Bioenergetics (1970) **1**, 379–386.

The Mechanism of Mitochondrial Swelling. VII. The Constant Topology of the Mitochondrial Inner Membrane During Swelling

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I. *Introduction*

In the previous papers in this series, studies were presented on both energized and pseudoenergized mitochondrial swelling.[1–6] Despite differences in the ways in which energized and pseudoenergized swelling are generated, the *configurational* changes which are seen in the mitochondrial inner membrane during both kinds of swelling were deduced to be identical.[4] The configurational changes seen during swelling are shown semi-diagrammatically in Fig. 1. Asai *et al.*[4] have proposed that both energized and pseudoenergized swelling can be described ultrastructurally as alterations of the cristal membranes, and that these alterations occur in a precise sequence. This sequence, as deduced from studies with isolated heavy beef heart mitochondria, involves an initial conversion of the mitochondrial inner membrane from the nonenergized (aggregated) configuration to the energized (aggregated) configuration, followed invariantly by a conversion to the energized (twisted) configuration. Thus far in the sequence, there has been only a minor increase in the matrix volume, as judged by inspection of electron micrographs and by matrix volume determination using C^{14}-sucrose.[7] The subsequent steps in the sequence, however, constitute the actual swelling process. Since the steps in actual swelling initiate from the invariant occurrence of the energized (twisted) configuration, any ultrastructural interpretation of the swelling process depends upon the interpretation of the ultrastructure of the mitochondrial inner membrane in the energized (twisted) configuration.

The energized (twisted) configuration has been interpreted in our laboratory by Penniston *et al.*[8] to result from first a comminution, i.e., a shredding, of the energized (aggregate) mitochondrial inner membrane into numerous fragments, followed by a resealing of the fragments into multiple, twisted, snake-like tubes with bulbous closed cul-de-sacs at both ends of each tube, with headpiece-stalk sectors oriented into the interior matrix space lumens of each tube. The "sidedness" of the membranes in these closed tubes was interpreted to be identical with that of the original intact mitochondrial inner membrane, i.e., with headpiece-stalk sectors extending into the enclosed matrix space in both cases. One could thus visualize the energized (twisted) configuration as

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† This investigation was supported in part by Program Project Grant No. GM-12847 from the National Institute of General Medical Sciences (USPHS), and also by a Training Grant GM-88 from the same source.

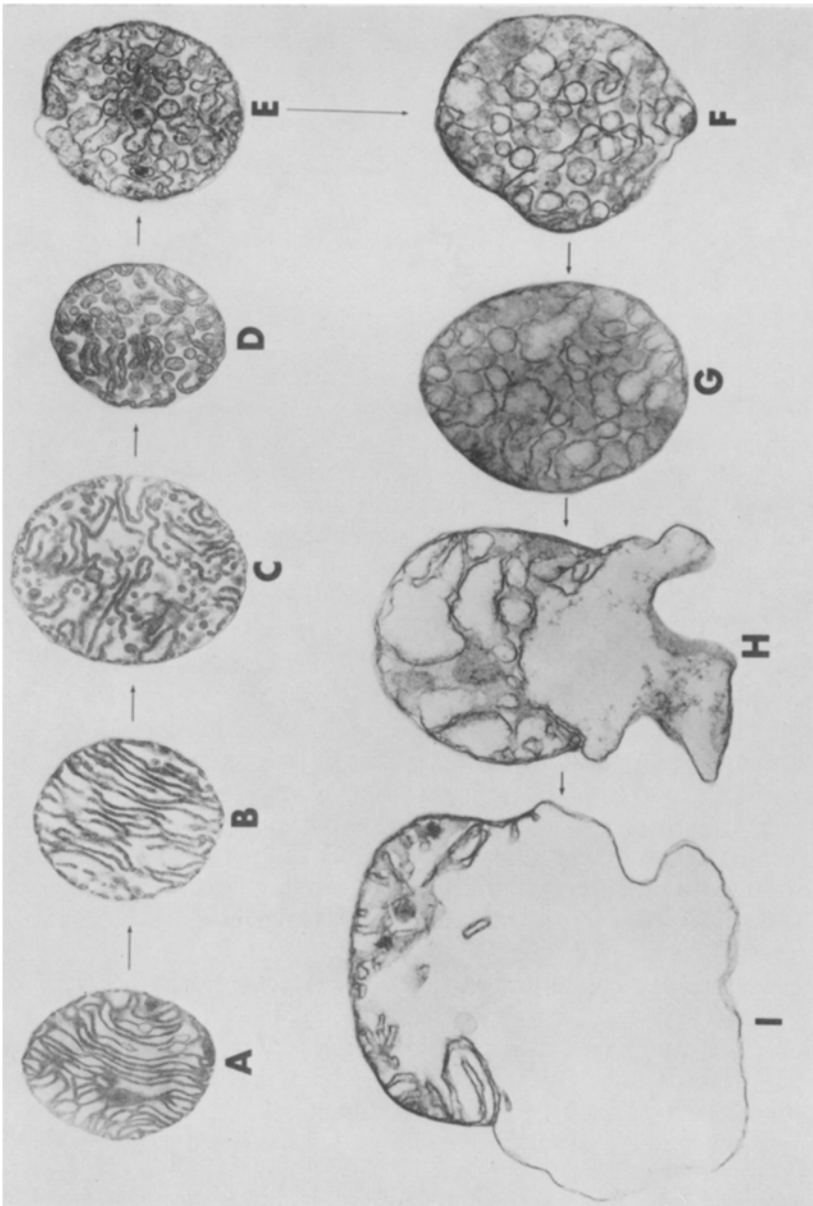

Figure 1. A semi-diagrammatic representation of the sequence of ultrastructural changes in beef heart mitochondria which occur during large amplitude swelling as proposed by Asai *et al.*[4] All *micrographs magnified* ×28,000.

one in which each individual mitochondrion has numerous miniature snake-like mitochondrial inner membranes enclosed by the mitochondrial outer membrane. On the basis of this interpretation of the ultrastructure of the energized (twisted) configuration, Asai *et al.*[4] proposed that the subsequent sequential steps in the actual swelling process consisted of (a) an increase in the matrix volume contained within individual snake-like tubes to give a collection of swollen small vesicles, follwed by (b) a coalescence of these swollen small vesicles to form larger swollen vesicles. This coalescence could be thought of as very like the coalescence of small oil droplets to give larger droplets. The electron micrographs taken during this swelling process seemed to indicate such a process was in fact going on, since what is seen initially is a great many quite small circular and tube-like structures which decrease in number but increase in diameter (see Fig. 1). In the limit, this process of coalescence ultimately leads to the re-formation of a single, intact, mitochondrial inner membrane surface continuum but in a vastly swollen state. The swelling process also results in (c) a rupture of the relatively inelastic outer membrane,[4,9] which allows the re-formed swollen inner membrane to spill forth through the rupture into the extramitochondrial space and to swell even further in volume. This total process was referred to as large amplitude swelling.

Although the proposal of Asai *et al.*[4] neatly accounted for the electron microscopic appearance of the mitochondrial inner membrane during the swelling process, it nevertheless presented one very serious problem which required a special assumption. The problem related to the fact that the matrix space retains its relative sucrose impenetrability during the swelling process,[2,3] despite the comminution and resealing of the mitochondrial inner membrane into multiple tubes, and the subsequent coalescence of multiple tubes back into a single intact mitochondrial inner membrane. A complex process involving the breaking and remaking of associations between enormous numbers of repeating units of the mitochondrial inner membrane is very hard to visualize without a loss in the impenetrability of the matrix space to sucrose. However, although there was an awareness that this serious problem existed, the special assumption was made that these processes could occur without opening the matrix space to the invasion of sucrose.

In a note added to their paper in proof, Asai *et al.*[4] referred to an alternative interpretation of the energized (twisted) configuration by Korman *et al.*, which has since been published,[10] in which the communication and resealing of the mitochondrial inner membrane is dismissed. The alternative interpretation given is based upon the underlying principles that the topology of the mitochondrial inner membrane is equivalent to that of a hollow sphere, and that the topology of that membrane never changes regardless of its configuration. Such a constant topology means that there can be no comminution of the mitochondrial inner membrane surface in going to the energized (twisted) configuration. Instead, a proposal was made, and experimental proof provided, that the energized (twisted) configuration arises from a process of "reverse invaginations" of the cristal membranes of the sheet-like energized (aggregated) configuration, to give rise to a mitochondrial inner membrane which has been ultimately converted into a labyrinth of interconnecting cul-de-sac tubes with bulbous ends, and with headpiece-stalk sectors lining the lumens of the tubes. Thus, as a surface, the mitochondrial inner membrane in the energized (twisted) configuration resembles the surface of an amoeba which has sent out an enormous number of pseudopodia which branch and rebranch. Sectioning of such a structure would result in a cross-section which has the appearance of multiple unconnected twisted snake-like tubes with bulbous cul-de-sac ends. This alternative interpretation of the energized (twisted) configuration of the mitochondrial inner membrane allows us to formulate a proposal for the sequence of ultrastructural events which occur during large amplitude swelling without recourse to any special assumptions about sucrose impenetrability.

II. *The Swelling of the Mitochondrial Inner Membrane*

Large-amplitude energized and pseudoenergized mitochondrial swelling, both of which processes go through the energized (twisted) configuration, can be completely rationalized structurally within the boundary conditions of the constant topology of the mitochondrial inner membrane. A diagrammatic representation of the sequence of structural changes which occur during swelling, starting with the energized (twisted) configuration, are given in Fig. 2. In Fig. 2(A) we see a representation of a mitochondrion in the energized (twisted) configuration. The outer membrane encloses an inner mitochondrial membrane which is in the form of a highly branched tubular system. The short straight lines on the surface of the inner membrane represent the headpiece-stalk sectors which extend into the matrix space. The matrix space, M, is contained within the lumens of the highly ramified tubes. The intracristal space is designated IC. The numbers 1 through 16 represent regions along the outer membrane into which the snake-like or finger-like tubes point. The letter T represents a branched tube (see region 1). In Fig. 2(B) we see a diagrammatic representation of the same mitochondrion which has begun to swell, i.e., in which the matrix space has increased somewhat in volume. The letter H refers to an outward herniation of the outer membrane. Such herniations of fairly large size are seen quite frequently in partially swollen mitochondria,* as seen in Fig. 3 (arrow). Notice that the tube labelled T in Fig. 2(A) has been reduced in length to a bud-like protuberance, B, in Fig. 2(B). Within the bud, the headpiece-stalk sectors are pointing toward each other. Such a transition from tube to bud can be thought of on a strictly structural basis as an inverse of tube formation which we have invoked earlier[10] to explain the change in configuration going from the energized (aggregated) to the energized (twisted). Thus the process of swelling can be described structurally as the "resultant" of two simultaneous changes, (a) an increase in the matrix volume, and (b) a loss of tubular structure. These two changes are, of course, only a convenient, if artificial, way for us to describe the overall changes. The two changes are not independent of one another. Thus, as the matrix expands, the diameters of the tubes increase concomitantly. Since the mitochondrial inner membrane has a constant surface area, such an increase in diameters of the tubes perforce requires shortening of the tubes. In the limit, the matrix expands with an eventual conversion of the tubes to non-tubular sheet-like surfaces. In Fig. 2(B) this combination of changes results in an alignment of regions of the cristal membrane basepiece-to-basepiece, with a concomitant "closing down" of the intracristal space and an "opening up" of the matrix space. In Fig. 2(C), the process of swelling has progressed even further. Some of the numbered regions along the inside of the outer membrane, which in the energized

* Relatively smaller outward herniations of the mitochondrial outer membrane are commonly seen in mitochondria fixed in a variety of configurational states. The herniation could thus be interpreted as an artifact of fixation and/or of staining. However, herniation can also be cited as evidence for the relative fragility of the mitochondrial outer membrane. Such fragility is consistent with our proposed sequence leading to large amplitude swelling.

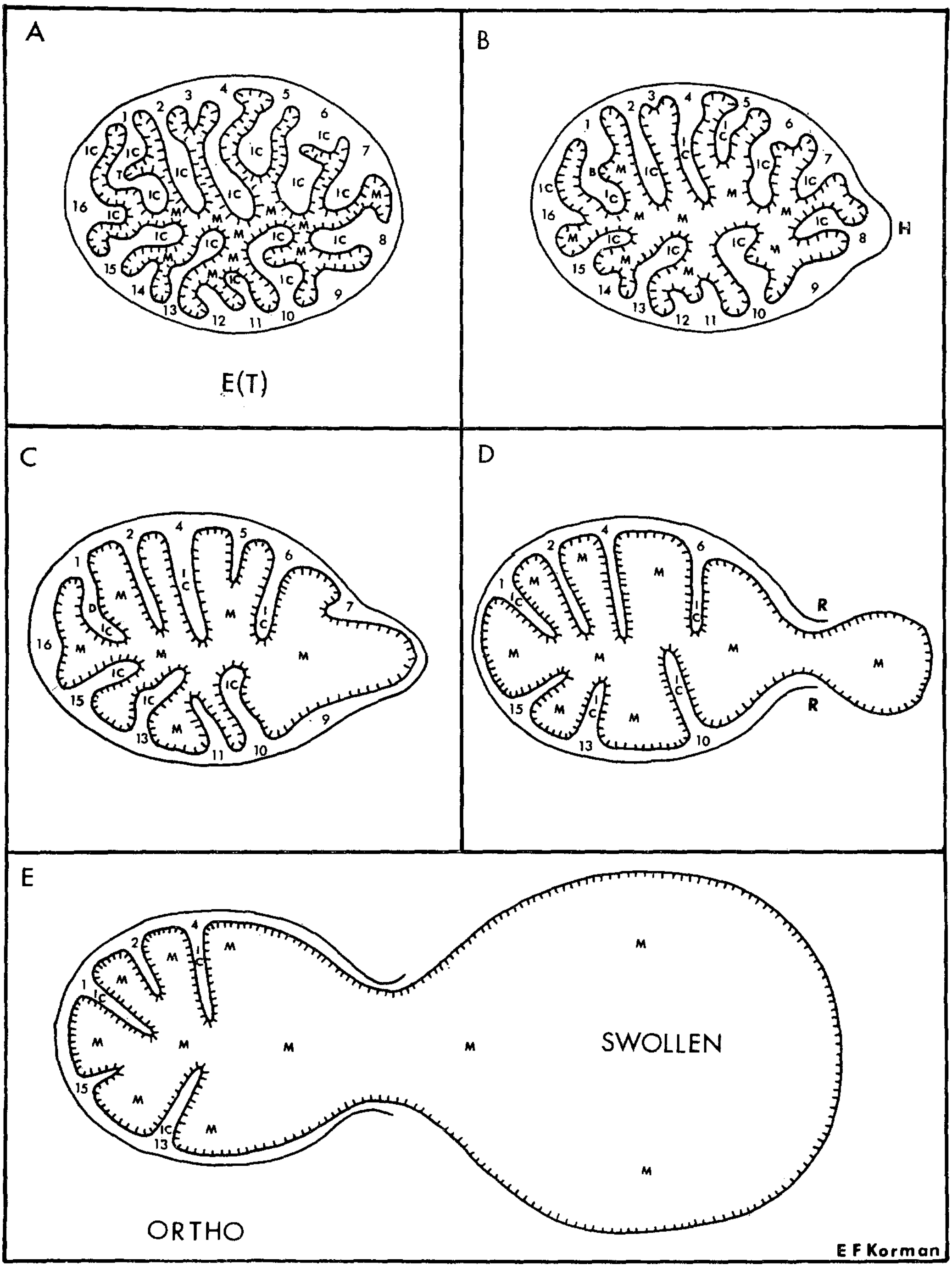

Figure 2. A diagrammatic representation of the process of large amplitude swelling in which the mitochondrial inner membrane is topologically constant.

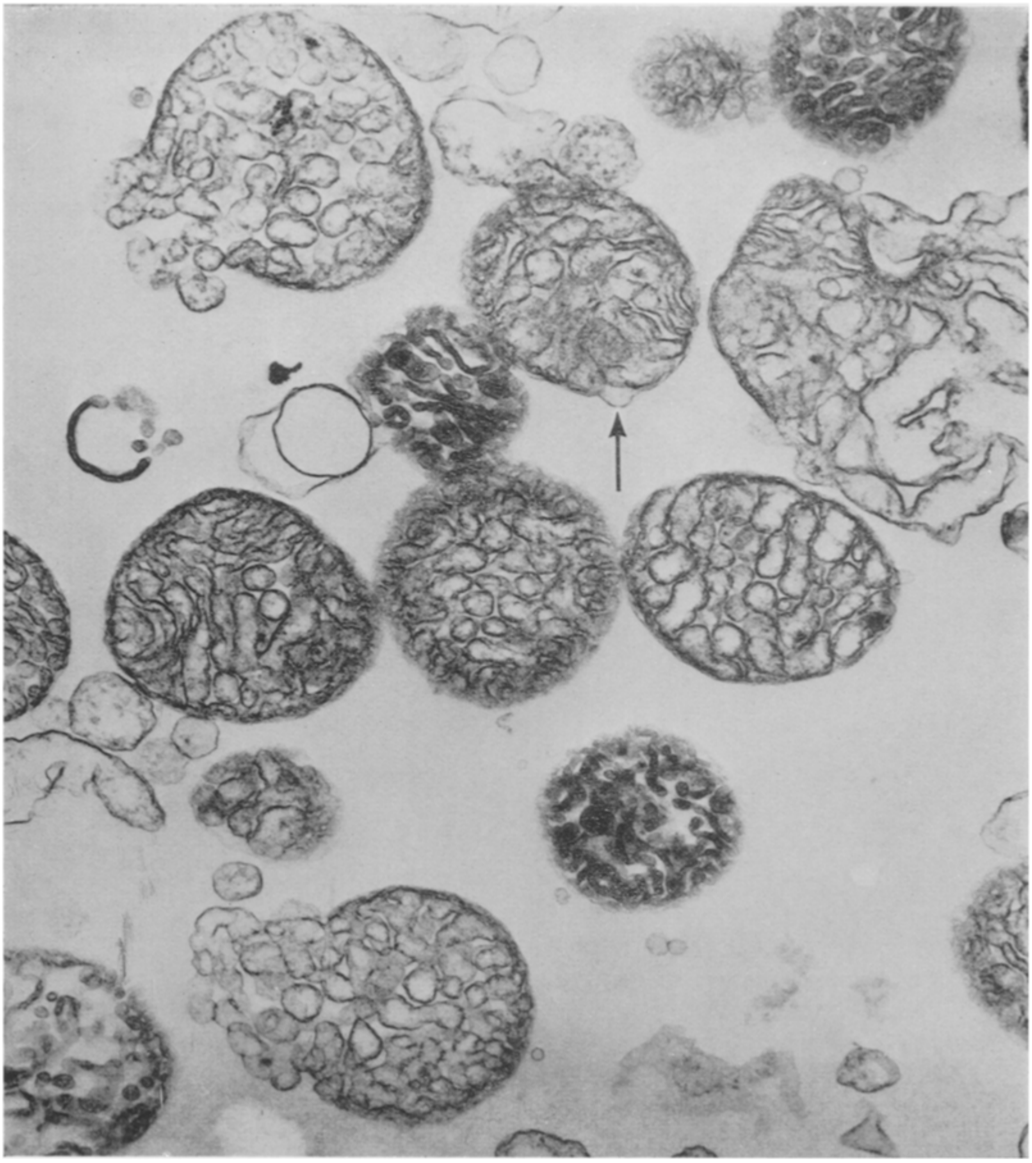

Figure 3. An electron micrograph of a field of beef heart mitochondria which are undergoing large amplitude swelling. There are mitochondria present with a tight energized (twisted) configuration of the mitochondrial inner membrane, as well as mitochondria with their inner membranes in various stages of swelling. Note the herniation of the outer membrane pointed to by the arrow. Several mitochondria have outer membranes which are ruptured, and with mitochondiral inner membranes which are spilling out into the extra-mitochondrial space. Magnification ×30,000.

(twisted) configuration were essentially filled with intracristal space and into which the finger-like portions of the highly ramified inner membrane were pointing, are now completely filled with mitochondrial inner membrane having an expanded matrix volume (regions 3, 12, 14, and 16). The herniation has enlarged, with a ballooning of the mitochondrial inner membrane into the herniation. The matrix is very enlarged, and the intracristal space is now quite small. Some regions of the mitochondrial inner membrane now have characteristically orthodox cristae. In Fig. 2(D), a rupture, R, in the outer membrane is depicted diagrammatically, with a spilling out of the inner membrane into the extra-mitochondrial space. The process of matrix space expansion and intracristal space reduction within the confines of the ruptured outer membrane has progressed still further, with essentially all that part of the mitochondrial inner membrane which has not spilled out into the extra-mitochondrial space but which has remained associated with the outer membrane, being unmistakably in the nonenergized (orthodox) configuration. Finally, in Fig. 2(E), we see an enormously swollen mitochondrion having a very large portion of the mitochondrial inner membrane protruding into the extra-mitochondrial space. The regions of the mitochondrial inner membrane still adhering to the ruptured outer membrane have a relatively tight, nonenergized (orthodox) configuration. The process of high amplitude swelling is structurally thus complete.

The ultrastructural changes which occur during swelling have been described here as continuous, i.e., with herniation and rupture of the outer membrane, then a spilling out of the mitochondrial inner membrane into the extra-mitochondrial space, all occurring simultaneously with an expansion of the matrix space and loss of tubular structure. However, it is possible that this process occurs discontinuously, i.e., with an expansion of the matrix space within a completely intact mitochondrial outer membrane. Such a swelling sequence would lead to the transformation of the mitochondria from the energized (twisted) configuration to the nonenergized (orthodox) configuration. Such nonenergized (orthodox) mitochondria are sometimes seen in the midst of mitochondria which have undergone large amplitude swelling. Such mitochondria may undergo subsequent rupture and large amplitude swelling.

III. *Discussion*

The process of mitochondrial swelling has been widely studied. Most of the descriptions of the ultrastructural changes which occur during this process have involved an interpretation that there is an expansion of the matrix space enclosed by an intact mitochondrial inner membrane.[11–14] In this regard, the description offered by our laboratory in the past[4] was essentially at variance. This difference arose because we observed the energized (twisted) configuration as an invariant structural intermediate during the swelling sequence. It was therefore necessary for us to postulate a sequence of structural changes during swelling which included that structure. Our former interpretation that the energized (twisted) configuration consisted of multiple tubes meant that the descriptions of the ultrastructural changes occurring during swelling was similar to that of others in certain details, but quite different from that of others in other details. With a re-evaluation of our interpretation of the energized (twisted) configuration, we now bring our interpretation of the structural changes which occur during the swelling process into essential topological agreement with the descriptions presented by other laboratories. We wish to make it quite clear, however, that this is so not because there has been any change in our conclusion that the energized (twisted) configuration is an invariant intermediate in the swelling sequence, a point on which we may still differ with others, but solely as a consequence of our reinterpretation of the topological structure of that configuration.

Swelling, whether energized or pseudoenergized, thus in no way violates the boundary conditions of constant topology of the mitochondrial inner membrane. In this regard, swelling is no different from other configurational changes which occur in mitochondria. The proposal given here rationalizes all the structural forms seen during swelling, with no need to invoke any special assumptions about sucrose impenetrability. Since the surface of the mitochondrial inner membrane is never broken during swelling, the need to explain sucrose impenetrability in special terms is obviated.

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