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ELECTRON MICROSCOPICAL AND HISTOCHEMICAL STUDIES ON THE TRANSVERSE STRIATED MUSCLES OF BIRDS AFTER PROLONGED HYPOKINESIS

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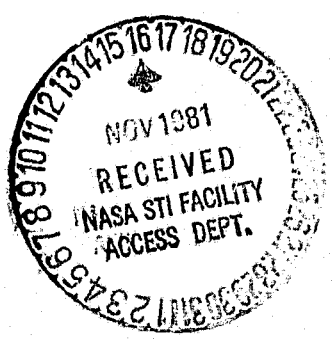

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16. Abstract: Electronmicroscopical and histochemical studies of the gastrocnemius muscle has been carried out in 4 month old cockerels of the laying hybrid after hypokinesis lasting 15 and 30 days. It was found that restricted movement resulted in dystrophic changes of myotibrils, enlargement of the sarcoplasmic reticulum and oedem of interfibrillar spaces. Histochemical studies revealed focuses of increased activity of non-specific esterase, decreased activity of dehydrogenase of lactic acid and a positive reaction of acid phosphatase.

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ELECTRON MICROSCOPICAL AND HISTOCHEMICAL STUDIES ON THE TRANSVERSE STRIATED MUSCLES OF BIRDS AFTER PROLONGED HYPOKINESIS

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*/37

In the previous studies (Belak et al, 1977), the ultrastructure of the mitochondria of the transverse striated muscles of birds after 100 days of hypokinesis and hypergravitation was described with reference to the effect of these stress states on the changes in the above mentioned organelles.

Since increasingly large lesions were always found in hypokinetic states, in our further study, we directed our attention to the effect of the limited movement on the overloaded tibia muscles.

I. Our studies

I.1. Material and methods

In the experiment, four-month old cockerels of laying hybrids were used. The test animals were kept in cages without possibility of movement. The food dishes and water containers were in the front part of the cage so that the animals had free access to the food. The cockerels were divided into three groups. The first group included 10 animals which were kept for 15 days in the above described cages while the second group included 11 animals kept under hypokinesis for 30 days. The third group represented control animals who were permitted free movement. The excisions were taken immediately after killing from the M. gastronemius and processed according to the methods indicated in earlier publications (Belak et al, 1977).

*Numbers in margin indicate pagination of foreign text.

In order to establish the activity of the acid phosphatase and non-specific esterase, in the histochemical study, assocopulation methods, dehydrogenasis of lactic acid and a modified water incubation medium were applied (Lojda and Papousek, 1970).

1.2. Results and discussions

In view of the fact that the mitochondria of the skeletal muscles form an accumulation center through their membrane arrangement, and their main function consists in cell respiration, it was possible in a disturbance of the normal processes in the first phase of hypokinesis to find quickly the unfavorable effect. The latter was manifested in the first group by the multiplication and concentration over very large areas and in a thickening of the internal membranes (Figure 1). Themitochondrial crests are very numerous and show a continuous variation. We find only in the later stages a mild transparency of the matrix with small vacuoles. Part of the mitochondria assume larger dimensions, while they are sporadically bloated. Often mitochondria are affected whose length /40 exceeded the sarcomere. The occurrence of increased, concentrated and gigantic mitochondria supports the hypothesis of increased energy needs and that of an increased metabolism in the damaged muscle. To this corresponded also the histological and chemical finding of an increased activity of the non-specific esterase (Figure 2). At the same time in this group we also established grey and as a rule round fat particles (Figure 3), regarding whose occurrence we know, that they represent a source of energy for muscular activity. The non-specific esterase hydrolyzes the esters of the lower fatty acids which are then burnt up in the Krebs cycle of the tricarbonic acids, taking place in the undamaged mitochondria. An overflow product of the fat disintegration is water and it is therefore possible that under certain conditions of increase decomposition of esters of the fatty acids and with insufficient fluid elimination from the tissues, there should be accumulation in the tissues and oedema should arise.

Figure 1. Mitochondria (Mi) concentrated over a very large area. They contain dense internal membranes so that their structure is not very striking. Enlargement: 30300

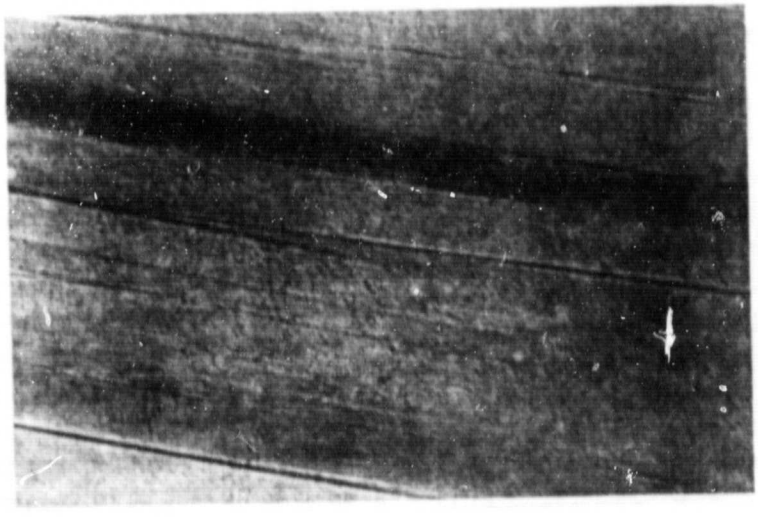

Figure 2. Increased activity of the non-specific esterase in dystrophic muscle fibers. Enlargement: Obj: 20, ocul., 3.2.

The dystrophic process developing in the 15 days od hypokinetic animals involved almost all the components of the muscle tissue. Extended cisterns of the sarcoplasmic reticulum (Figure 4) were observed, also changes in the myofibrils.

Figure 3. Squeezed fat particles (F) are found between closely arranged p 39 mitochondria (Mi); enlargement: 30300

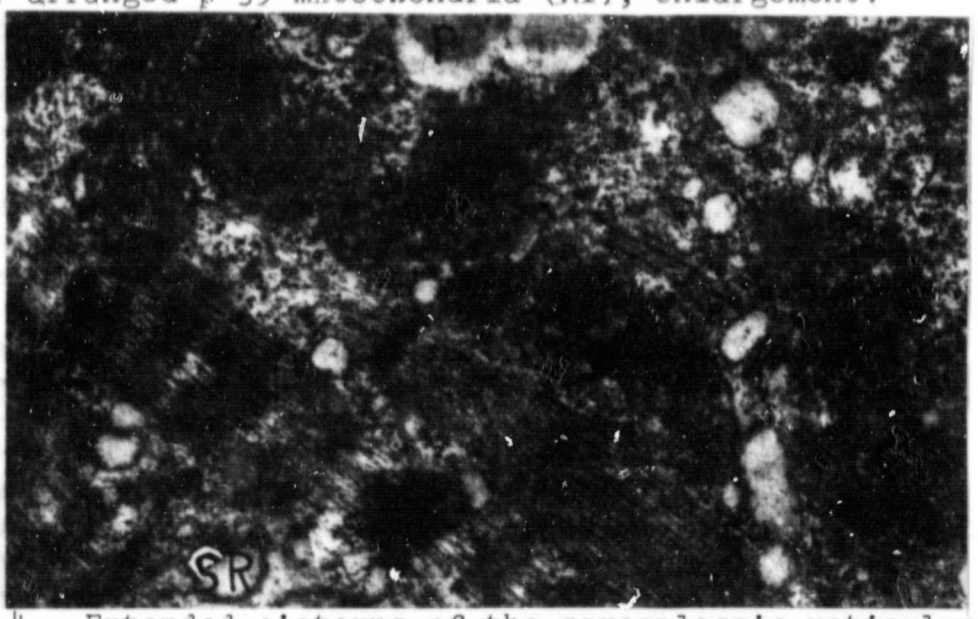

Figure 4. Extended cisterns of the sarcoplasmic reticulum (SR) are visible near the Z-line. Fat particles (P) Enlargement: 30300

Figure 5. Disturbed Z lines (arrow) designated as zig-zag line and vague boundary between I and A lines. Enlargement 30300 x

Figure 6. Reduced activity of dehydrogenase of lactic acid in the dystrophic muscle focus (arrow). Enlargement: Obj. 16, ocul. 4

The loss of transverse striation with no established boundary of actin and myocin fibers was particularly striking. The Z-lines bounding the individual sarcomeres lost their straight course (Figure 5) which was described by many authors as an accompanying phenomenon of the dystrophic processes and is designated as the so-called zig-zag (Z-) line. The differences described by us in the myofibrils, sarcomeres, Z-lines and mitochondria, which differed from the normal undamaged muscular tissue are still reversible. This is also confirmed by the reduced activity observed by us in the dehydrogenase of lactic acid (Figure 6). Portugalov (1971, 1976) and Ilyina Kakeuva et al.(1976) describe similar focus like dystrophic changes in animals exposed to hypokinesis induced and other stress states which were not defined as specific, short term and reversible. The dystrophic foci which we found are reversible, since they occurred as a rule only in isolated muscle fibers with preserved sarcolemma so that a relative extensive regeneration of the transverse striated muscles is possible (Novotny, 1966). This hypothesis is also favored by the studies of Jozsa (1974) who after traumatization of the skeletal muscles in rats found, similarly to our results, a reduced dehydrogenase activity of the lactic acid. /41

In the group of animals who were kept for 30 days in conditions of hypokinesis it was possible to observe a considerable development of dystrophic changes, manifested in the disintegration of the muscle fibers (Figure 7). The myofibrils concerned exceeded in number the undamaged fibers while they were mostly concentrated in the process over large areas. Frequent interspaces were also noted between the myofibrils, while the latter were oedematic (Figure 8). The sarcomeres remained preserved but the Z-lines showed less visible outlines, while the I-bands lost their characteristic structure. This ended in a destruction of the muscular tissue. In the oedematous area there were fragments of the sarcotubular system with isolated myosin fibers (Figure 9). The described changes also included modifications in the tubular system.

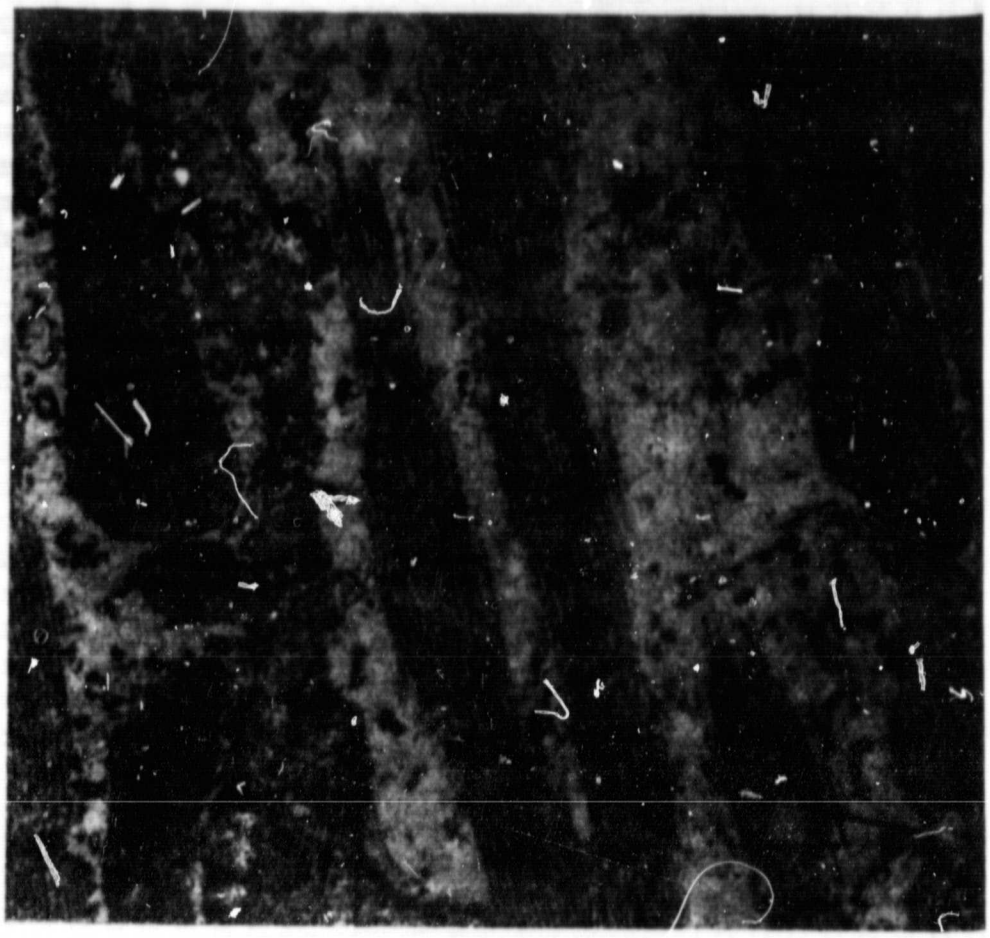

Figure 7. Focus-like decay of the muscle fibers, mitochondria (Mi) vacuolized (arrow). Enlargement: 30300 x

The positive reaction of the acid phosphatase was observed in the hypokinetic animals in regularly defined areas in the muscle tissue and was manifested in a darker color of the cell elements which were generally arranged radially. Some of them formed foci penetrating irregularly into the surrounding medium (Figure 10). It seems that there is decomposition of the basic muscular substance. In muscular dystrophies, several authors (deRobertis, 1965; David, 1967; Pitt, 1975) describe, beside increased number of mitochondria, a multiplication of the lysosomes, whose missions appears more clearly in the tissues with damaged function. Acid phosphates /42

Figure 8. Separated myofibrils (My) with oedematous intermediate spaces (arrow). Enlargement: 30300

is located in these organelles and its increased activity supports the hypothesis of its effect in the dystrophic muscle.

Morphometric calculations gave data concerning the number and size of the mitochondria surfaces in the individually followed time intervals. Thus, the average number of mitochondria was lower for 15 days old hypokinetic birds, while the area was larger, whereas the contrary happened in the group of 30 days old animals. This finding is explained by the fact that in animals exposed to hypokinesis for 30 days, which was manifested in increased requirements on the organism, a multiplication of young mitochondrial forms is obtained (Portugalov, 1971; Frolov, 1972) which can testify to an adaptation in the effort at compensation of this stress situation. /43

All the changes described here reached their maximum on the 30th day of hypokinesis which proves that the organism has dealt with the new effects and compensated for them, adjusted to them and gradually reached the same level. Portugalov (1976) and Melechin

and Grigin (1977) established that these changes observed have disappeared and the muscles have been restored morphologically and histochemically to their initial state when the animals return to normal conditions.

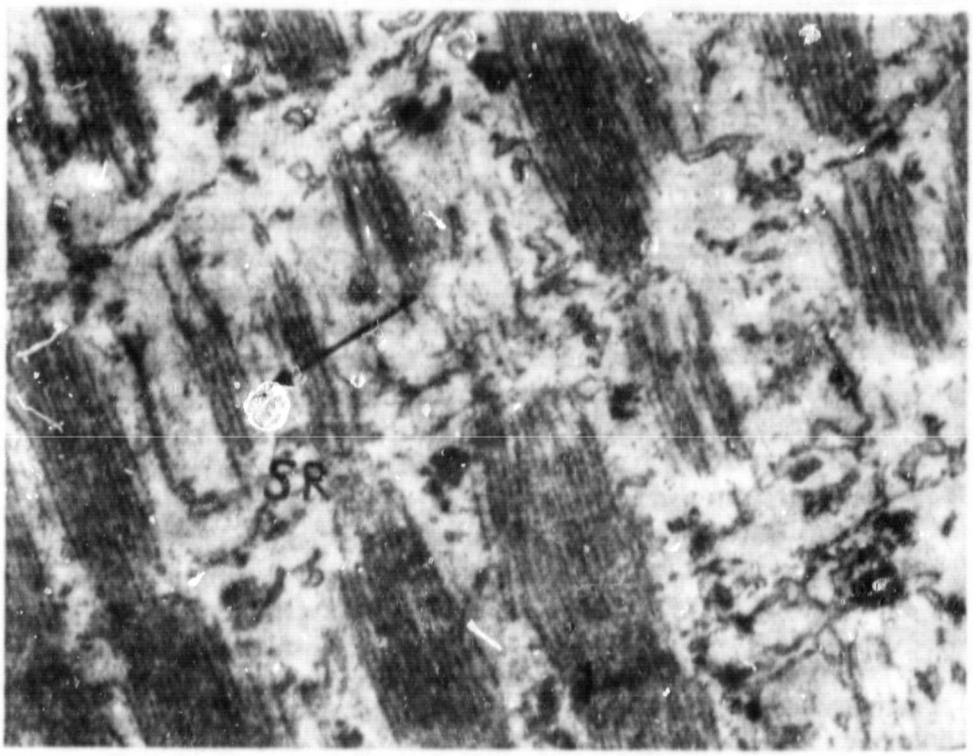

Figure 9. I-bands (I) in the damaged sarcomeres lost their striking structure, in the oedematous area there are fragments of the sarcotubular system (SR) with isolated myosin fibers (arrows)
Enlargement: 30300

In conclusion, it may be stated that our observations correspond to the typical picture of a dystrophic process, as was described by many authors for different nutritional deficiencies (Cheville, 1966; Shafiq, 1971), hereditary dystrophies (Julian, 1973), myopathies (Jones, 1974) and in the administration of toxic substances. The lack of activity of an organ or its weakened function caused by limitation of movement or total hypokinesis,

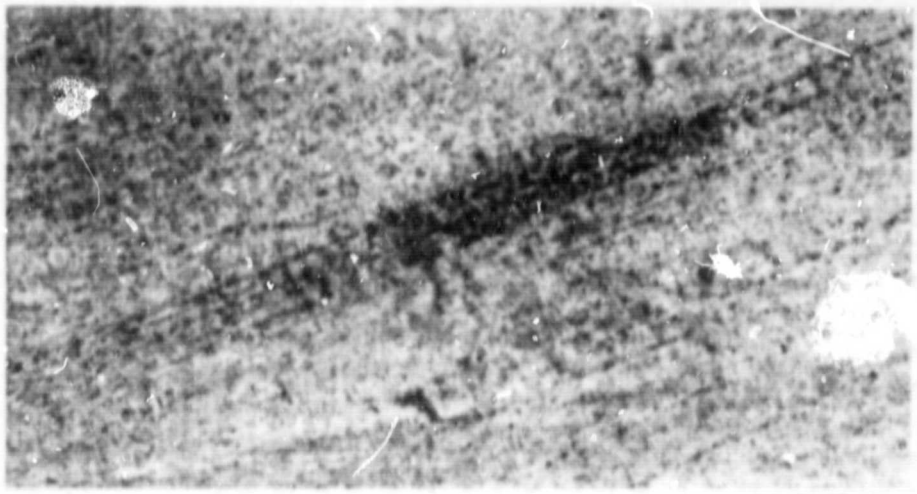

Figure 10. Increased activity of the acid phosphatase in the dystrophic muscle focus
Enlargement: Obj. 20; ocul. 3.2

cause a large number of cytochemical indices, which is manifested in the last phase in morphological changes. The primary cause resides probably in the inadequate or interrupted perfusion of the muscle which when continued very long leads to the development of pathological changes. It is necessary to find the limits, for which the changes are still reversible since we were not able to find any similar stage in the degree of the pathological changes. In some cases, these alterations encompassed larger areas of muscle tissues, or again only individual groups of fibers.

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