrix space, we now have examples of patterns involving every possible mitochondrial

structure, i.e., the outer membrane, the inner membrane, the intracristal space plus contents, and the matrix space plus contents. We are now in a postion to formulate a general picture of mitochondrial paracrystalline array patterns.

V. *Discussion*

In this communication we have not attempted to give an exhaustive survey of paracrystalline array patterns seen in mitochondria. Rather, we have presented only a few examples of such patterns which seemed to us not only especially bizarre and difficult to understand, but which, more importantly, illustrate the underlying principles of a general picture of mitochondrial paracrystalline array patterns.

This general picture invokes the intrinsic structures of the mitochondrion to account for paracrystalline array patterns. When an explanation for the paracrystalline array patterns is failing on this basis, than and only then, do we believe an explanation should be sought in terms of extra-mitochondrial structures. Within this restriction, then, we have illustrated how the mitochondrial inner membrane *per se* gives rise to paracrystalline array patterns, as shown in the case of the Star of David pattern seen in the *in situ* mitochondria of the jumping muscle of the migratory locust and rat heart. In both of these cases, it has been deduced that the pattern derives from the energized (twisted) configuration of the mitochondrial inner membrane. Since the energized (twisted) configuration is only one of the numerous possible configurations of that membrane, we would like to suggest that other configurations of that membrane may give rise to paracrystalline array patterns in like fashion. A consideration of those configurations may prove fruitful in rationalizing some of the as yet unexplained paracrystalline array patterns.

In addition to the mitochondrial inner membranes *per se*, other mitochondrial structures have been invoked to explain mitochondrial paracrystalline array patterns. The intracristal space has been invoked as the site of paracrystalline array patterns in heavy beef heart mitochondria, in *Phaseolus vulgaris* mitochondria, and in guinea pig liver mitochondria. In all these cases there is "structured material", whose nature is as yet unknown, which is visualized in the intracristal space. Both the mitochondrial inner and outer membranes participate in the formation of these paracrystalline array patterns, if only indirectly, by modulating the volume of the intracristal space. Their participation may, however, be more than indirect. In the case of beef heart mitochondria, the periodicity of the structured material within the intracristal space stands in a one-to-one size relationship to that of the dimensions of the basepieces of the cristal membrane. The structured material has a period of approximately 110–120 Å, while the basepieces of the repeating units of the cristal membrane are calculated to be approximately 110–115 Å center to center.[21] This fact suggests the possibility that the repeating units of the cristal membrane may act as a template upon which materials within the intracristal space may align.

The paracrystalline array patterns which are seen in the intracristal space occur only when there are extended areas of cristal membrane in close basepiece-to-basepiece apposition. This condition is to be expected most frequently in the nonenergized (orthodox) configuration of the mitochondrial inner membrane. This is exactly what is found in the cases of *Phaseolus vulgaris* and guinea pig liver mitochondria. Curiously enough,

however, in heavy beef heart mitochondria the paracrystalline array patterns seen in the intracristal space are seen only in the energized configuration, but then only in regions where the energized cristal membrane has extensive areas of close basepiece-to-basepiece apposition *in addition to* close headpiece-to-headpiece apposition of cristal membranes. Since we see paracrystalline array patterns in both the nonenergized (orthodox) and energized configurations, we should, therefore, expect paracrystalline array patterns within the intracristal space to arise in other configurations as well, so long as there is a possibility for extensive regions of close basepiece-to-basepiece apposition of cristal membranes in any of those configurations. In fact, even energized (twisted) tubes could give rise to such patterns if the tubes in question align basepiece-to-basepiece. Here again, we would like to suggest that a careful consideration of such possibilities may provide the rationalization of as yet unrationalized mitochondrial paracrystalline array patterns.

The matrix space has also been invoked as a site for mitochondrial paracrystalline array pattern formation. Structured material in that space definitely gives rise to patterns, as illustrated in the *in situ* mitochondria of the astrocyte of the Syrian hamster. In that case, we would deduce that the mitochondrial inner membrane is in the nonenergized (orthodox) configuration. However, here again, it certainly is possible to envisage the formation of paracrystalline array patterns within the matrix space as the result of the modulation of the volume of that space by the various configurations of the mitochondrial inner membrane. We again suggest this as yet still another useful vantage point within our general picture from which to examine mitochondrial paracrystalline array patterns which have as yet not been resolved.

In conclusion, we would like to reiterate our general thesis, namely, that the structure of many mitochondrial paracrystalline array patterns seen *in situ* can be made completely comprehensible by invoking only the intrinsic structures of the mitochondrion, i.e., the mitochondrial outer and inner membranes and the two mitochondrial compartments plus their contents. Since the mitochondrial inner membrane can have a wide variety of cristal invaginations depending upon the tissue source of the mitochondrion, e.g., pillow-case type in beef heart mitochondria,[14, 15] linear tubes in the case of kidney mitochondria,[22] and scalloped tubes in the case of adrenal cortex mitochondria,[23] and since the mitochondrial inner membrane can exist in a multiplicity of configurational states,[14, 15] e.g., in the nonenergized (orthodox), the energized (aggregated), the energized (twisted), and the energized (zigzag) configurations,[14, 19] the number of possible ways in which mitochondrial paracrystalline array patterns could arise and be attributed solely to the mitochondrial inner membrane *per se* seems to be very large. In addition, there is the possible modulation in all such cases of the two mitochondrial compartments All in all, we feel that the intrinsic structures of the mitochondrion may very well provide the explanation for a great many of the still unexplained mitochondrial paracrystalline array patterns.

The question of extra-mitochondrial entities as participants in mitochondrial paracrystalline array patterns cannot, however, be dismissed. In our analysis, the only *materials* which we can state are *unequivocally* of mitochondrial origin are the mitochondrial inner and outer membranes *per se*. The contents of the two mitochondrial compartments can conceivably be either intrinsic to the mitochondrion or of extramitochondrial origin, or both. In our analysis, it should be noted, the intracristal and matrix

spaces were discussed as intrinsic mitochondrial *sites*. Thus, in the analysis of mitochondrial paracrystalline array patterns, the growing body of evidence for the involvement and function of both intra- and extra-mitochondrial materials must be dealt with. We have attempted to treat some aspects of that evidence and the interesting interpretations one can reach from it elsewhere.[24]

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