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Studies on Development and Differentiation of Muscle. VII. An Expression of the Myogenic Cell Fusion by Using ^{3}H-thymidine Autoradiography in Chick Embryos

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Summary

We attempted to express the mode of myogenic cell fusion by ^{3}H-thymidine autoradiography. This phenomenon was compared the M. complexus with the M. biceps femoris at 10 and 16 days of incubation. At 10 days of incubation, when myogenic cells in the M. complexus were expected to fuse frequently, many labeled cells stopped to divide and entered into fusion process limiting at G_1-phase of their mitotic cycles. On the other hand, the majority of myogenic cells in the M. biceps femoris kept up their proliferation. At 16 days of incubation, when the M. complexus takes PI values of higher order, many satellite myoblasts situated arround matured myotubes continued to divide and frequently did not enter the cell fusion process. Since myotubes formed in the M. biceps femoris were still immature, some satellite myoblasts were in need of the cell fusion with other myogenic cells.

Introduction

There have been many observations on growth and regeneration of muscle *in vivo*, but the main arena in which the mechanism of myogenesis have been investigated has been the *in vitro* one. Here myoblasts of mammalian and of chick origin have been found to form myotube by cell fusion (1～6). The question is whether this behavior reflects the situation in the organism, or is an *in vitro* peculiarity.

The occurrence of myoblasts fusion *in vivo* was rigorously demonstrated in experiments by Mintz and Baker (7). They analyzed electrophoretically muscle tissues of allophenic mice for isocitrate dehydrogenase isozymes and demonstrated that the hybrid enzyme was present in skeletal muscle, thus conclusively demon-

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strating the *in vivo* origin of these syncytium by myoblast fusion, rather than by repeated nuclear division in nondividing cell body. Unfortunately, there were no observations in various embryonic muscle tissues comparing the degree of myogenic cell fusion.

In this study, we attempted to express the mode of myogenic cell fusion and compare their proliferating pattern among embryonic muscles by using ^{3}H-thymidine autoradiography.

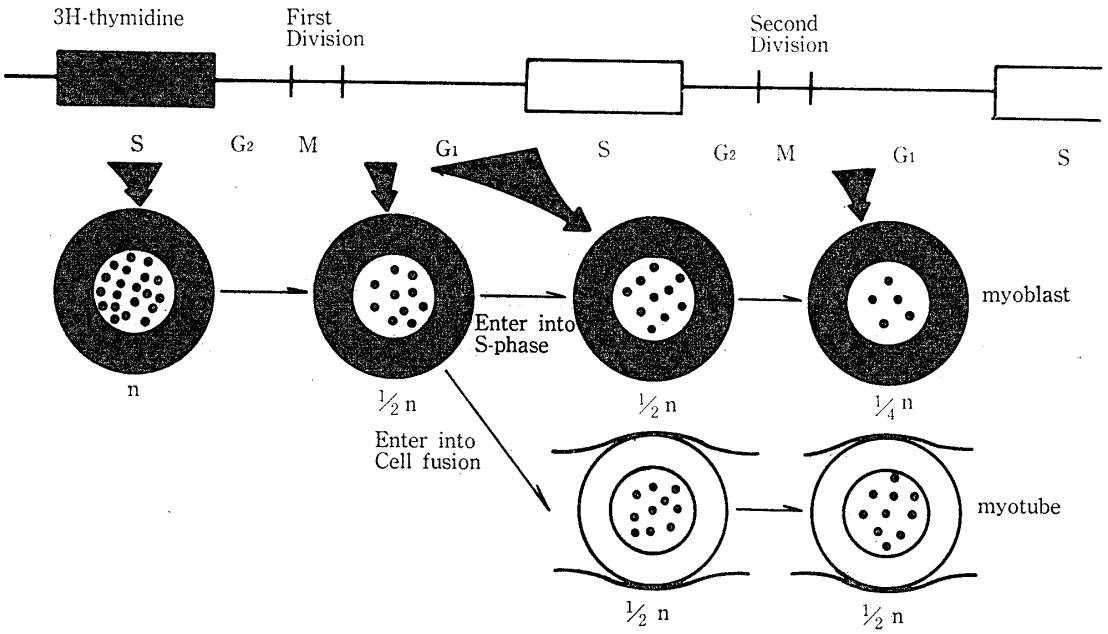


FIG. 1. A schematic drawing illustrating the theoretical silver grain counts over labeled nuclei at various stages during the course of mitotic cycle after pulse labeling of ^{3}H-thymidine. The cells labeled with ^{3}H-thymidine at S-phase have silver grain counts, n, as a theoretical number. They were divided in two daughter cells at the first mitotic phase. Here the grain counts of these cell nuclei decreased by one-half, $1/2n$. The cells which destined to fuse with other myogenic cells in the following G_1-phase maintained their grain counts at the same value, $1/2n$. On the other hand, the cells which destined to continuously proceed the next cycle divided their grain counts into four, $1/4n$.

Materials and Methods

Details of the ^{3}H-thymidine autoradiography in this study have been reported previously (8). The cells labeled with ^{3}H-thymidine at S phase shifted to the mitotic phase and then were divided into two daughter cells. Here the grain counts of these cell nuclei decreased by one-half. The cells which destined to fuse with the other myogenic cells in the following G1-phase maintained the grain counts of cell nuclei at the same value. On the other hand, the cells which were destined to continuously proceed to the next cycle divided the grain counts into four (Fig. 1). From these theoretical viewpoints, the replication and cell fusion

pattern of the labeled presumptive myoblasts were demonstrated in histograms of the percentage distribution of grain counts over nucleus at the various time after injection of ^{3}H-thymidine and were compared to the M. complexus with the M. biceps femoris at 10 and 16 days of incubations. A square field outlined an area on the slide approximately 0.10 mm on a side. This field usually contained between 200 and 250 nuclei. The number of silver grain over nuclei within the standard field was randomly calculated.

Results and Discussions

In previous report, we analyzed the durations of mitotic cycle in the M. complexus, the M. biceps femoris and the M. pectoralis at various developmental stages of chick embryos (8). It could be concluded from these results that there were no differences in the periods of the generation time and its component phases among these muscles at certain incubation days. The reasons why the labeling index (LI), the mitotic index (MI) and the proliferation index (PI) showed more remarkable decline in the M. complexus than in the other muscles during from 10 to 13

TABLE 1. *Theoretical Silver Grain Counts over Labeled Nuclei at Various Stages in the Mitotic Cycle after Pulse Labeling of ^{3}H-thymidine*

Various stages during the course of mitotic cycle after pulse labeling of ^{3}H-thymidine	Theoretical silver grain number over nuclei	10-days of incubation	The times for experiments	16-days of incubation	The times for experiments
A labeled myoblast finishes the first cell division for the first time	n	hours 1.87	hours	hours 1.79	hours
All labeled myoblasts finish the first cell division and some of them enter into G_1-phase	$1/2n$	8.66	8.00	8.08	8.00
A labeled myoblast finishes the first G_1-phase for the first time	$1/2n$	3.57		7.20	
All labeled myoblasts finish the first G_1-phase and some of them enter into the next S-phase, while others enter into cell fusion	$1/2n$	10.37		13.50	
A labeled myoblast finishes the second cell division for the first time	$1/2n$	12.24	14.00	15.20	18.00
All labeled myoblasts finish the second cell division and some of them enter into the G_1-phase	$1/2n+1/4n$	19.03	20.00	21.49	24.00

days of incubation might be dependent on the transition of myoblasts from proliferation phase to non-proliferation phase, and on the acceleration of cell fusion.

In order to determine whether the proliferating myoblasts labeling with ^{3}H-thymidine really fused with the other myogenic cells at G_1-phase during the first and second cell divisions, we analyzed DNA synthetic and mitotic pattern by the method of silver grain counts over cell nuclei. Consulting the changes of LI and PI values during embryonic days, the making of histograms was done at 10 and 16 days of incubation. It could be presumed that, in the M. complexus, the majority of myoblasts did fuse with the other myogenic cells at the former days. The majority of myoblasts finished the course of cell fusion and entered, one after another, into the following cell divisions at the later days.

The durations after ^{3}H-thymidine injection for a purpose of calculation were determined by our previous data of the mitotic cycle (Table 1). If the grain count of nuclei one hour after injection suppose n as a theoretical value, it decreased approximately by one-half after the first cell division, *i.e.*, $1/2n$. This periods were about 8 hours at 10 and 16 days of incubation in both muscles. During the course of the following mitotic cycle, the cell fusion of some labeled cells occured especially limiting at G_1-phase. The grain counts over nuclei retained its values $1/2n$ at that time. On the other hand, the labeled cells which did not enter into cell fusion

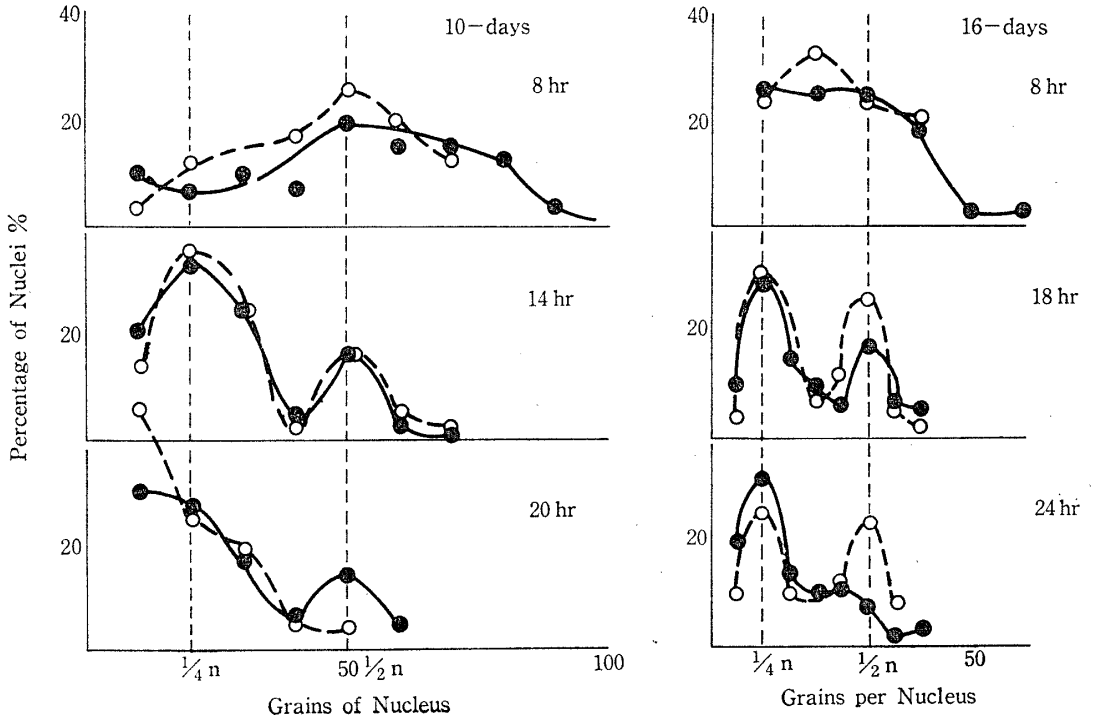
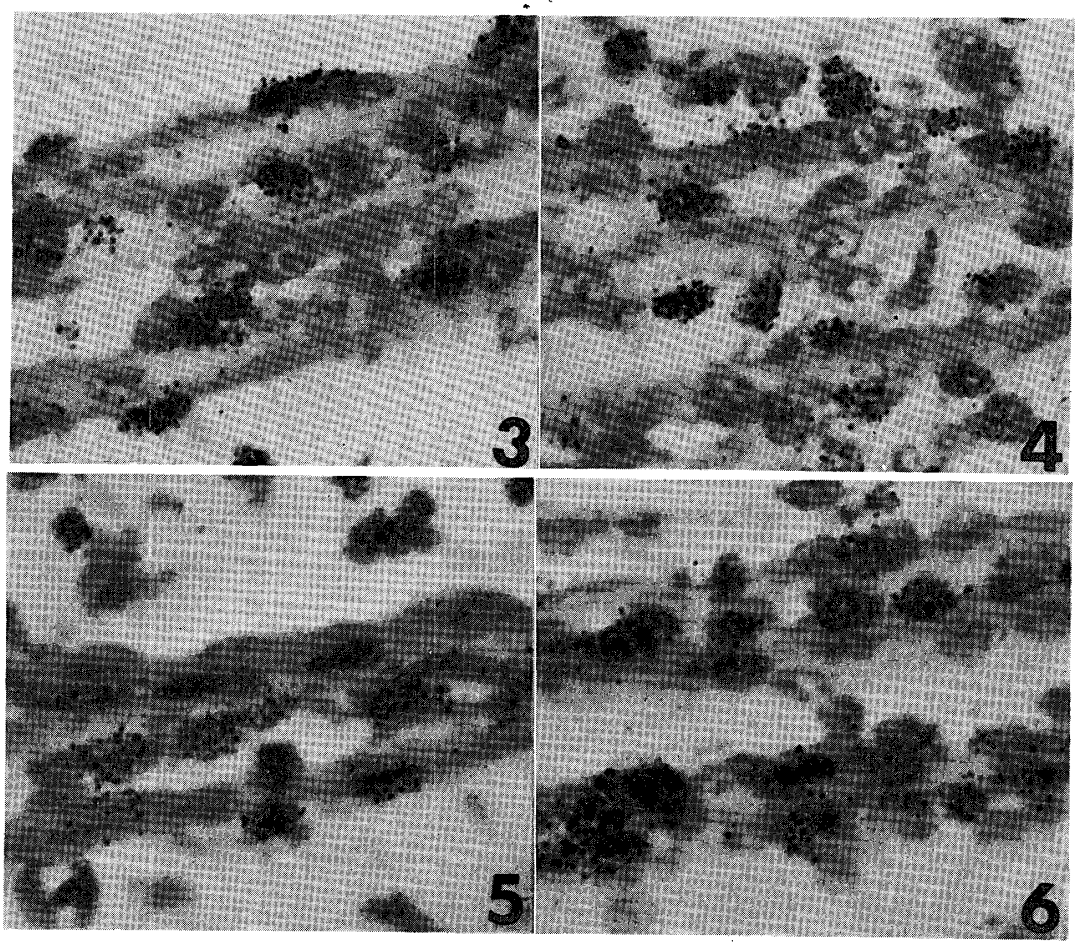


FIG. 2. Experimental results of the percentage of nuclei with various silver grains over nuclei. The counting was examined 8, 14, and 20 hours after pulse labeling of ^{3}H-thymidine at 10 days of incubation, and 8, 18, and 24 hours after pulse labeling of ^{3}H-thymidine at 16 days of incubation. •—•, *M. complexus*; ○—○, *M. biceps femoris.*

process began to go on to the next cell division after 12.24 hours at 10 days and after 15.20 hours at 16 days of incubation, respectively. Then their grain counts reduced to $1/4n$. All labeled nuclei finished the next cell division after 19.03 hours at 10 days and after 21.49 hours at 16 days of incubation, respectively. The theoretical grain counts of labeled nuclei were expected to be completely grouped into $1/2n$ and $1/4n$. As shown in a histogram, the distribution of nuclei by labeling intensity obtained in this experiments agreed well with this hypothesis (Fig. 2). At 10 days of incubation, there was an increase in the percentage of nuclei with $1/2n$ and $1/4n$ grain counts at 14 hours after injection in both muscles. The highest and the lower peaks appeared in this curve were restricted at the order of grain



These autoradiographic photomicrographs were taken from embryonic muscles at 10 days of incubation.

FIG. 3. The M. complexus sumpled 8 hours after pulse labeling of ^{3}H-thymidine. Hematoxilin stain, ×1000.

FIG. 4. The M. biceps femoris sumpled 8 hours after pulse labeling of ^{3}H-thymidine Hematoxilin stain, ×1000.

FIG. 5. The M. complexus sumpled 20 hours after pulse labeling of ^{3}H-thymidine. Hematoxylin stain, ×1000.

FIG. 6. The M. biceps femoris sumpled 24 hours after pulse labeling of ^{3}H-thymidine. Hematoxylin stain, ×1000.

counts over nuclei in $1/2n$ and $1/4n$. As the most characteristic point, the labeling intensity illustrating after 20 hours appeared in $1/2n$ was greater in the M. complexus than in the M. biceps femoris.

The photomicrographs representing in Figure 3 and 4 show the labeling intensity over nuclei after 8 hours and compare the M. complexus with the M. biceps femoris. There are few differences in the distribution of silver grain counts over nuclei. The lower photographs shows the labeling intensity over nuclei after 20 hours and compares expressions of the cell fusion of the M. complexus with that of the M. biceps femoris. Since the majority of labeled nuclei in the M. biceps femoris undergo the next mitotic phase and divide in two daughter cells, they take their silver grain counts into four (Fig. 6). On the other hand, many myoblasts in the M. complexus maintain their silver grain counts at the same value (Fig. 5). These results showed that the cell fusion of the presumptive myoblasts during G_1-phase occured more frequently in the M. complexus than in the M. biceps femoris.

Contrary to the results examined at 10 days of incubation, a curve peak appeared at the grain count of $1/2n$ remained higher in the M. biceps femoris than in the M. complexus at 16 days of incubation. These results showed that the majority of the presumptive myoblasts in the M. complexus passed through G_1-phase without cell fusion process and entered into the following proliferation phase (Fig. 2).

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