A Microphotometric Study on the Proliferation Pattern of Muscle Cell Nuclei in Chickens with Hereditary Muscular Dystrophy

Tohoku Journal of Agricultural Research
Vol. 36, No. 2, November 1985
Printed in Japan

A Microphotometric Study on the Proliferation Pattern of Muscle Cell Nuclei in Chickens with Hereditary Muscular Dystrophy

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(Received, August 20, 1985)

Summary

Using a Feulgen-DNA photometry, the activity of DNA synthesis of muscle cell nuclei of biceps muscle was studied in chick embryos and chickens with hereditary muscular dystrophy. In dystrophic chickens, the number of muscle nuclei increased more rapidly than in normal ones after hatching. The photometry on DNA-Feulgen complex showed that the relative number of S and G_2 nuclei per total muscle nuclei remained at 5-6% in the period from hatching to 8 weeks of age. On the other hand the value in normal chickens decresed to 1% at 4 weeks after hatching.

Hereditary muscular dystrophy in the chick is characterized by selective destruction of a specific fiber type (α-fiber) in skeletal muscle (1, 2). The pectoralis and biceps muscle are typically and severely involved. The histological disorder in muscle fibers in the dystrophic chickens is characterized by wide variability in size with a large number of fibers which are smaller or larger than normal ones, an increased number of myonuclei, and vacuolization of the muscle fiber (3, 4, 5).

In the muscles of chickens with herediatry muscular dystrophy, the dislocation of nuclei from a peripheral to an internal position and the marked increase of nuclear number in muscle fibers occurs with the progression of the muscle abnormality. In this study, the rate of DNA synthesis and the proliferation pattern of myofiber nuclei in the biceps muscle was investigated by using DNA microphotometry, of Feulgen stained nuclei.

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Materials and Methods

Normal (NH 412) and dystrophic (NH 413) chickens and chick embryos were used in this experiment. Two or three chick embryos and chickens of each genotype were sacrificed at 12 and 16 days of incubation, at hatching, and at 1, 2, 4 and 8 weeks after hatching. The muscles (*M. biceps brachii*) were fixed in 10% phosphate buffered formalin for 48 hours and embedded in paraffin wax. The 5 μm sections were stained with the modification of Van Gieson's picrofuchsin method (6) to count the mean number of the myofiber nuclei. The mean number of nuclei per cross-sectioned muscle fiber was calculated from observation of 50 muscle fibers in each chicken at hatching and at 1, 2, 4 and 8 weeks of age.

Feulgen staining for the photometric measurement of DNA was performed as follows. Hydrolysis was carried out in 5N HCl for 45 min at 18°C. Schiff's reagent (Merk, Art. 9033) was diluted to 10% in glycine buffer adjusted to pH 2.3. Schiff's reaction was carried out at 18°C for 45 min. The sections were then transfered to 1.5% sulfite rinse (glycine buffer, pH 2.3) and washed, dehydrated, and mounted in Entellan New (Merk).

The amount of Feulgen-DNA stain complex was measured with a microphotometer (MMSP-TK, Olympus Optical Co. Ltd., Tokyo). Total extinction at 560 nm was used as a measure of the amount of Feulgen-positive material in the nucleus. The measurements were carried out on 100 nuclei at peripheral and internal regions of myofibers in each chick embryo and chicken. Data of two or three birds at each stage were pooled and the histograms of relative DNA content per nucleus were illustrated. The relative numbers of S and G_2 nuclei of the cell cycle were approximated from the histograms of Feulgen photometry.

Results

The mean values of nuclear number in myofibers increased during growth in normal and dystrophic chickens as shown in Fig. 1. At hatching and 1 week after hatching, there were no significunt differences in the number of myofiber nuclei between the two genotypes. After 2 weeks of age, the number of myofiber nuclei in dystrophic chickens increased more rapidly than in normal chickens (Fig. 1), reaching 3.9 ± 0.6 at 8 weeks. On the other hand in control muscle, the number increased only to 1.5 ± 0.3.

As shown in Fig. 2, the histograms of DNA Feulgen microphotometry showed a relatively broad peak representing nuclei with a diploid (2c) DNA content (G_1 phase in cell cycle). Some nuclei contained the tetraploid (4c) DNA value and represented G_2. The nuclei in S phase were considered between the DNA values of 2c and 4c. The nuclei which were regarded to have DNA in the amount of S and G_2 phase, are illustrated by shading in Fig. 2. There were no nucli in the internal location of myofibers showing a high amount of DNA-Feulgen stain

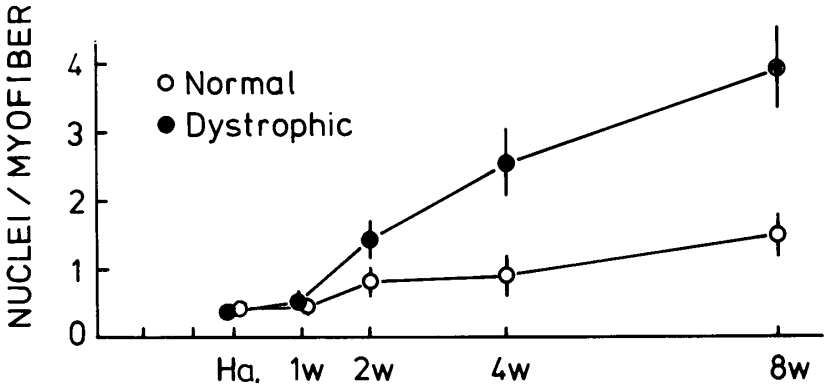

FIG. 1. Nuclear number per crosscut myofiber in M. biceps brachii of developing normal and dystrophic chicks. Dots and vertical bars eqal mean and standard deviation.

complex.

The relative number of S and G_2 nuclei is shown in Fig. 3. These values were relatively high in the embryonic stages and showed no differences between normal and dystrophic genotype. These values decreased in normal chickens after hatching and reached about 1% at 4 weeks after hatching. However, in dystrophic chickens, the relative number of S and G_2 nuclei still remained at 5-6% in the period from hatching to 8 weeks of age.

Discussion

The dystrophic disorders of skeletal muscle in chickens are largely confined to the fast twitch myofibers. The pectoralis muscle (*M. pectorales superficialis*) is affected more severely at any given age than the biceps muscle. Necrotic fibers, spritting fibers and other phenomena of severe muscle destruction were often observed in the pectoralis muscle at earlier stages ex ovo than in other fast twitch muscles. To avoid the difficulty in the microphotometric analysis of muscle disfunctions in embryonic and young chickens, the present study focused on the biceps muscles which show a milder progression of histophathologic abnormalities.

Yorita et al. (7) reported that in the muscle of the pectoralis superficialis of dystrophic chickens (NH 413), the number of true muscle nuclei and satellite cell nuclei per cross-section of the muscle fiber increased with age. Ashmore and colleagues (8, 9) found that the patigialis muscle of dystrophic chicks has a DNA concentration nearly three times that found in the normal muscle. In this study, also, the myonuclear number of the biceps muscle of muscular dystrophic chickens showed marked increase with age, and at 8 weeks it was 2.5 times that of the normal muscle.

The microphotometric study on DNA content of the muscle nuclei showed that the nuclei at the peripheral region of dystrophic myofibers were synthesizing DNA and proliferating after hatching. The nuclei in the peripheral region of

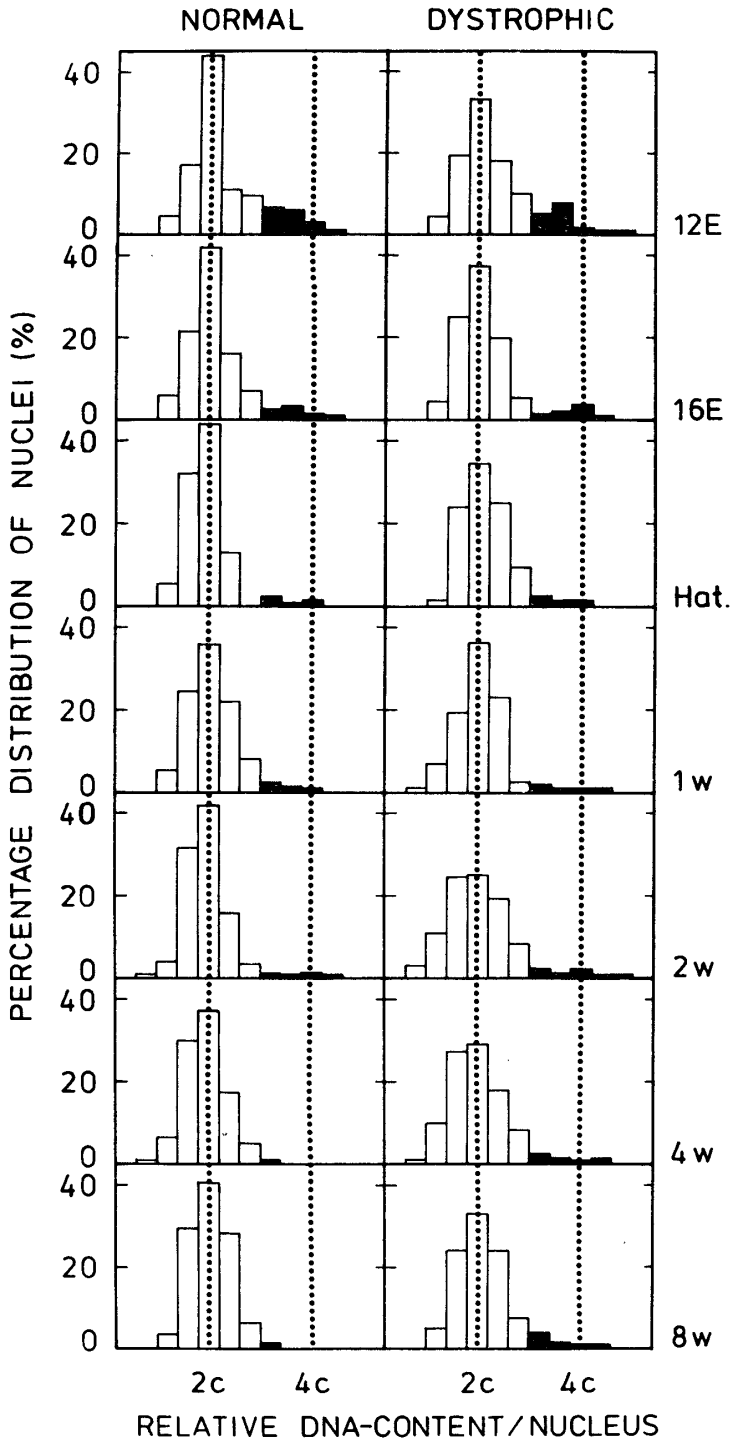

FIG. 2. Histograms from Feulgen microphotometry showing distribution pattern of DNA content (arbitrary unit) of muscle fiber nuclei of the M. biceps brachii of deveoping normal and dystrophic chicks. Culms with shading indicate the nuclear population with DNA in the amount of S and G_2 phase.

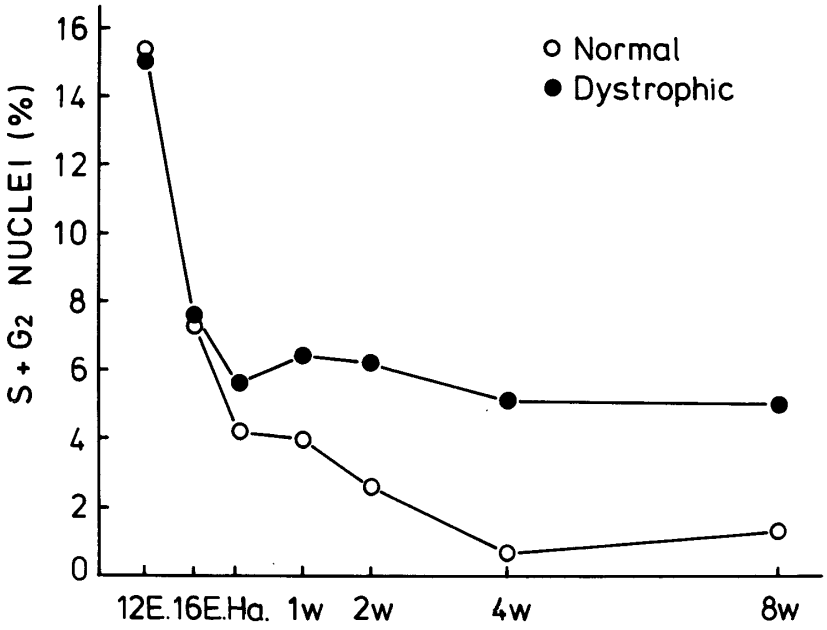

FIG. 3. Rate of $S+G_2$ nuclei in cell cycle per total muscle fiber nuclei in the M. biceps brachii of normal and dystrophic chicks.

myofibers consist of both true muscle fiber nuclei and satellite cell nuclei. The satellite cells are small cells located between the basement membrane and the plasma membrane of the muscle fiber, so they are idientifiable only by electron microscopy. In this photometric study, therefore, we could not distinguish between these two nucleus types.

Using radioautography it is shown that myonuclear increase was achieved by the fusion of post-mitotic satellite daughter cells with the muscle fiber during postnatal growth (10, 11) and in compensatory hypertrophy (12). By electron microscopy, Yorita et al. (7) reported that many satellite cells maintained the activated feature at least during the first 40 days after haching in dystrophic muscles. The nuceli synthesizing DNA as shown by our photometric study, therefore, are identical to satellite cell nuclei.

In pectoralis muscle of dystrophic chickens, the number of true muscle nuclei increased more than that of satellite cell nuclei (7). The rate of satellite cell nuclei per total muscle fiber nuclei decreased from 23% at 5 days after hatching to about 15% beween 12 and 40 days (7). If the data on the pectoralis muscle (7) apply also to the biceps muscle, the activity of DNA synthesis and/or proliferation of satellite cells is higher than that shown in Fig. 3.

Data presented here indicate that, in the biceps muscles of dystrophic chickens, the nuclei at the peripheral region of myofibers, which should be myosatellite cell nuclei, are capable of synthesizing DNA and increasing nuclear number continuously at a rate higher than those of normal chickens after hatching. It seems likely that a large number of myosatellite cells are activated to

increase nuclear number which are then incorporated in myofibers in dystrophic chickens.

Ashmore (8) recently reported that stretch of muscle applied to newly hatched dystrophic chickens was a powerful deterrent to the development of symptoms characteristic of hereditary muscular dystrophy. He proposed a new possibility that a large percent of myofibers in affected muscles of young dystrophic chicks do not grow or devolop appreciably beyond the myotube stage, if stretch tension is not applied to the fibers at the early developing stage. We think the data, presented here and by Yorita (7), also indicate that myofibers of dystrophic chicks are in the myotube stage and showed a high activity of proliferation of satellite cells.

Acknowledgements

This study was supported by a grant No. 85-04-05 from the National Center for Nervous, Mental and Muscular Disorders (NCNMMD) of the Ministry of Health and Welfare, Japan.

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