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Naturally-occurring degeneration in chick muscle development: ultrastructure of the M. complexus

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INTRODUCTION

Interest in the chick's complexus muscle stems from reports suggesting it plays an important role in the hatching process (Fisher, 1958; Smail, 1964; Hamburger & Oppenheim, 1967; Rigdon *et al.* 1968; Bock & Hikida, 1968, 1969; Brooks & Garrett, 1970). Several of these studies (Fisher, 1958; Bock & Hikida, 1968; Rigdon *et al.* 1968) have indicated progressive histological alterations in the muscle with the approach of hatching. Other studies (George & Iype, 1963; Brooks & Ungar, 1967; Klicka, Edstrom & Ungar, 1969; Ramachandran, Klicka & Ungar, 1969; Klicka & Kaspar, 1970) have shown biochemical changes which could well be related to the preparation of the M. complexus for its postulated role in the hatching process. Such considerations have led to the complexus being referred to as 'the hatching muscle'.

Among the histological observations was that, at 14 days of incubation, many of the chick's complexus fibres contained swollen fibrils. These fibres were of larger diameter than surrounding ones and were more densely stained. The swollen fibres are believed to break up and disintegrate later (Bock & Hikida, 1968). Cells with swollen myofibrils are most abundant during the fourteenth day of incubation. It was decided to study these fibres more fully with the electron microscope.

MATERIALS AND METHODS

Fertilized eggs of White Rock (Heavy) chickens, obtained from Ehrler's Hatchery (Lancaster, Ohio) were incubated at 37·5 °C to the desired developmental ages. Embryos and chicks were examined at 12, 14, 15, 16 and 18 days of incubation and at 4 days after hatching. At least four embryos from each incubation period were killed by decapitation. Both the M. complexus and the pectoralis major were fixed *in situ* in Karnovsky's (1965) solution, post-fixed in phosphate buffered osmium tetroxide, dehydrated, and embedded in an Epon–Araldite mixture. The pectoralis major muscle served as a control. Sections were made on a Reichert Om U2 ultramicrotome, contrasted in aqueous uranyl acetate and lead citrate, and examined on a Siemens Elmiskop I modified for high resolution.

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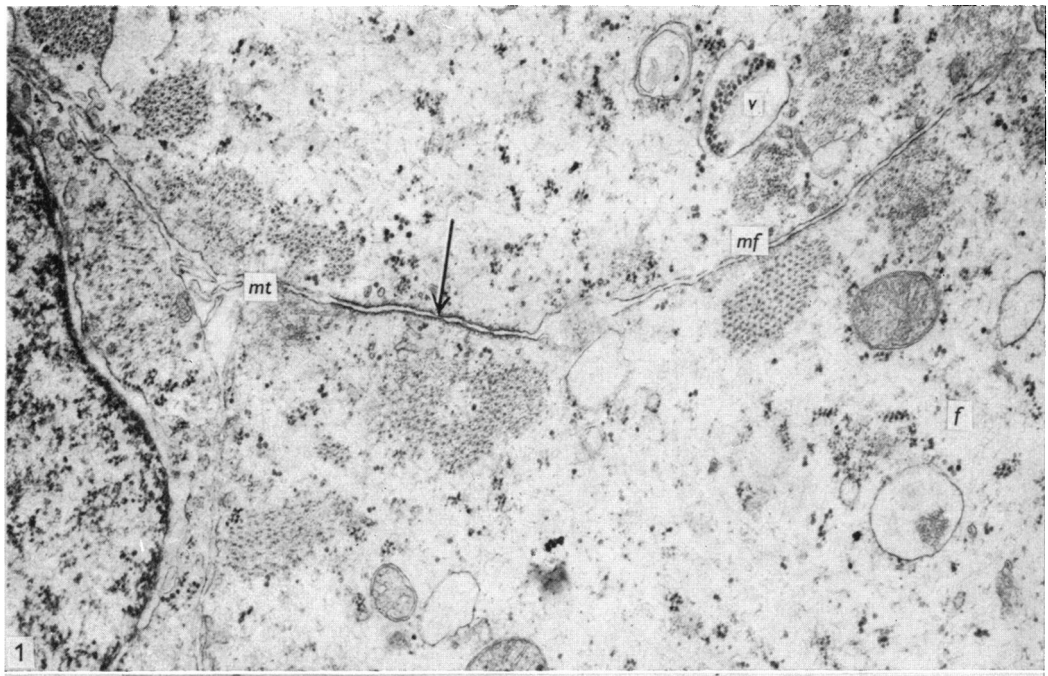


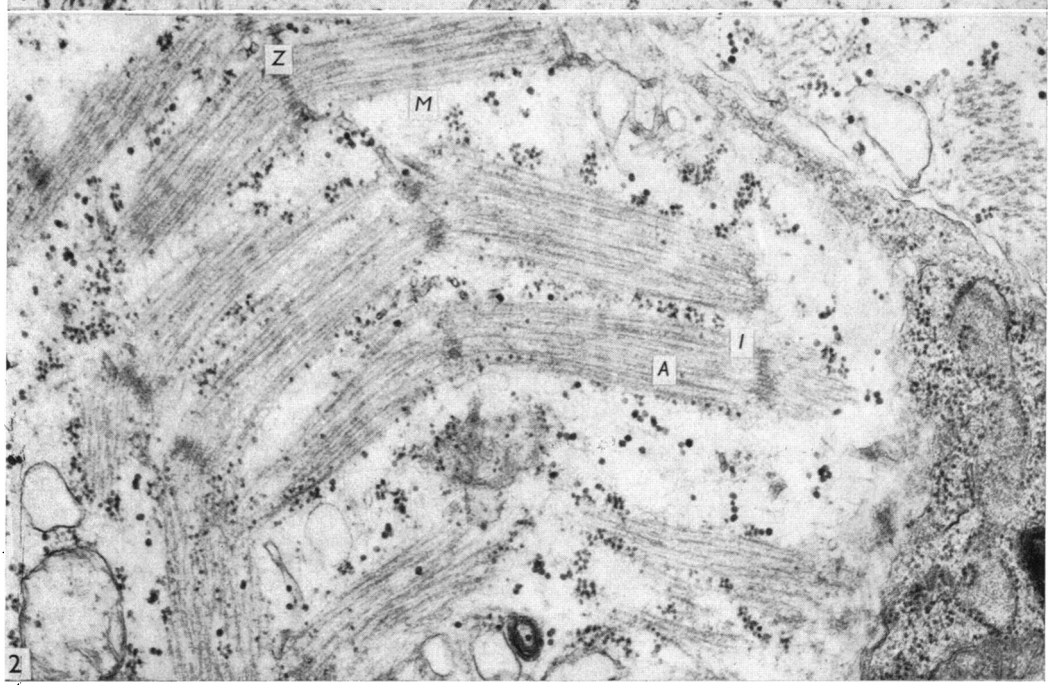


Fig. 1. M. complexus of a 12 day chick embryo. Glycogen-containing vesicles or autophagic vacuoles (*v*), microtubules (*mt*) and filaments (*f*) are present in the cytoplasm.The myofibrils (*mf*) are located at the periphery of the myotubes, indicating early myofibril formation. Adhering junctions (arrow) are visible between two myotubes. ×27000.

Fig. 2. M. complexus of a 12 day chick embryo. The myofibrils show well developed Z (*Z*) and M (*M*) lines, and clear A (*A*) and I (*I*) bands. The irregular arrangement of myofibrils is common at this stage of development ×34000.

RESULTS

Development of the fibres of the M. complexus is asynchronous. Staging the embryos by days of incubation served merely to increase the probability of observing early muscle development in the younger embryos, and well developed fibres in the older embryos. Although various stages of muscle development could be observed at most incubation periods, the description of fibres containing contracted myofibrils is based on 14–16 day old embryos, when contracted myofibrils were most commonly observed.

12 *days of incubation*

Typical early muscle development was observed in the M. complexus at this incubation period. Because of asynchrony of development, various stages of myofibrillar assembly and differentiation were to be seen in different fibres. Many fibres showed the typical early developmental form recognizable by the peripheral location of the myofibrils (Fig. 1), the sarcoplasm otherwise containing only microfilaments and ribosomes. Many fibres showed what appeared to be isolation bodies or autophagic vacuoles (Fig. 1). The better developed fibres contained myofibrils with distinct Z and M lines, and A and I bands (Fig. 2).

Microtubules were common in the peripheral sarcoplasm (Fig. 1). The cells typically showed enlarged vesicles, which were drawn into long tubular shapes as they approached the Z line areas of the developing sarcomeres. The myofibrils were irregular in cross section, and the sarcoplasmic reticulum invaded the various crevices formed by this irregular shape. The sarcolemma showed fuzzy-coated vesicles (Lipton & Konigsberg, 1972), and when the sarcolemma was highly convoluted, closely juxtaposed cells were correspondingly convoluted.

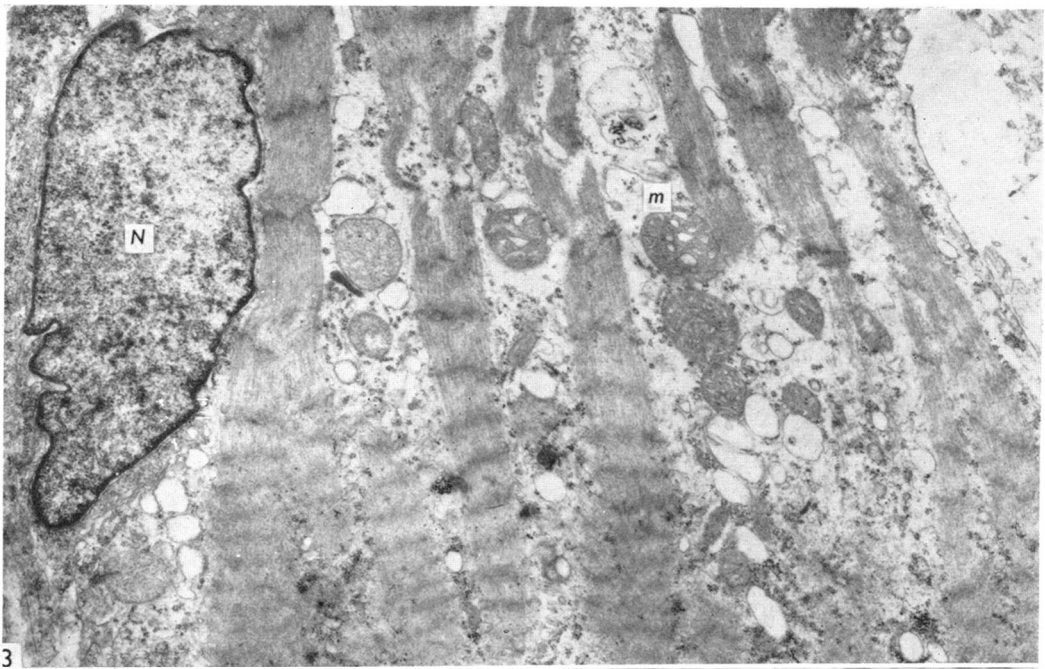
Closely associated with many of the fibres or myotubes were cells (Fig. 2) which had the general appearance of mesoblasts (Fischman, 1970). Mesoblasts are characterized by their sparse content of endoplasmic reticulum and by large numbers of ribosomes which are often clumped and are not associated with membranes; they show areas of clear cytoplasm and plenty of glycogen. The nucleus is large and ovoid, with a conspicuous nucleolus. Most distinctive is the absence of a basal lamina between the myofibre and the mesoblast cell.

Some of the developing fibres showed pronounced contraction of their myofibrils particularly fibres which had only peripheral sarcomeres. When the contraction zones were near the cell surface, the sarcolemma was highly convoluted. Filaments extended from the contracted myofibrils, and many of them extended into the convoluted areas of the sarcolemma. The mitochondria of these cells, although still maintaining an elliptical shape, had enlarged cristae.

14 *days of incubation*

At this stage two types of fibre were present in the M. complexus. The more numerous were similar to those described for the earlier period of incubation and were like the fibres found in the control pectoralis muscle. The fibres showed a highly developed sarcoplasmic reticulum, and triadic junctions were common. Caveolated vesicular systems were observed near the Golgi complex and nucleus.

The other type of fibre found at this stage showed contracture in varying degree,



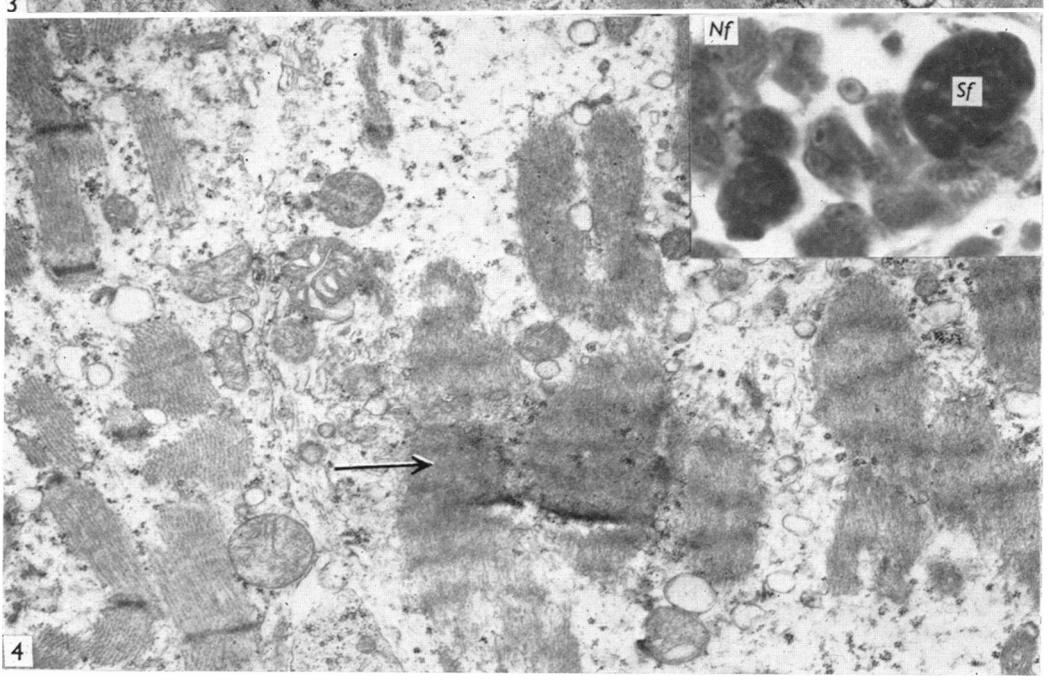


Fig. 3. M. complexus of a 14 day chick embryo. Various degrees of contraction are observed along a myofibril. Some of the mitochondria (*m*) show enlarged cristae. The nucleus (*N*) is located at the periphery, suggesting a mature muscle fibre. × 11000.

Fig. 4. M. complexus fibres of a 14 day chick embryo showing contracted myofibrils (arrow), and an adjacent cell with a more typical sarcomere length. × 15000. Insert. A paraffin-embedded light microscopic photograph of a 14 day M. complexus muscle containing normal (*Nf*) and swollen (*Sf*) fibres. × 960.

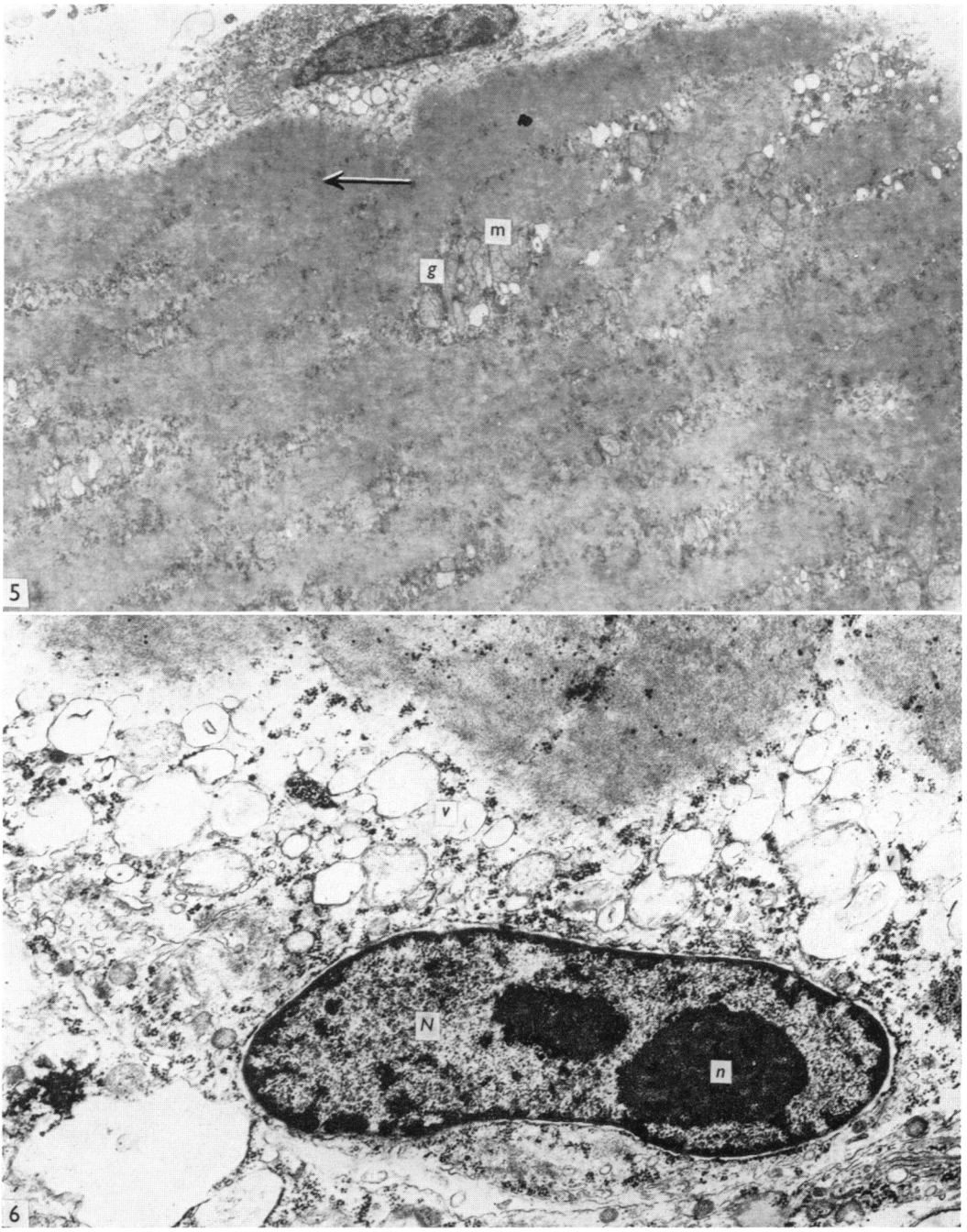


Fig. 5. M. complexus of a 14 day chick embryo. Extremely contracted myofibrils, with indistinct Z lines (arrow). Mitochondria (*m*) and glycogen (*g*) are compressed into small areas in the amorphous mass of myofibrils. ×8400.

Fig. 6. M. complexus of a 16 day chick embryo. The nucleus (*N*) contains a dark condensed nucleolus (*n*), and an alignment of vesicles (*v*) is seen medial to the nuclear region. The myofibrils are highly contracted, and the Z lines are indistinct. ×22300.

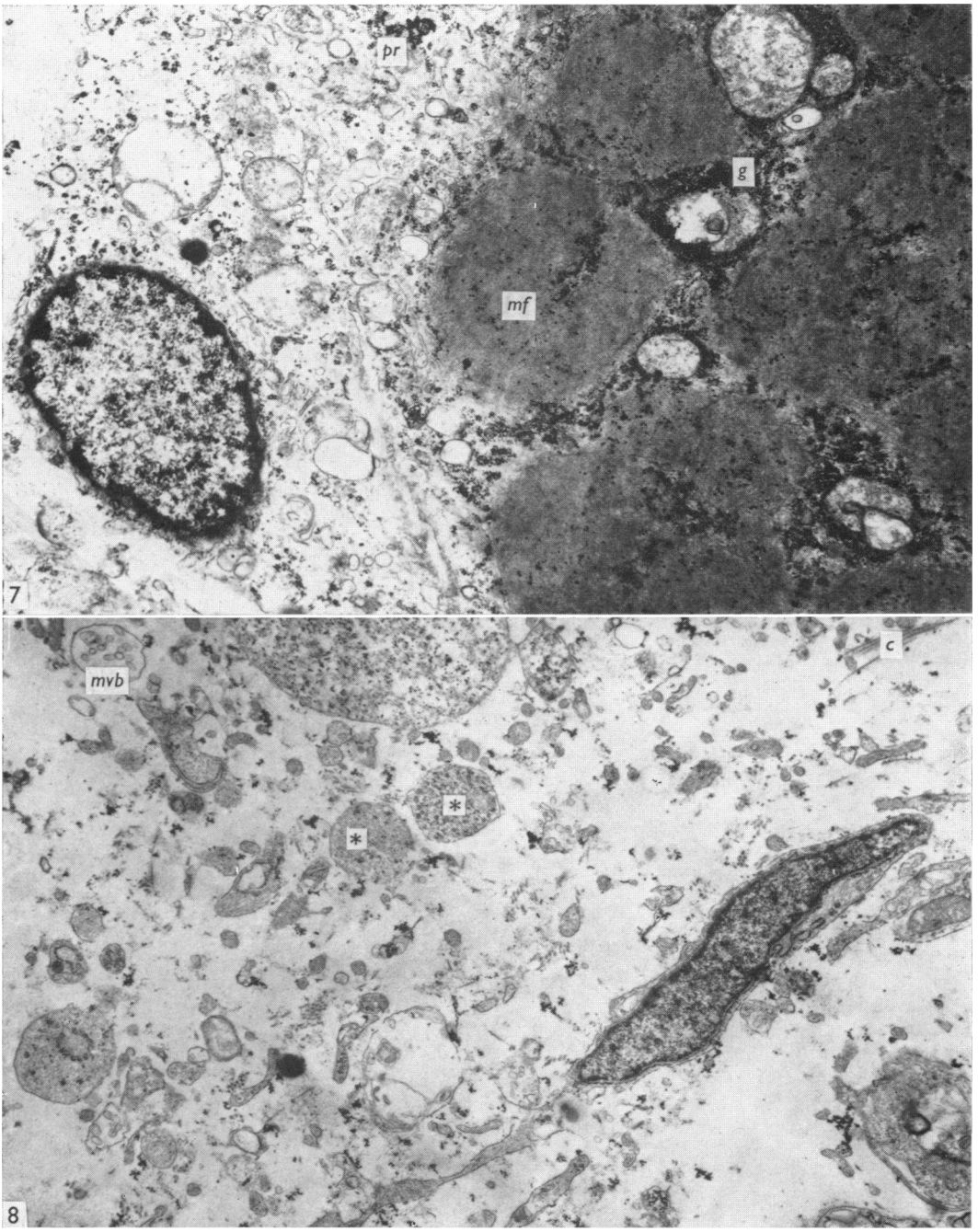


Fig. 7. M. complexus of a 16 day chick embryo. A more complete separation line appears between the amorphous myofibrils (*mf*) and nuclear area. Concentrations of what appear to be glycogen granules (*g*) are in the myofibrillar areas, although the granules are not much larger than ribosomes; ribosomes are seen as polyribosomal aggregates (*pr*) at the periphery of the cell. ×11500.

Fig. 8. An area of what appears to be cellular debris, from sections of the *M.* complexus muscle of a 16 day chick embryo. Collagen (*c*), small packets of cytoplasm (*), and structures resembling multivesicular bodies (*mvb*) are distributed randomly through the area. ×16000.

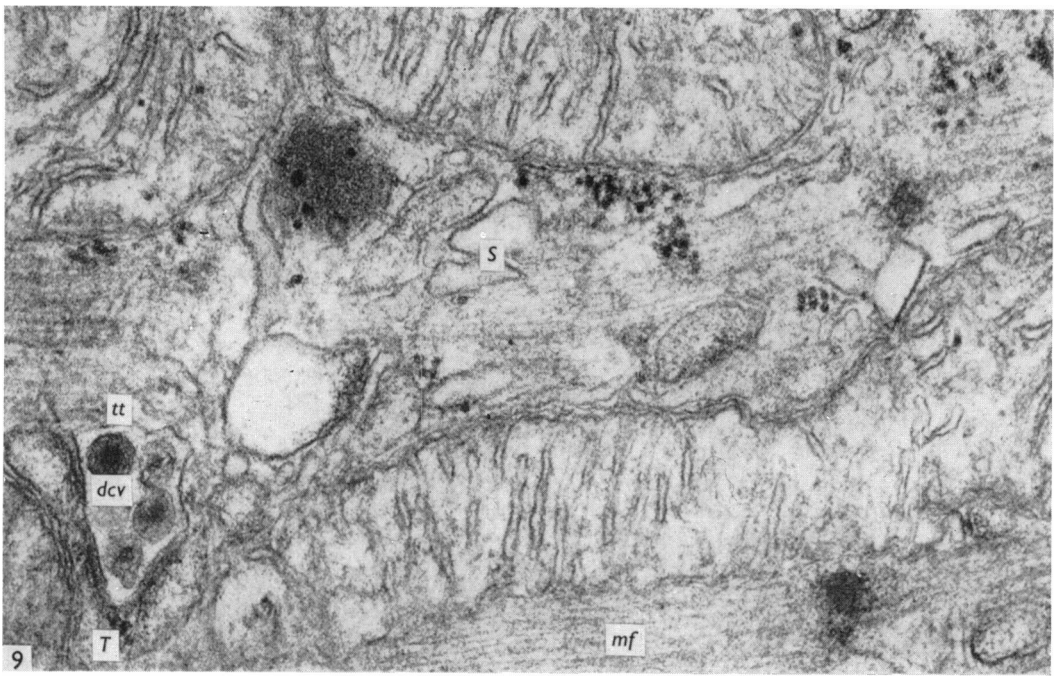


Fig. 9. M. complexus muscle of a 4 day chick. Well developed sarcoplasmic reticulum (*S*) and myofibrils (*mf*) are seen at this stage of development. The triadic junctions (*T*) contain horizontal as well as transversely oriented transverse tubules (*tt*). Dark core vesicles (*dcv*) are common in the transverse tubules and intercellular spaces at this time of development. × 73400.

even along a single myofibril (Fig. 3), and cells containing contracted myofibrils were often surrounded by cells with relaxed sarcomeres (Fig. 4). When most contracted, the banding pattern of a myofibril was no longer visible (Fig. 5). In these fibres the cell organelles were compressed into tight bundles between the myofibrils. Contracted myofibrils were found in both myotubes and muscle fibres. The sarcolemma near the contracted myofibrils did not show the highly convoluted form that was observed at 12 days of incubation. In the contracted fibres, the mitochondria had swollen cristae and had more rounded profiles than in more 'normal' fibres.

16 *and* 18 *days of incubation*

Many fibres still showed contracture, and in some of these the nucleus appeared separated from the contracture area by an alignment of enlarged vesicles (Fig. 6), such as would be present in the formation of myoblasts or satellite cells. In other fibres a more continuous line of separation appeared just medial to the peripheral nucleus (Fig. 7). Often material which was interpreted as cellular debris was found between muscle fibres (Fig. 8).

4 *days post-hatching*

All cells appeared normal, with well developed myofibrils and sarcoplasmic reticulum (Fig. 9). At this stage of development the M. complexus showed no fibres with contracture zones.

Closely associated with the sarcolemma were numerous structures resembling dark core vesicles which were often seen within the lumen of the transverse tubular system of the myofibres (Fig. 9). Where the dark core vesicles were present in the transverse tubules, the tubules were greatly distended to accommodate them. At the surface of these cells the dark core vesicles were often found in association with a pinocytotic area. The large number of these bodies was striking at this stage. From reviewing other studies of chick muscles, such bodies would appear to be characteristic of certain stages of development.

The control muscle, the pectoralis major, which was removed with the M. complexus and carried through the same procedures, showed none of the features occurring in the contracted fibres of the complexus, 'hatching' muscle.

DISCUSSION

During late embryonic development the M. complexus becomes enlarged because of an increase in fluid content. Fluid uptake begins 3 or 4 days before hatching, and may be increased by progesterone (16 hydroxyprogesterone and 17 hydroxyprogesterone; Brooks & Ungar, 1967). Acid mucopolysaccharide concentration increases during the final four days of incubation, being highest on the day of hatching. Klicka *et al.* (1969) suggested that the mucopolysaccharides, along with water, enable the M. complexus to act as a cushion during hatching. Daily assays of the M. complexus and gastrocnemius during pipping and hatching showed water imbibition and a decreased glycogen content in the hatching muscle (Ramachandran *et al.* 1969). They showed that myosin ATPase and acid phosphatase also increased at hatching, and these enzyme changes preceded the muscle fibre breakdown.

On histological grounds Bock & Hikida (1968) suggested that the oedema of the complexus was due to the osmotic effect of the breakdown of products of specialized fibres during the last days of incubation. Our electron microscope studies support the suggestion of muscle degeneration occurring prior to hatching in the M. complexus of the chick. The early stages of myofibril contraction closely resembled muscle degeneration. The fibre degeneration of the M. complexus most closely corresponds to Tomanek & Lund's (1974) description of degeneration in fast-twitch-oxidative-glycolytic fibres of the guinea-pig hind limb. In the guinea-pig there is little Z line streaming, and the myofibrils appear to maintain a parallel arrangement of filaments and Z lines during the initial degenerative stages. Later stages of degeneration, however, show only an amorphous mass of myofibrils.

The presence of these highly contracted myofibrils could be determined during the sectioning of the blocks. As the sections were made, they showed the same puckered appearance as sections containing a large amount of collagenous material. As the muscle did not contain an excessive amount of collagen, this appearance was probably caused by the density of the tightly packed myofilaments in the contracture zone. It might also be due to the presence of large mineral deposits in association with the filaments. A high mineral content within the sarcoplasm is perhaps indicated by the swollen appearance of the mitochondria (Green & Baum, 1970) such as is often found in the contracted fibres. Schmalbruch (1973) found contraction zones in muscles of normal individuals, mechanically injured muscles, and in Duchenne

dystrophy. He termed the contraction area a 'contracture knot', and suggested that the enlarged area of the muscle was due to a segmental swelling, and not to a hypertrophy of the entire muscle fibre. In the less contracted state of the M. complexus the entire fibre was not involved; but in the highly contracted fibres, most or all of the fibre appears contracted.

In many of the degenerating fibres it appeared that nuclei were separating off from the muscle cell; however, actual separation could not be observed with the techniques used in this study. Areas of mesoblast fusion (those where early myofibril formation can be observed in the fusing cell) showed the cytoplasmic characteristics described by Fischman (1970), and the fusing mesoblast cytoplasm was distinct from myotube or muscle fibre cytoplasm. Early mesoblast fusion characteristics were not seen in the cleavage areas, so a splitting, rather than a fusion, is considered more likely.

The nuclei appeared normal during all stages of degeneration except for the prominent appearance of the nucleolus in late degeneration. An abnormal nuclear appearance has been reported in degenerating muscle fibres following denervation (Muscatello, Margreth & Aloisi, 1965; Pellegrino & Franzini-Armstrong, 1969; Tomanek & Lund, 1973). Large vacuoles were scattered through the contracted fibres of the hatching muscle, typically around the nuclear areas. These enlarged vacuoles were probably endoplasmic and sarcoplasmic reticular which, in the case of the sarcoplasmic reticulum, have been reported previously to be enlarged after denervation-induced degeneration (Gori, 1972; Pellegrino & Franzini, 1963; Muscatello *et al.* 1965). Although enlarged vesicles are not uncommon in developing muscle, the large size of many of the vacuoles in the hatching muscle was atypical. The largest vacuoles were usually near the nucleus and they often contained glycogen.

A caveolated vesicular system was observed; however, in opposition to the view that the system develops from the sarcolemma (Ishikawa, 1968), these tubules were most often closely associated with the Golgi complex and nucleus. In some cases they appeared to be continuous with the nuclear envelope.

Although the changes occurring in the contracted fibres of the M. complexus closely resembled those of the fast-twitch-oxidative-glycolytic fibres of the guinea-pig after immobilization, there were some differences, such as the presence of vacuoles. A comparison of these two systems does not lead to any hypothesis concerning the reason for, or the mode of initiation of, the proposed degeneration of selected fibres of the complexus.

SUMMARY

Ultrastructural study of the M. complexus muscle of the chick embryo has demonstrated two populations of fibres: the more common is the normal myotube or muscle fibre, which was observed in various stages of development; the other shows myofibrillar contractions which, at their greatest degree, produce the appearance of an amorphous mass of myofilaments. The contracted fibre had rounded and swollen mitochondria, and vacuoles (autophagic) containing glycogen, and it exhibited a cleavage of the cell which isolated the nuclear region from the main body of the fibre.

When the fibres were less contracted they resembled degenerative mammalian fast-twitch-oxidative-glycolytic fibres after immobilization.

The more normal fibre population was identical with that of the pectoralis muscle, which was used as a control.

These results suggest that the contracted fibres are degenerating, which agrees with conclusions by earlier investigators using light microscopy.

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