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ULTRASTRUCTURAL STUDIES OF THE MITOCHONDRIAE IN THE STRIATED MUSCLES OF BIRDS WITH REGARD TO EXPERIMENTAL HYPOKINESIS

M. Belak, J. Kocisova and K. Bod'a

Translation of "Studium der Ultrastruktur von Mitochondrien in der quergestreiften Muskulatur der Vögel unter Berücksichtigung der experimentell hervorgerufenen Hypokinese", Archiv fuer Experimentelle Veterinaermedizin (Leipzig), Vol. 31, July 4, 1977, pp. 537-546.

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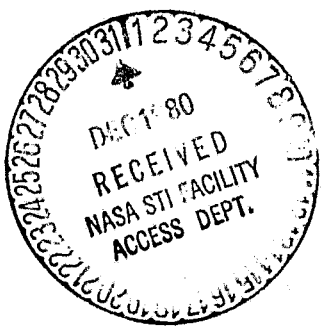

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16. Abstract: Electron microscopic studies have been carried out on the mitochondria of the transversely striated muscles with regard to experimental hypokinesis. As compared to the central group the mitochondria of m. pectoralis thoracicus and the m. iliotibialis posterior in hypokinetic birds reveal marked changes. In filamentous and ovoid mitochondria vacuoles can be observed which in some cases produced larger light formations with following disappearance of the cristae and destruction of mitochondria. Fat particles located at the poles of the altered mitochondria, sporadically occurring also laterally, presented another finding. The Z-lines of the sarcomere did not form a continuous line, but were somewhat shifted.

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Ultrastructural Studies of the Mitochondria in the Striated Muscles of Birds with Regard to Experimental Hypokinesis

M. Belak, J. Kocisova and K. Bod'a

The striated muscles of birds has been a subject of recent electron microscope investigations. In particular, the elements of myofibrils have been studied, such as, for example, the differences in the organization of the T-system between the front and back muscles of the backs of chicks (Page, 1969), and the breast muscles of poultry (Mendel, 1971). Hikida (1972) studies the structure of metapatagial muscles in pigeons, Adal (1973) studies the structure of the intrafusal muscle, and Hegarty, et al. (1973) studied the sarcomeres in birds and piglets in rigor mortis. Zelena and Jirmanova (1973), and Jirmanova and Zelena (1973) investigated neutrophil interactions and muscle integrity in the muscles of chicks.

The amount of mitochondria differs among the different types of muscles. The relation of mitochondria and myofibrils to each other contains information about the frequency of muscle contractions and their strength. The more mytochondria in the muscle fibers, the more frequent the contractions. When more myofibrils are present, the capacity increases, but the frequency is reduced. In general, mytochondria are concentrated perinucleate in striated muscles, but they are also found in cell membranes and myofibrils. The little ovoid mytochondria are usually found in pairs, and are located symmetrically near the Z-lines in the layer of the I-bands (McNaughtan, 1974). Most of the larger, filamentous mitochondria are situated without regard to their relation to the different sarcomere

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units.

In view of the fact that the mitochondria of the skeletal muscles, through their membraneous structure, form an energetic accumulation center and, in that they are highly sensitive to the influence of changed physiological states, it became the goal of this study to investigate the ultrastructure of these organelles in toto in the area of the thigh and breast muscles with regard to experimental hypokinesis.

INVESTIGATIONS

Methods and Materials

177-day old Japanese quails were used for the electron microscope study. At the age of 77 days, they were placed in cages for six hours of each in the following 100 days, in such a way that all movement was inhibited. 177 quails were raised normally and were used for control purposes. The necessary excisions were taken from the M. pectoralis thoracicus and from the M. iliotibialis posterior. One muscle strip was soaked for a period of two hours in a 3.5% fixing solution (after Sabatini, 1963). Excisions measuring 1 x 1 mm in size were subsequently taken from these strips. They were washed in a 0.1 m cacodylate buffer, fixed in a 2% OsO_4 solution, dehydrated in a graded series of ethanols, and finally embedded in Durcupan. Ultra-thin sections were produced with the Ultrotom LKB; these were colored with a combination of uranyl acetate (Watson, 1958) and lead citrate /538
(Reynolds, 1963), and then examined under a Tesla 513 electron microscope and photographed.

Results

The myofibrils of the striated breast and thigh muscles of the control animals formed repeating uniform sarkomers, which are defined as segments between the two following Z-lines, and which

contain the A-bands and half of the connected I-bands. The H-zone with the narrow M-line runs through the middle of the A-band (Figure 1). Two types of myofilaments were distinguishable: one comprised thin actin filaments running from the Z-lines to the I-bands; the second type consisted of thicker myosin filaments which ran into the A-bands. The interfibrilar space contained tubular and vesicular structures.

Figure 1. Longitudinal section through the thigh muscle of a quail from the control group. The H-zone (H) with a narrow M-line runs through the middle of the A-band. The filamentous mitochondria maintain a well-preserved and clearly defined internal structure. Magnification: 28500x.

Two shapes of mitochondria appeared in our results. Those in the breast muscles were mainly filamentous, while those of the thigh muscles ranged from small and round to ovoid shapes. Their inner structure contained a large number of closely aligned cristae, which ran vertical to the longer axis of the mitochondria.

In comparison to the control group, the mitochondria of the breast muscles of hypokinetic quails showed distinct changes.

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Both small and larger enclosed vacuoles and a fading matrix were found in some of the filamentous mitochondria, which were often perinucleated (Figure 2, 3). Sometimes these formations filled the entire organelle. The larger vacuoles are probably generated through the fusion of several small and lighter formations. Destruction and even disappearance of the mitochondrial cristae occurred at the location of the vacuole opening. Finally, a total decay of the mitochondria occurred as a consequence of these alterations (Figure 5).

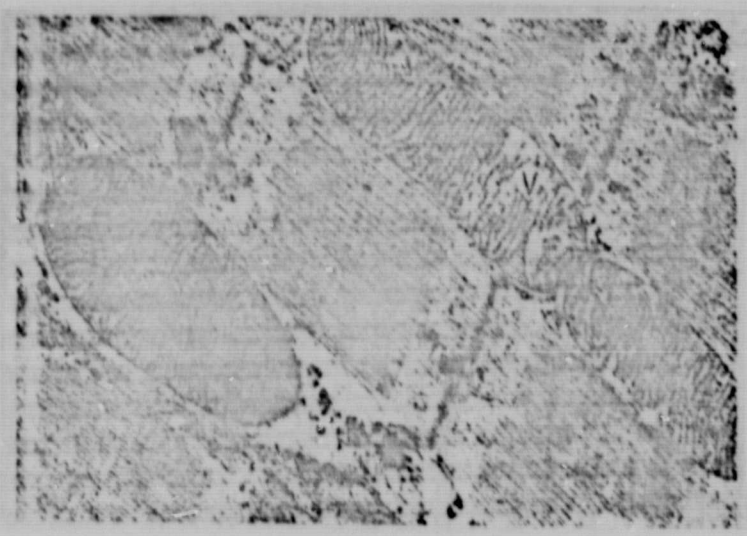

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Figure 2. Longitudinal section through the breast muscle of hypokinetic quails. The filamentous mitochondria (Mi) show a clearly defined internal structure which is only occasionally disturbed by little vacuoles. Magnification: 19650x. /539

Figure 3. Longitudinal section through the breast muscle of hypokinetic quails. The large mitochondria (Mi),

which are squeezed in between the myofibrils are larger than the sarcomeres and do not suffer damage in their outer membranes. These are large vacuoles in the matrix and the cristae show occasional changes. The Z-line (Z) is slightly shifted. Mag.: 19650x.

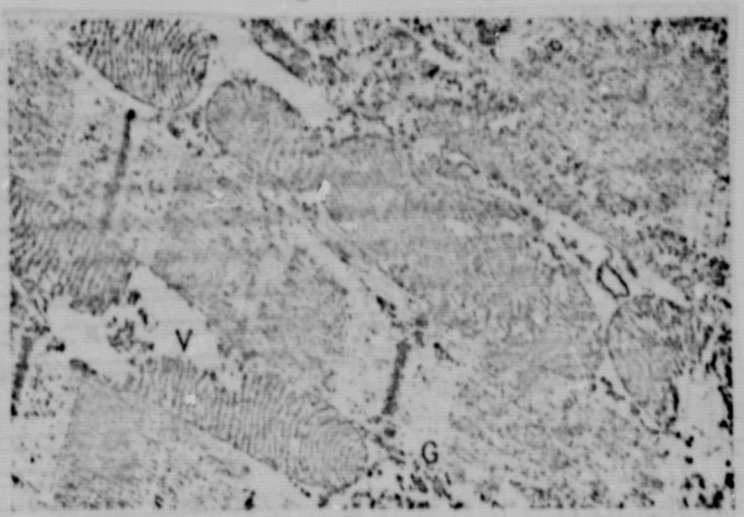

Figure 4. The cristae of the filament and ovoid mitochondria lose their continuity. The mitochondria (Mi) grow in size and are filled by large vacuoles (V), which probably generate from the fusion of smaller vacuoles. Among the mitochondria and myfilaments are numerous osmiofile granula (G). Mag: 19650x. /540

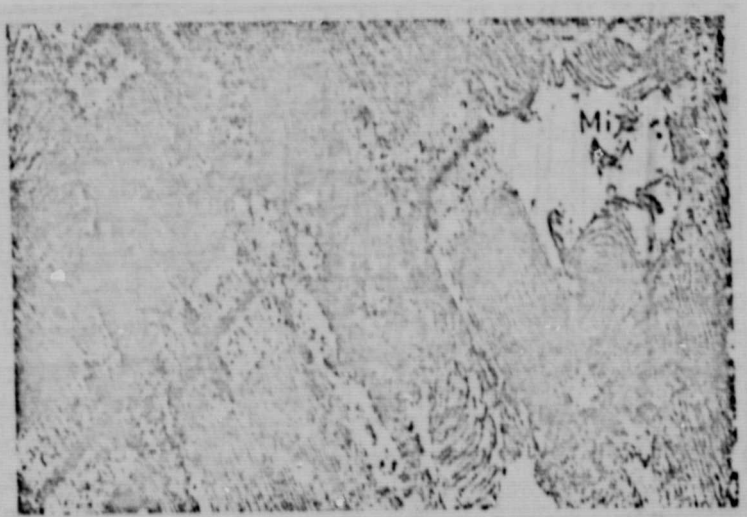

Figure 5. Longitudinal section through the breast muscle of hypokinetic quails. The membranes of the cristae within the mitochondria (Mi) lose their characteristic internal structure; the outer membrane is damaged as well. Mag.: 19650x.

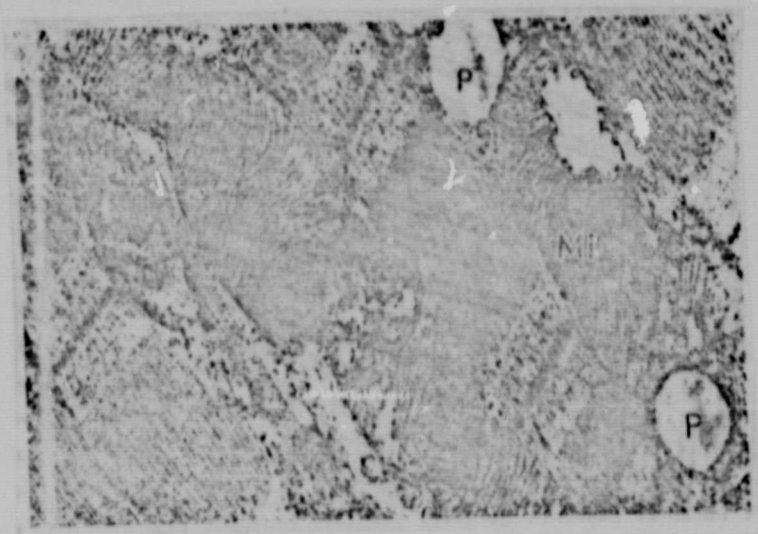

Figure 6. Longitudinal section through the breast muscle of hypokinetic quails. On both poles of the altered mitochondrion (Mi) are fat particles (P) measuring 0.8 µm. Mag: 19650x. ./541

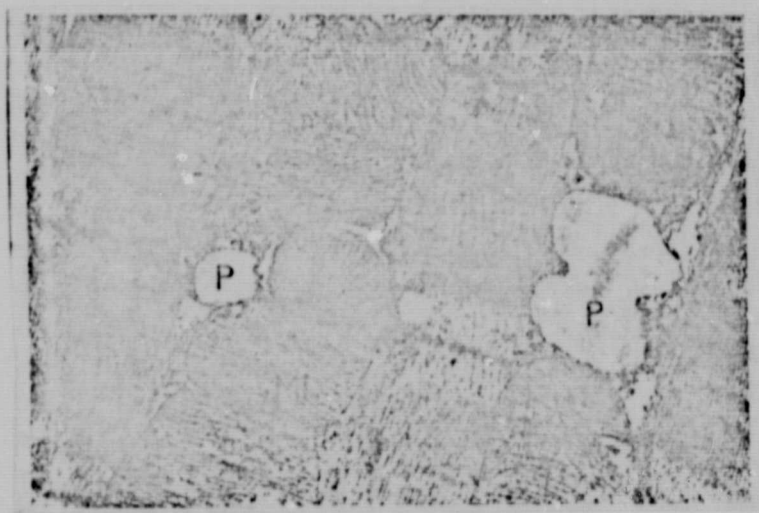

Figure 7. Longitudinal section through the breast muscle of hypokinetic quails. The inner membrane of those mitochondria which lie in close proximity to each other (Mi) are vacuolated. The large fat particles (P) are occasionally located in a lateral position. Mag.: 20750x.

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Figure 8. Longitudinal section through the thigh muscles of hypokinetic quails. The cristae disappeared in the middle of the altered mitochondrion (Mi), but the outer membrane is still intact. The H-zone is characterized by the alternating presence of light and dark filaments. A slight shift of the Z-lines is noticeable. Mag.: 16000x. /542

Figure 9. Longitudinal section through the thigh muscle of hypokinetic quails. There is a destroyed mitochondrion (Mi), the outer membrane of which is not destroyed. There is a triad (T) in the vicinity of the Z-lines. Mag.: 16000x.

A further interesting finding concerned the fat particles, which were approximately 0.8 μm in size, and were localized primarily at the poles of altered mitochondria (Figure 6). Occasionally, they were also observed in lateral locations.

The mitochondria of the thigh muscles of the test animals /543 showed changes similar to those of the breast muscles. The following characteristic features were observed: illumination of the matrix, disappearance of cristae, and the destruction of cristae without damage to the outer membranes (Figure 8, 9).

The sarcomeres of the breast and thigh muscles of the hypokinetic quails were characterized by the constant exchange of A- and I-bands. The Z-lines, however, did not form a coherent zone, but showed a slight shift in some cases.

Discussion

Our investigations showed that the mitochondria in the M. pectoralis thoracius of Japanese quails are numerous and consist of large filamentous and small ovoid mitochondria. They are located perinucleate and in the cell membrane; sometimes they are between the myofibrils. The M. iliotibialis posterior contains fewer mitochondria than the M. Pectoralis thoracicus, and contains more small round and ovoid mitochondria. These are mainly localized in the layer of the isotrop zone of the sarcomere.

In the longitudinal sections of the thigh muscles of hypokinetic quails, the Z-lines which separate the sarcomeres (Allen, 1973) could not be found in one layer. Rather, they made the impression that they were shifted. This phenomenon could stem from an adaption of the myofibrils to altered conditions (Zelena and Jirmanova, 1973). The function of the mitochondria, defined by their enzymic content, is their role in cell respiration, or oxidation, i.e., the transport of electrons from the different substrata to the final oxygen source. Aside from their job of delivering energy for cell functions, these organelles also use energy to keep the cell structures in their normal state (Brozman, 1968). They are very complicated units and are highly sensitive to the impact of alterations. Diculesca and Popesca (1973) suggest that they also form an accumulation zone

for calcium in the striated muscle system. Barta, 1973, Devine, et al., 1973, think that this happens when there is a surplus of stored calcium in these muscles. The calcium is probably transported through the transversal channels of the mitochondria.

The electron microscope studies of the mitochondria of the breast and thigh muscles of Japanese quails with regard to experimental hypokinesis have shown that the changes which are caused by this state can be observed on single mitochondria without all of them being affected.

During hypokinesis, the breast muscles exhibited mainly orthodox forms of organelles, whose typical structure was maintained, and which was characterized by the formation of numerous long mitochondria cristae, the latter filling the gap between the outer membranes. The formation of cristae is a measure of the magnitude of energy production, shown in the amount of ATP formed by the mitochondria.

In some of the mitochondria, small light vacuoles could /544
be observed; these were generally located in the inner periphery of the outer membrane. A comparison of the electrograms proved that the alterations probably manifest themselves as a fusion of small vacuoles into larger ones, which means that the continuity of the mitochondrial cristae was disturbed and the matrix illuminated. It is interesting that, in this state, no disturbance of the outer cell compartment occurs. The only alteration observed in these mitochondria was a different grade of hydration. The final result of the alterations was, in some cases, a total destruction of the mitochondria whereby the outer cortex disappeared entirely.

It appears that as long as the outer membrane of the mitochondrion is maintained, this process is reversible, and can

therefore be regarded as the minimal damage which can occur through lack of oxygen caused by inactivity.

It is assumed that particularly the breast muscles of flying birds are metabolically much more active, and have a higher rate of oxidation activities. Similar conditions, although less active, seem to prevail in the thigh muscles. Therefore, after lengthy subjection to hypokinetic conditions, energy insufficiency and, as a consequence, hydration of the mitochondria, will occur. The initial alterations concern the mitochondria cristae, where the enzyme systems of the oxidation phosphorylation and of the electron transport are located. The penetration of water into the mitochondria removes or destroys these enzyme systems. Consequently, the required energy can not be produced, which causes further damage of the mitochondria (David, 1967, Krolenko and Svinka, 1974).

During extensive restricted lifting movements of the limbs of dogs, Kuprjanov et al. (1975) observed a reduction in the size of the mitochondria, whereby small round forms with a reduced number of cristae and a thickened matrix were the majority. The mitochondria only decayed in places where myofibrils underwent the largest alterations.

A moderate increase of particles normally located at the poles of the mitochondria, or at lateral locations, also indicates a certain lack of oxygen.

It can be concluded from our investigations that the alterations of mitochondria of the breast and thigh muscles of Japanese quails with regard to experimental hypokinesis are probably caused by energetic insufficiency followed by hydration of the organelles. Because of the damage of the mitochondria cristae and the matrix, the function of the enzyme system of the mytochondria is disturbed. As a consequence, certain catabolic

alterations in the oxidation and reduction process in muscle activity can occur.

Summary

Electron microscopic studies have been carried out on the mitochondria of the transversely striated muscles with regard to experimental hypokinesis. As compared to the central group the mitochondria of m. pectoralis thoracicus and the m. iliotibialis posterior in hypokinetic birds reveal marked changes. In filamentous and ovoid mitochondria vacuoles can be observed which in some cases produced larger light formations with following disappearance of the cristae and destruction of mitochondria. Fat particles located at the poles of the altered mitochondria, sporadically occurring also laterally, presented another finding. The Z-lines of the sarcomere did not form a continuous line, but were somewhat shifted.

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