

MITOCHONDRIAL ULTRASTRUCTURAL AND ATPASE CHANGES DURING THE LIFE CYCLE OF *ASCARIS SUUM*

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Ultrastructural morphology and ATPase specific activities of mitochondria isolated from 1-celled fertilized egg, 10-day embryo, 21-day infective larvae and adult body wall muscle of Ascaris suum and rat liver were determined and compared. Although cristae of both muscle and egg mitochondria contained numerous elementary particles with head pieces of conventional diameter (85 Å), each muscle mitochondrion contained relatively few, short cristae with a diminished frequency of elementary particles and associated ATPase activity.

These morphological relationships are related to the previous conclusion that the transition from an aerobic to an essentially anaerobic metabolism is intimately associated with the mitochondrion and is a normal and mandatory feature of development.

The intestinal nematode *Ascaris suum* undergoes a transition between aerobic and anaerobic metabolisms during the course of its life cycle. Specifically, the eggs and larvae of *Ascaris* develop aerobically (Kmetec, Beaver & Bueding, 1963; Oya, Costello & Smith, 1963; Passey & Fairbairn, 1955). There is evidence that migrating larvae in host tissues are also aerobic (Saz, Lescure & Bueding, 1968). Mitochondria from fertilized eggs and developing larvae contain significant amounts of cytochromes a, b and c (Lee & Fairbairn, 1973). Fatty acids are oxidized and both the tricarboxylic acid and glyoxylate cycles are present and functional (Ward & Fairbairn, 1970).

In contrast, the adult metabolism is primarily fermentative even in the presence of oxygen (Laser, 1944). Mitochondria from the adult body wall muscle contain small amounts of cytochromes (Cheah & Chance, 1970; Cheah, 1976). Identification of some

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tricarboxylic acid cycle enzymes have not been possible, and palmitic acid is not oxidized (Ward & Fairbairn, 1970; Barrett & Körting, 1976). Mitochondrial respiration is only partially inhibited by cyanide and azide (Rodrick & Fairbairn, 1973; Lee, 1974; Lee & Fairbairn, 1973; Köhler & Bachman, 1980). Also, mitochondrial oxidative phosphorylation that occurs is inefficient and reduced (Rodrick & Fairbairn, 1973; Cheah, 1974).

Because of these mitochondrial metabolic differences, it was of interest to compare the morphology and ATPase activity of mitochondria isolated from 1-celled eggs, 10-day embryo, 21-day infective larvae and adult body wall muscle of *Ascaris suum*.

MATERIALS AND METHODS

Biological Materials

Adult female *Ascaris suum* were collected at a slaughterhouse, washed and maintained overnight at 37°C in a balanced salt solution (Baldwin & Moyle, 1947). Dissections were made within 24 hours.

Isolation, decoating, storage and incubation of eggs were carried out by established methods (Costello, 1961; Kaulenas & Fairbairn, 1968). Adult body wall muscle was obtained by dissection (Laser, 1944).

Isolation of Mitochondria

The chitinous shell was removed from 1-celled fertilized eggs and eggs containing 10-day vermiform embryos (Fairbairn, 1961). These eggs were then washed five times by centrifugation in 0.04 M Tris, pH 7.5 and suspended in buffer (3.3×10^6 eggs/ml) diluted with an equal volume of 0.5 M sucrose. The suspension was homogenized at 0-4°C by a Ten Broeck homogenizer.

Ascaris adult muscle strips and rat liver were rinsed three times with 0.25 M sucrose, suspended in two volumes of sucrose, and homogenized with three five second bursts at low speed and 0°C in a Waring blender. The crude homogenate was then diluted with three volumes of sucrose.

Homogenates were centrifuged at 3000xg for ten minutes at 0°C (in a Sorvall RC5B centrifuger, rotor 34). The supernates were decanted and centrifuged at 12,000xg for 20 minutes, 0°C. The resulting pellet was then resuspended in 0.25 M sucrose and recentrifuged at 20,000xg, 0°C twice.

All supernates and pellets were checked for mitochondrial bound succinate dehydrogenase activity (King, 1963) and protein was determined by the method of Lowry et al (1951).

Counting Mitochondria

Serial dilutions of the second 20,000xg mitochondria pellet resuspended in 0.25 M sucrose were counted under phase contrast microscopy in a Petroff-Hauser bacterial chamber (Parsons & Simpson, 1973; Rodrick et al, 1977). At least 370 mitochondria were counted in not less than two serial dilutions, the results being reproducible within $\pm 2\%$ in 97% of the experiments.

Electron Microscopy

The second 20,000xg mitochondrial pellets were resuspended in 8% (w/w) sucrose and 20 ml portions were centrifuged at 100,000xg for 45 minutes. These pellets were fixed for 60 minutes at 50°C in 2% glutaraldehyde buffered with 0.1 M Sorensen's phosphate buffer, pH 7.4. They were then cut into 1 mm³ pieces, washed in buffer for 15 minutes, and fixed in buffered 2% osmium tetroxide for 60 minutes. The fixed mitochondria were dehydrated in increasing concentrations of aqueous ethanol and immersed twice in propylene oxide for 15 minutes. The dehydrated pellets were then immersed in propylene oxide: epon (1:1) for one hour, followed by propylene oxide: epon (2:1). Twelve to 24 hours later they were embedded in epon and heated at 65°C for 24 hours. Silver and gold sections mounted on 300 mesh grids were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined with an RCA-EMU-3H microscope.

Negative staining with 1% phosphotungstic acid, pH 7.6, was carried out by the established techniques of Chance & Parsons (1963). The preparations were examined with a Philips 200 electron microscope at magnifications of 18-20,000.

Determination of Oligomycin Sensitive ATPase activity

ATPase activity was determined by the method of Pullman et al (1960) in the second 20,000xg mitochondrial pellets.

RESULTS

Mitochondria isolated from fertilized 1-celled egg, 10-day embryo, 21-day larvae and adult body wall muscle of *Ascaris suum* were intact and free from contamination with other organelles and fragments excepting a little glycogen in the muscle preparation (Fig. 1, A and B). A double membrane and well-defined cristae were present. Mitochondria from the fertilized eggs and larval stages were found in both the condensed and the orthodox conformational states. Mitochondria isolated from muscle were in the condensed and the enlarged semiorthodox states. In all mitochondrial preparations, which are typical of many, the condensed state was most common. Adult muscle mitochondria were much larger (diameter 0.35 μ m to 0.41 μ m) than those of the eggs, larval stages or rat liver (0.21 μ m 0.27 μ m). Also, they possessed relatively few cristae, which extended only short distances in a highly granular matrix (Fig. 1, B).

In all mitochondria, elementary particles were attached to the cristae (Fig. 1, C and D). The headpieces were spherical in shape and averaged 85 Å for rat liver, 1-celled fertilized egg, 10-day embryo and 21-day larvae while the body muscle averaged 80 Å in diameter (Table I). The number of subunits per micrometer cristae was also determined (Table I), and ranged from 85 to 110 for all preparations except for the adult body muscle. In the body muscle mitochondria, the number of subunits per micrometer cristae was reduced and ranged from 75 to 80.

ATPase activity, which is situated in the headpiece of the subunit, was present in all mitochondria preparations. However, the ATPase activity was some 10 times less in muscle mitochondria when compared with mitochondria from 10-day embryos and rat liver (Table I).

Results based on the determination of protein and ATPase activity and on mitochondrial counts are presented in Table II. In general, mitochondria from fertilized 1-celled eggs, 10-day embryos and 21-day larvae resembled those from rat liver. However, the body muscle mitochondria were strikingly different. Compared with those from the eggs, body muscle mitochondria contained 79% less protein per mitochondrion and 87.5% less ATPase per mitochondrion (Table II).

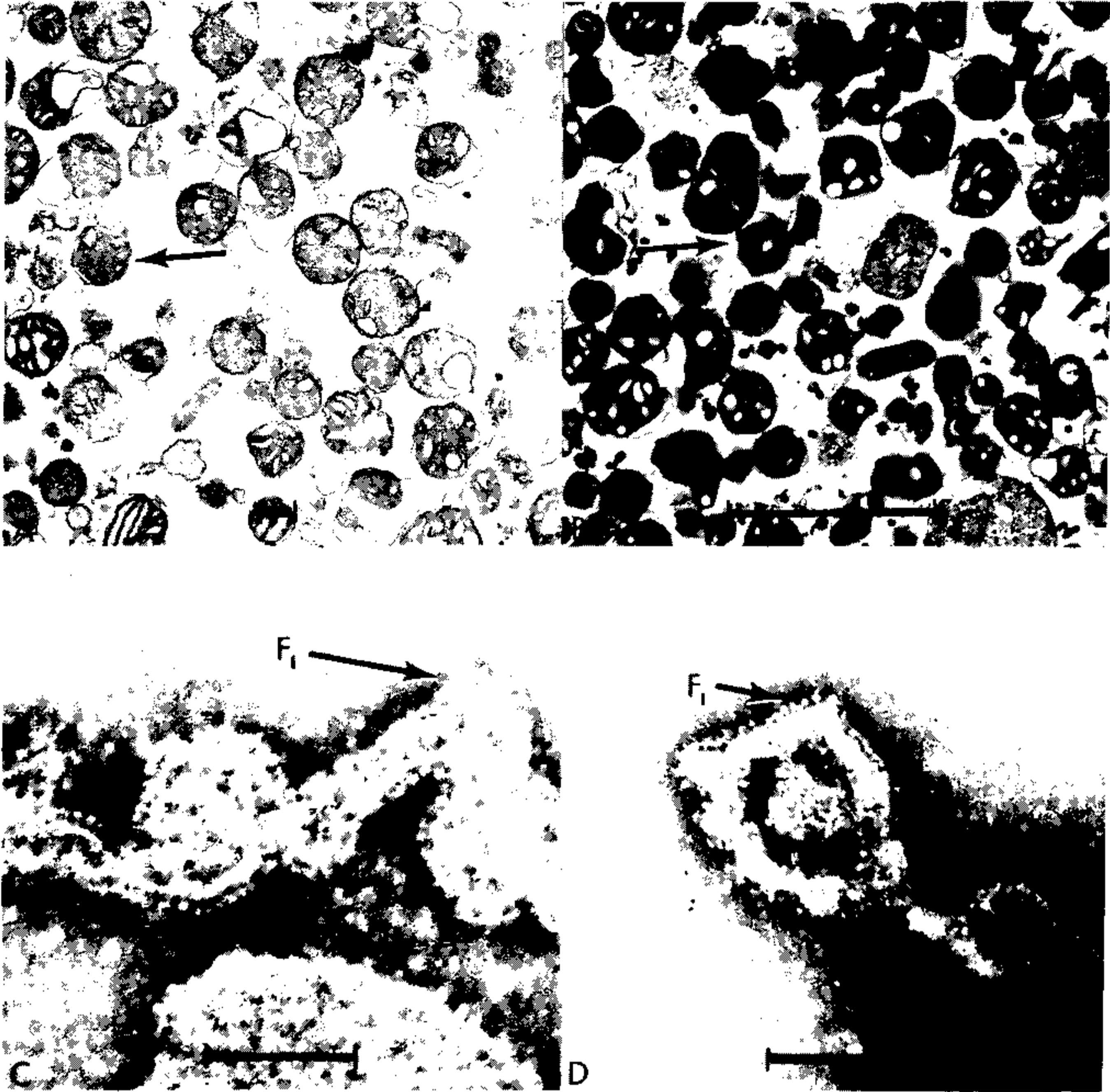


Fig. 1 — A and B. Mitochondria isolated from 1-celled fertilized eggs (x35,000) and adult body wall muscle (x24,000) of *Ascaris lumbricoides* var. *suum*. Arrow points to mitochondria in the condensed configuration and the lengths of the scales are 0.1 μm and 1.0 μm , respectively.

C and D. Negatively stained cristae from eggs (x175,000) and muscle (x112,500) showing the elementary particles (F₁). The length of each scale is 0.1 μm .

DISCUSSION

The parasitic intestinal nematode, *Ascaris suum*, undergoes an aerobic to anaerobic metabolic transition during the course of its life cycle (Fairbairn, 1970). The adult possesses an essentially anaerobic metabolism and resides in the anaerobic environment of the small intestine while producing millions of aerobic eggs. Thus, a molecular mechanism causing dramatic changes in mitochondrial metabolism is functioning in *Ascaris*.

Our study indicates that dramatic changes in both the mitochondrial ultrastructure and associated ATPase activities accompany this aerobic to anaerobic transition. Specifically, mitochondria isolated from the adult body wall muscle were 1.6 times larger and possessed a highly granular matrix with only a few short cristae extending into it, when compared to mitochondria isolated from 1-celled eggs, 10-day embryo, 21-day larvae and rat liver.

TABLE I

Dimensions and Number of Mitochondrial Subunits per Micrometer Cristae in Rat Liver, 1-Celled Fertilized Egg, 10-Day Embryo, 21-Day Larvae and Body Muscle of *Ascaris lumbricoides* var. *suum*.

Source of Mitochondria	Head Size Å	Spacing between Subunits Å	Subunits/Micrometer Cristae
Rat Liver	85	115	90-110
<i>Ascaris</i> 1-celled fertilized egg	85	115	88-110
<i>Ascaris</i> 10-day embryo	85	115	90-110
<i>Ascaris</i> 21-day larvae	85	120	85-110
<i>Ascaris</i> body muscle	80	150	75-80

TABLE II

Interrelationships between Mitochondrial ATPase Specific Activity and the Number of Mitochondria per Milligram Protein and ATPase per Mitochondria in Rat Liver, 1-Celled Fertilized Egg, 10-Day Embryo, 21-Day Larvae, and Adult Body Muscle of *Ascaris lumbricoides* var. *suum*.

Source of Mitochondria	ATPase Specific Activity ¹	Number of Mitochondria/ mg Mitochondrial Protein $\times 10^{-9}$	ATPase/ Mitochondrion
Rat liver	0.061	2.45	2.49×10^7
Fertilized 1-celled egg	0.018	2.28	0.79×10^7
10-day embryo	0.051	2.31	2.21×10^7
21-day larvae	0.021	2.69	0.78×10^7
Adult body muscle	0.005	4.93	0.10×10^7

¹ ATPase activity is expressed as $\mu\text{moles/mg protein/min}$

High resolution electron microscopy of negative stained isolated mitochondria from the 1-celled egg, 10-day embryo, 21-day larvae, adult body muscle of *Ascaris* and rat liver revealed similarities in the diameter of the stalk headpiece ranging from 80 to 85 Å. This value is in agreement with Chance & Parsons (1963) and Rew & Saz (1974). However, differences were noted in the spacing of headpieces and the frequency of headpieces (number per micrometer cristae). It is well known that negative staining of membranes can cause stretching. The inner membrane of the Ascarid mitochondrion is quite elastic. In an attempt to rule out preferential stretching of the inner mitochondrial membrane, which would account for the observed spacing differences, the mitochondrial bound ATPase activity was determined. It is well known that ATPase is located in the headpiece of elementary particles and that these elementary particles are intimately associated with the three mitochondrial sites at which oxidative phosphorylation occurs (Racker & Horstman, 1967). ATPase is essential for such phosphorylation (Racker & Horstman, 1967). Distinct differences in the specific activity of ATPase and ATPase activity per mitochondrion exist between the aerobic egg and larval stages and the adult body muscle. The changes of spacing and frequency of the mitochondrial elementary particles may be asso-

ciated with the deficiencies in ATPase reported in this study and electron transport and oxidative phosphorylation (Köhler & Bachmann, 1980; Rodrick et al, in press). Van den Bossche (1974) has reported low activities of *Ascaris* body muscle ATPase and has suggested that those low activities may be regulated by a cytoplasmic protein ATPase inhibitor. However, further proof is needed.

In conclusion, the fertilized 1-celled egg, 10-day embryo, and 21-day larvae of *Ascaris suum* contain mitochondria that closely resemble those of rat liver and are morphologically and functionally quite unlike those of the adult body muscle. It is clear that the observed differences are developmental and that regulation of protein synthesis in muscle mitochondria may be mediated in part by genes that control the synthesis of mitochondrial DNA (Rodrick et al, 1977). The mandatory developmental features of the mitochondrial morphology and biochemistry in *Ascaris* are worth emphasizing since previous work seems to have been limited to organisms in which similar changes in mitochondrial morphology and physiology were environmentally induced.

RESUMO

Foram determinadas e comparadas a morfologia estrutural e as atividades específicas da ATPase de mitocôndrias do *Ascaris suum* (isoladas do ovo unicelular fertilizado, do embrião de 10 dias, da larva infectante de 21 dias e do músculo da parede do corpo do adulto) e do fígado do rato. Embora as cristas das mitocôndrias do músculo e do ovo contivessem numerosas partículas elementares com cabeças de diâmetro convencional (85 Å), cada mitocôndria do músculo continha cristas curtas e em número relativamente pequeno, com diminuição da frequência das partículas elementares e da respectiva atividade de ATPase.

Estas relações morfológicas são vinculadas à conclusão prévia de que a transição do metabolismo aeróbio para o metabolismo essencialmente anaeróbio está intimamente associada à mitocôndria e constitui um característico normal e obrigatório do desenvolvimento.

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