Muscle Lesions in Embryos and Chicks on the Day of Hatching

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Abstract. Histopathological studies were carried out on skeletal muscles of 1,723 individuals consisting of embryos, and abnormal and normal chicks which had been collected on the days of hatching during the same year. Muscle lesions were readily found in 360 (21%) of the specimens at low-power magnification microscopy.

Muscle fibers in the lesions showed mainly focal swelling, focal rarefaction with a lack of myofibrils and sarcoplasm, and the appearance of basophilic substance. Some of the lesions were associated with mild reactive changes in the interstitial connective tissue.

Based on the findings of rarefied areas in the affected muscle fibers, a presumption was made that these muscle lesions might have occurred in immature muscle fibers and be regarded as a hypoplastic change.

There are several papers published on spontaneously occurring muscular degeneration in chickens. This change has been observed in adult chickens [10, 14], young chickens [9, 10], newly hatched chicks before taking food [8, 10–13] and chick embryos [11]. The following histological changes have been described in the reports cited: muscular degeneration [8], "hyalin-schollige Degeneration" [9], Zenker-like necrosis [10], acute myopathy [11], focal necrosis [12, 13] and muscular dystrophy [14]. The etiology of these conditions was not determined. On the other hand, degenerative changes almost identical with what has been mentioned to occur in spontaneous cases were reported in skeletal muscles of young chickens fed diets deficient in vitamin E [2–4, 6].

The present authors and their co-worker previously conducted histopathological studies on aerocystitis erosiva observed in embryos and chicks collected on the day of hatching [15]. A fairly high incidence of skeletal muscle lesions was recognized in such specimens. This article describes the histopathological findings of these lesions in detail.

Materials and Methods

The materials used for the present investigation included some specimens that had been used for the previous investigation by the present authors and their co-worker on aerocystitis erosiva observed in embryos and chicks on the day of hatching [15]. They were composed of 1,658 abnormal individuals, which consisted of 766 not pipping embryos, 397 pipping embryos, 55 dead chicks, and 440 culled
chicks. All of them were collected from two parental flocks, A and B, on the day of hatching twelve times during the same year (Table 1). Not pipping embryos mean embryos which did not yet pip even on the expected day of hatching. They were dead, except some which were alive. Pipping embryos mean embryos which pipped partially on the expected day of hatching. They were alive, except some which were dead. Dead chicks mean those which died in the incubator after they had hatched completely on the expected day of hatching. Culled chicks mean those which were inadequate for rearing because of physical weakness or abnormalities on the day of hatching.

In addition, 65 normal day-old chicks were collected from the hatch of flock A on August 5, 1964. They were Barred Plymouth Rock and White Cornish hybrids. No abnormalities were seen in the hatchability of any flock from the same hatchery during the experimental periods.

Each individual was fixed in 10% formalin solution after removing the skin. Cross sections cut at the levels shown in Fig. 1 and designated A, B and C were made for histological examination from each case without decalcification. In addition, cross sections designated D to H, as shown in Fig. 1, and a longitudinal section of the thigh muscles were made in cases which were collected on August 5, 1964. All the sections were embedded in paraffin and stained with hematoxylin and eosin.

Fig. 1. Schema showing levels of cross sections for histological examination

Results

1. Lesions of striated muscle tissue

Muscle fibers in the lesions showed mainly focal swelling, focal rarefaction with a lack of myofibrils and sarcoplasm, and the appearance of basophilic substance.

The swollen areas of muscle fibers were variable in size and shape (Figs. 2–6). Many of them were square-like in shape, forming a right angle to the longitudinal direction of each affected muscle fiber. The areas were accompanied by sarcoplasm, and stained usually deep and sometimes rather poorly with eosin. Occasionally, myofibrils with cross striations were demonstrable within the swollen areas. In some parts, the muscle fibers appeared to disintegrate into fragments of variable sizes (Figs. 5 and 6) and sometimes showed a myolytic change (Fig. 15).

Such swollen areas frequently presented a rosary-like arrangement in each affected fiber. Muscle fibers between the swollen areas showed focal rarefaction with a lack of myofibrils and sarcoplasm, and were stained quite faintly or sometimes appeared almost empty (Figs. 2–4 and 6). Usually, the sarcolemna was not involved and remained intact in these areas.

In the cross sections of muscle bundles involved, muscle fibers varied in thickness and stainability (Figs. 7, 10, 12 and 14). Empty spaces, which were surrounded by the intact sarcolemna, resembled adipose tissue (Figs. 7 and 10). A few myofibrils were frequently recognized within these spaces.

Lesions were found readily when the focal swollen and rarefied areas of muscle fibers were arranged clearly and alternately simulating a rosary. On the other hand, it was difficult to find such lesions when they were not common (Fig. 9).

Occasionally, two kinds of basophilic substance were present in affected muscle fibers. They were distinguished from each other by morphology and stainability. One of them was stained poorly with hematoxylin and presented as a pin-point-like or water-drop-like form (Figs. 12–15). It occurred chiefly in the junctions of the swollen and rarefied areas of muscle fibers, or was scattered within the empty spaces of these fibers. The other was granular in shape and stained intensely with hematoxylin (Figs. 16 and 17). It was frequently dense in the swollen areas of muscle fibers.
Table 1. Incidence of muscle lesions among experimental groups

<table>
<thead>
<tr>
<th>Date of hatching</th>
<th>Parent flock</th>
<th>Incidence of muscle lesions* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not pipping embryo</td>
</tr>
<tr>
<td>Aug. 5, 1964</td>
<td>A</td>
<td>8/28 (29)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8/35 (23)</td>
</tr>
<tr>
<td>Aug. 24, 1964</td>
<td>A</td>
<td>10/31 (32)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6/31 (19)</td>
</tr>
<tr>
<td>Sept. 23, 1964</td>
<td>A</td>
<td>4/30 (13)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2/28 (7)</td>
</tr>
<tr>
<td>Nov. 9, 1964</td>
<td>A</td>
<td>8/33 (24)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3/37 (8)</td>
</tr>
<tr>
<td>Dec. 2, 1964</td>
<td>A</td>
<td>4/25 (16)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1/12 (8)</td>
</tr>
<tr>
<td>Jan. 26, 1965</td>
<td>A</td>
<td>8/32 (15)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0/19 (0)</td>
</tr>
<tr>
<td>Feb. 18, 1965</td>
<td>A</td>
<td>2/25 (8)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8/31 (26)</td>
</tr>
<tr>
<td>Mar. 20, 1965</td>
<td>A</td>
<td>5/52 (10)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2/17 (12)</td>
</tr>
<tr>
<td>Apr. 25, 1965</td>
<td>A</td>
<td>5/70 (7)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1/24 (4)</td>
</tr>
<tr>
<td>May 20, 1965</td>
<td>A</td>
<td>6/66 (9)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2/24 (8)</td>
</tr>
<tr>
<td>June 17, 1965</td>
<td>A</td>
<td>2/33 (6)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>July 15, 1965</td>
<td>A</td>
<td>2/30 (7)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1/16 (6)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>98/766 (13)</td>
</tr>
</tbody>
</table>

Remarks.
*
: Number of individuals with lesions/Number of individuals examined.

In the interstitial connective tissue of the affected muscles there were occasional edema (Figs. 2 and 15) and small hemorrhages (Fig. 8). An infiltration of a few heterophils (Figs. 6, 8 and 17) and a proliferation of histiocytes and fibroblasts (Figs. 8 and 11) were rarely present in a small number of specimens. Several nuclei of the muscle fiber showed a row arrangement, without being accompanied by any distinct regenerative picture.

The lesions were variable in size, consisting of small foci affecting a single muscle fiber (Fig. 5) and large ones involving a few muscle fiber bundles (Fig. 8). In each case, the former foci tended to be scattered and the latter ones were inclined to be multiple.

There was no definite anatomical pattern in the distribution or consistency of muscle lesions. Lesions extended to the muscles of the entire body, including the muscles around the trachea.

2. Incidence of muscle lesions

Table 1 shows the incidence of muscle lesions among the experimental groups. It indicates the number of individuals with muscle lesions which were readily found in sections cut at levels A, B and C of each case by low-power magnification microscopy.

Lesions occurred in most of the experimental groups collected during the same year. The mean percentage of incidence of lesions in each experimental group was 47% for the dead chicks, 25% for the culled chicks, 22% for the pipping embryos and 13% for the not pipping embryos. Lesions were found in 26 chicks (40%) of the 65 normal ones which were collected from flock A.
on August 5, 1964. This percentage ranked second among the experimental and control groups combined.

**Discussion**

The muscle lesion observed in the present study was regarded as the muscular degeneration described in the previous report by the present authors and their co-worker [15], in which the swollen areas of affected muscle fibers were noticed. If the muscle lesions are observed with emphasis placed on the swollen areas, they will be regarded as foci of Zenker’s hyaline degeneration. As a result of the present detailed examination, however, more attention was paid to the focal rarefaction which was represented by a lack of myofibrils and sarcoplasm within the muscle fibers between the swollen areas. The rarefied areas were regarded as composed of immature muscle fibers which corresponded to myotubes, exhibiting one of the stages seen in the normal development of the muscle fiber [1]. These changes seemed to be hypoplastic ones. Furthermore, hypoplasia of the spinal cord and retarded growth were noticed in a fairly high percentage of the individuals examined in this study, as described in the previous report [15]. These results may lend support to the hypothesis that the muscle lesion may be a partial sign of systemic hypoplasia.

Rigdon et al. [10] observed muscle necrosis in a chick killed 24 hours after hatching. They found spaces in muscle fibers microscopically. These spaces may be identical with the rarefied areas recognized by the present authors.

The etiology of the condition described in this study was unknown. Such myopathy of the leg as characterized by atrophy of the muscles of the leg has been induced experimentally in embryos by using eggs laid by hens fed a diet deficient in vitamin B_{12} [7].

A frequent occurrence of necrosis in the “pipping” muscle (M. complexus) has been reported in embryos and newly hatched chicks [11–13]. This muscle is active in making the chick break up the egg-shell at the time of hatching [5]. Therefore, the produced lesion may be a cause for the failure of the embryo to hatch [13]. A marked reduction in hatchability has been described in a case in which chicks were affected with muscular degeneration [8].

The present authors have no suggestions concerning the influence resulting from the muscle lesion observed. It is significant that the muscle lesion was observed in normal chicks, as well as embryos and abnormal chicks, on the day of hatching. The findings reported in this paper will contribute to studies on the histopathogenesis of the muscle lesion and other myogenic disorders in baby chicks.

**References**


Explanation of Figures

All the photomicrographs were taken from sections stained with hematoxylin and eosin.

Fig. 2. Longitudinal section of musculi (Mm.) osis hyoidei. A large number of muscle fibers of variable thickness are seen showing focal swelling or attenuation. The interstitial connective tissue is edematous. Not pipping embryo (case No. 17). ×125.

Fig. 3. Longitudinal section of Mm. abdominis. Myofibrils, many of which have cross striations, are distinct within muscle fibers, but there is little sarcoplasm between myofibrils. Within the fibers are scattered foci variable in shape and size. Stained deep with eosin. Many of the foci form a right angle with each fiber. Not pipping embryo (No. 222). ×253.

Fig. 4. Longitudinal section of Mm. cinguli extremitatum thoracicae. Within muscle fibers are a large number of areas showing focal swelling, stained deep with eosin and accompanied by sarcoplasm. Among these areas there are distinct myofibrils with cross striations, but sarcoplasm is lacking between these myofibrils. Not pipping embryo (No. 652). ×495.

Fig. 5. Longitudinal section of musculus gluteus maximus. Two muscle fibers (arrows) are affected. Of them, the left one is swollen and has disintegrated into three fragments within which there are demonstrable cross striations and spots looking like worm-eaten ones. The right one is irregular-shaped and contains a granular substance. Weak chick (No. 187). ×495.

Fig. 6. Longitudinal section of Mm. dorsi. A large number of muscle fibers show focal swelling or disintegration into fragments which are variable in size and shape and stained deep with eosin. A few heterophils are present in the interstitial connective tissue. Weak chick (No. 676). ×253.

Fig. 7. Cross section of Mm. dorsi. Some normal muscle fibers are seen among others which are variable in thickness and show a lack of myofibrils and sarcoplasm. The sarclemma of affected fibers is not involved. Not pipping embryo (No. 158). ×253.

Fig. 8. Cross section of Mm. colli. Small (lower right) and large (upper left) foci are present. They consist of swollen muscle fibers stained rather poorly with eosin. A few heterophils, histiocytes, and hemorrhages are seen in the interstitial connective tissue. Weak chick (No. 55). ×125.

Fig. 9. Oblique section of Mm. dorsi. A large number of muscle fibers are slightly swollen and stained rather poorly with eosin. Weak chick (No. 1418). ×125.

Fig. 10. Cross section of muscle around the trachea. Muscle fibers very in thickness. Some of them are empty and surrounded by an intact sarcolemma. Normal chick (No. 72). ×253.

Fig. 11. Cross section of Mm. colli. A marked proliferation of histiocytes and fibroblasts is present. In this lesion there are scattered swollen muscle fibers, some of which contain fine granules stained poorly with hematoxylin. Normal muscle fibers are seen in the upper and lower right corners of the photomicrograph. Normal chick (No. 96). ×253.

Fig. 12. Cross section of Mm. osis hyoidei. Some muscle fibers are swollen and contain scattered granules stained poorly with hematoxylin. Some fibers show a lack or a disintegration of myofibrils and sarcoplasm. Not pipping embryo (No. 156). ×495.

Fig. 13. Oblique section of Mm. dorsi. All the muscle fibers show a diminution in the density of myofibrils. Some of them are slightly swollen. Focal water-drop-like areas stained with hematoxylin (dark spots) are scattered within the fibers. Not pipping embryo (No. 1140). ×253.

Fig. 14. Cross section of Mm. dorsi. Some muscle fibers were stained completely and deep with hematoxylin (dark areas). Other fibers are slightly swollen or empty. Not pipping embryo (No. 458). ×495.

Fig. 15. Longitudinal section of Mm. thoracis. Muscle fibers contain scattered areas which were stained homogeneously and poorly with hematoxylin. Some muscle fibers disintegrated into small pieces stained poorly with hematoxylin or eosin. The interstitial connective tissue is edematous. Not pipping embryo (No. 310). ×253.

Fig. 16. Oblique section of Mm. osis hyoidei. Almost all the muscle fibers contain a granular substance stained intensely with hematoxylin. A few fibroblasts are present in the interstitial connective tissue. Pipping embryo (No. 171). ×495.

Fig. 17. Oblique section of Mm. dorsi. Variable quantities of a granular substance stained intensely with hematoxylin are scattered within some muscle fibers. A few heterophils and histiocytes are seen in the interstitial connective tissue. Pipping embryo (No. 1411). ×253.