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Isolation and Clonal Analysis of Satellite Cells from Chicken Pectoralis Muscle

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Satellite cells, liberated from the breast muscle of young adult chickens by sequential treatment with collagenase and trypsin, were fractionated by Percoll density centrifugation to remove myofibril fragments and cell debris which otherwise heavily contaminate the preparation. This procedure allowed direct measurements of cell yields ($1.5–4 \times 10^5$ cells/g tissue), plating efficiencies (27–40%) and identification of single cells in culture. In mass cultures, satellite cells gave rise to myotubes on the fifth day, and the progeny of these cells were sequentially passaged several times without losing myogenic traits. In clonal studies, over 90% of the satellite cells gave rise to large clones of which more than 99% were myogenic as demonstrated by the appearance of myotubes. The results obtained with satellite cells differ from observations made using embryonic muscle cell preparation from chicks. In the embryonic system massive formation of myotubes was observed following the third day of culture; sequential subculturing led to overgrowth of fibroblast-like cells following the first passage; and cells gave rise to both small myogenic clones (up to 16 terminally differentiated cells per clone) and non-myogenic clones in addition to large myogenic clones. We conclude that the isolated satellite cells represent a homogeneous cell population and reside in a stem cell compartment.

INTRODUCTION

Adult skeletal muscles of higher vertebrates are capable of regeneration following injury. A large body of literature has indicated that satellite cells are the myogenic precursor cells in mature muscle (Bischoff, 1975, 1986; Konigsberg *et al.*, 1975; Allbrook, 1981; Campion, 1984). These mononucleated cells are identified in terms of their position between the basement membrane and the plasma membrane of the differentiated muscle fiber (Mauro, 1961, 1979). At some stage of development, depending on the species, satellite cells become mitotically quiescent, but can reinitiate proliferative activity following muscle injury. Their progeny can fuse into multinucleated fibers which express muscle specific proteins (Matsuda *et al.*, 1983; Campion, 1984). *In vitro* studies by Cossu and co-workers suggest that satellite cells are not identical to embryonic myoblasts (Cossu *et al.*, 1983, 1985). However, this issue is not yet clearly resolved.

Cultures of satellite cells from various species have been described; e.g., mouse (Kagawa *et al.*, 1977), rat (Jones, 1977; Allen *et al.*, 1984), human (Blau and Webster, 1981), chicken (Matsuda *et al.*, 1983), and hamster (Yasin *et al.*, 1976). However, direct quantitation of the number of satellite cells isolated was not possible in many of these studies because of the large amounts of myofibril fragments and cell debris in the final cell suspensions from both adult muscle (Young *et al.*, 1978; Blau and Webster, 1981; Matsuda *et al.*, 1983) and from muscles of older embryos (Hauschka, 1974). In such studies, the number of cells in the initial suspension was estimated following the early culturing phase which permitted cell attachment and removal of most of the debris from the culture (Blau and Webster, 1981; Matsuda *et al.*, 1983). The inability to assess accurately the number of satellite cells derived from a given tissue sample or in a culture innoculum has been a major obstacle to determining the effects of physical or pharmacological agents on satellite cell yield or activation. Furthermore, contamination with debris has not permitted studies in which the isolated cells are to be directly analyzed biochemically without culturing.

In this article we describe a method for the isolation of muscle precursor cells from the pectoralis muscle of young adult chickens employing Percoll density centrifugation. Using this approach, which completely removes myofibril fragments and other debris from the isolated cells, we have begun to characterize the adult myogenic precursor cells both in mass and clonal cultures. Currently, we have no way of clearly identifying the myogenic precursor cells isolated from the adult muscle as those satellite cells which have been identified in terms of their position beneath the basal lamina of the intact muscle fiber. However, as commonly accepted by other investigators, we refer to the myogenic precursor cells isolated in this study as satellite cells. We conclude that with respect to their proliferative potential, the satellite cells represent a homogeneous cell population. The vast majority of the cells can generate many progeny before fusion occurs. This finding is in

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striking contrast to the behavior of myogenic cells from the embryo where the precursors vary greatly in their proliferative potential and reside in different compartments of the myogenic lineage (Quinn *et al.*, 1984). In accord with the lineage model proposed for myogenesis in the chicken embryo (Quinn *et al.*, 1985), we suggest that most satellite cells (>90%) reside in the stem cell compartment.

MATERIALS AND METHODS

Source of cells. Cells were prepared from the pectoralis muscle of 10- to 11-week-old chickens (White Leghorn; Biological Supply, Bothell, Wash.). Chickens were sacrificed, the skin at the chest area was removed, and the breast muscle processed immediately for cell isolation.

Cell isolation. About 5–10 g of muscle tissue were minced with sharp scissors into fragments about 0.1–0.2 cm^3. The fragments were washed twice with Eagle's minimal medium (MEM) and were treated with collagenase at a final concentration of 0.2% (Sigma, type 1A, 482 unit/mg) in MEM for 45 min at 37°C. The ratio of collagenase solution to muscle tissue was kept at 4–5 ml enzyme/g tissue and the preparation was constantly stirred. The treated muscle tissue was then forced five times through a 10-ml pipet, collected by centrifugation (300*g* for 5 min), washed once with MEM, and the fragmented tissue was redigested with 5 vol of trypsin (GIBCO, final concentration 0.1% in MEM) for 45 min at 37°C with constant stirring. At the end of the digestion, the suspension was diluted with 4 vol of standard medium (85 parts MEM, 10 parts horse serum, 5 parts embryo extract, penicillin and streptomycin at 10^5 units/liter of medium each, Fungizone and Gentamicin at 2.5 and 5.0 mg/liter of medium, respectively). This was followed by a centrifugation at approximately 300*g* for 10 min to collect liberated single cells and fragments. The dilution of the trypsin suspension prior to the centrifugation allowed the recovery of additional cells which otherwise were trapped in the viscous trypsin supernatant. The final pellet was resuspended in 5–10 ml of standard medium, and tissue fragments were mechanically dissociated by a series of five repeated passages through a 10-ml pipet, followed by a 5-ml pipet, and finally a Pasteur pipet. The resulting suspension was filtered through a double layer of lens tissue. Cells were then collected by centrifugation (300*g*, 10 min), resuspended in 2.5 ml of standard medium and triturated by five passages through an 18-gauge needle to dissociate cell aggregates. The suspension was then fractionated on 20% Percoll in a 15 ml Corex tube as described for embryonic muscle (Yablonka-Reuveni and Nameroff, in press). Briefly, 11.5 ml of 20% Percoll in MEM was layered over 1.5 ml of 60% Percoll in a Corex tube that was first treated for about 2 hr with horse serum to minimize adherence of cells to the glass walls. The cell suspension (about 2 ml) was layered over the 20% Percoll and was centrifuged for 5 min at 15,000*g* at 8°C with brakes off in a fixed angle rotor (Sorvall SS-34). Following centrifugation, the cells at the 20/60% Percoll interface were collected and diluted with 5 vol of standard medium and recovered by centrifugation (300*g*, 10 min, room temperature). The cell pellet was resuspended in 2 ml of standard medium by repeated passage through a Pasteur pipet and additional passages through an 18-gauge needle. Cells were then counted in a hemocytometer and used for cultures.

Vital staining of isolated cells. Fluorescein diacetate (Sigma) was used as described by Persidsky and Baillie (1977) at a final concentration of 5 μg/ml. Cells were exposed to the stain for 1–3 min, collected by centrifugation, resuspended in MEM, and analyzed with a Zeiss photomicroscope equipped for epifluorescence.

Cell culture. Cells were plated into tissue culture dishes which were coated with 2% gelatin and were preincubated for 3 hr with 25% horse serum in MEM to promote cellular adherence (Quinn and Nameroff, 1983). Cultures were maintained at 37.5°C in a water saturated atmosphere containing 5% CO_2 in air. For mass cultures, cells were plated at 2×10^5 cells per 35-mm dish. For sequential passage of the cells, the culture medium was removed and the cells were treated with 0.05% trypsin (GIBCO) in MEM at 37°C for 5 min. Cells were then collected, centrifuged, and the cell pellet was resuspended in standard medium. After repeated passages through a Pasteur pipet and an 18-gauge needle, the cell suspension was filtered through a double layer of lens tissue to eliminate myotubes, and cells were plated at a concentration of 2×10^5 per 35-mm dish. Photographs of the live cells were obtained with a Zeiss inverted microscope and Kodak Tri-X film. Clonal cultures were prepared, fed, and the individual clones were identified and marked as previously described (Quinn and Nameroff, 1983; Quinn *et al.*, 1984). Briefly, short-term clones (initiated with 500 cells per 60-mm dish) were kept in culture for 4–5 days then fixed for immunofluorescence assays as described below. Long-term clones (initiated with 100 cells per 60-mm dish) were kept in culture for 10 days, then fixed with absolute methanol for 1–2 min and stained for 5 min with 1% toluidine blue in 30% methanol. The presence of myotubes in individual clones was then determined. In some instances long-term clones were fixed for immunofluorescence as described below.

Antibody staining. Immunolabeling of cells in culture was performed with the indirect immunofluorescence technique as previously described (Robinson *et al.*, 1984; Yablonka-Reuveni and Nameroff, 1986; Yablonka-Reu-

veni and Nameroff, in press). A guinea pig antiserum against chicken muscle myosin heavy chain and a rabbit antiserum against the muscle isozyme of creatine phosphokinase (M-CPK) were those described elsewhere (Quinn and Nameroff, 1983; Robinson *et al.*, 1984). Fluorescein-labeled secondary antibodies were obtained from Cappel. Cultures were rinsed (3×) with MEM warmed to 37°C, fixed for 30 sec with ice-cold AFA (70% ethanol:Formalin:acetic acid, 20:2:1) and rinsed (3×) with ice-cold MEM. Following fixation, cultures were kept at 4°C in sterile Tris-buffered saline containing normal goat serum (TBS-NGS; 0.05 *M* Tris, 1% NaCl, 1% normal goat serum, pH 7.6). Prior to adding the antibodies, cells were rinsed with Tris-buffered saline containing Tween 20 (TBS-T20; 0.05 *M* Tris, 1% NaCl, 0.05% Tween 20, pH 7.6). Cultures were then exposed to the primary antibody (1:60) for 30 min at room temperature, rinsed (3×) with TBS-T20, and exposed to the secondary fluorescent antibody (1:60) for an additional 30 min at room temperature. This was followed by another rinsing cycle with TBS-T20 and a final rinse with Tris buffer (TB; 0.05 *M* Tris, pH 7.6). Cultures were mounted in 90% glycerol containing *p*-phenylenediamine as an antifading agent (Johnson and Nogueira Araujo, 1981). Observations were made with a Zeiss photomicroscope equipped for epifluorescence, and Kodak EL 135 film (400 ASA) was used for photography.

Autoradiography. Cultures were exposed for 2 hr to [^{3}H]thymidine (1 μCi/ml final concentration; sp act 6.7 Ci/mmole; Amersham). Cultures were then fixed for immunofluorescence and reacted with the desired antibody as described above, coated with NTB2 emulsion in 1% glycerol, stored in the dark for about a week at 4°C, and developed with Microdol-X.

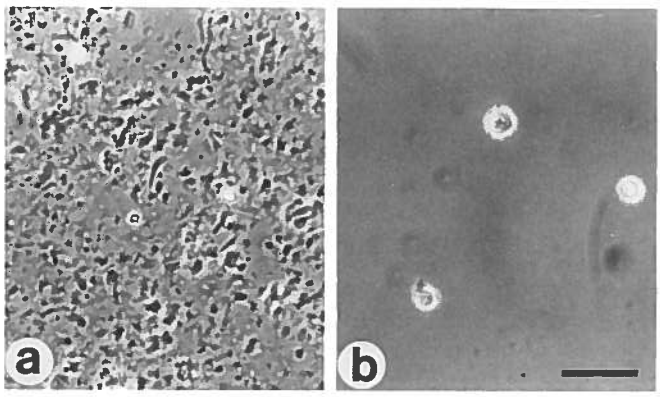

FIG. 1. Phase micrographs of satellite cell preparations obtained from the pectoralis muscle of young adult chicken. (a) Cell suspension following collagenase and trypsin digestion only; (b) cell suspension following enzyme digestion and Percoll density centrifugation. Bar = 48 μm.

TABLE 1
YIELDS AND PLATING EFFICIENCIES OF MONONUCLEATED CELLS FROM SKELETAL MUSCLE OF ADULT CHICKEN

Expt[a]	Cells/g muscle × 10^5	Plating efficiency (%)
1	3.1	40.5
2	2.9	33.0
3	1.9	28.5
4	1.5	27.0
5	4.0	33.5

[a] Cells were plated at 1–2 × 10^5 cells per 35-mm dish.

RESULTS

Density Gradient Separation of Cells

The enzymatic digestion procedure and Percoll centrifugation yielded mononucleated cells that were free from myofibril fragments and debris (Fig. 1). Over 95% of the isolated cells incorporated fluorescein diacetate, indicating that, after Percoll isolation, most of the cells were viable. Based on the number of cells isolated from the 20/60% interface, we recovered 1.5–4 × 10^5 cells/g of muscle tissue (Table 1). Prior to the Percoll centrifugation, the crude cell preparation was reacted with fluorescein diacetate to distinguish between cells and debris. About 85% of cells in the crude preparation could be recovered from the 20/60% Percoll interface following density centrifugation. After the centrifugation, the myofibril fragments and debris were detected primarily in the top third of the separating medium. Some cells could be recovered from the Percoll regions above the 20/60% interface. These cells amounted to about 1–3% of the recovered cells. In mass and clonal cultures about 40% of these lower-density cells resembled the nonmyogenic, fibroblast-like cells that were purified from chicken embryos (Yablonka-Reuveni and Nameroff, in press) while the rest were myogenic and fused into myotubes. Erythrocytes, which contaminate most satellite cell preparations, were pelleted at the bottom of the 60% Percoll and thus eliminated from the myogenic cell preparation.

Cultures of Percoll Isolated Cells

The cells from the 20/60% Percoll interface were cultured onto gelatin-coated culture dishes. When plated at high cell densities, 27–40% of the cells attached to the dish after about 15 hr in culture. Prolonging the incubation time (up to 48 hr) prior to the first medium change did not result in higher plating efficiency. The results from several experiments are summarized in Table 1. The morphology of the cells at different stages of culture is shown in Fig. 2. On the second day of culture the iso-

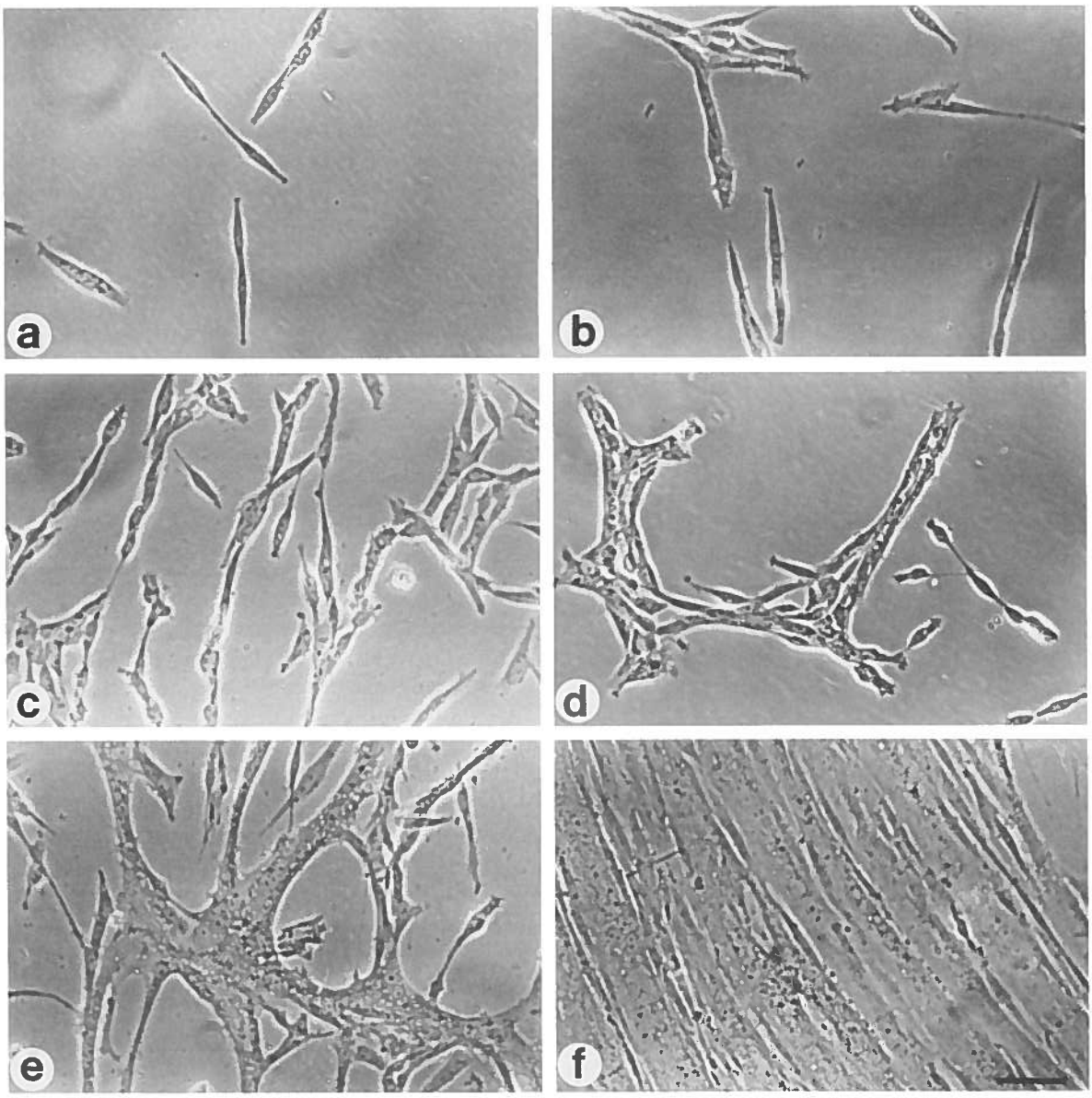

FIG. 2. The development of muscle cultures derived from satellite cells isolated from the pectoralis muscle of 10-week-old chicken. Cell were plated at 2×10^5 cells per 35-mm dish and unattached cells were removed from the dish 15 hr after initial plating. (a) Day 1 (17 hr); (b) Day 2 (40 hr); (c) Day 3 (64 hr); (d) Day 4 (85 hr); (e) Day 5 (110 hr); (f) Day 6 (132 hr). Bar = 48 μm.

lated mononucleated cells were relatively homogeneous in their appearance. Cells with flattened, fibroblast-like morphology were rarely detected. On the third day of culture many of the cells were aligned as strings. On the fourth day, in about 50% of the fields examined, cells were closely associated or clustered in a formation that sometimes resembled multistrings or sheets. In the rest of the fields cells were more widely spaced. Myotube formation was minimal on Day 4 but some differentiated muscle cells could be detected with the aid of indirect immunofluorescence with antibodies to M-CPK or skeletal myosin (data not shown). Myotube formation was very prominent by the fifth day of culture and further enlargement of myotubes took place on the sixth day. Mononucleated cells and myotubes were very dense on the sixth day and it became increasingly difficult to distinguish between mononucleated cells and myotubes. Therefore, to determine the percentage of nuclei within myotubes, immunofluorescence with antiskeletal myosin or anti-M-CPK was employed to clearly demarcate myotubes. By this procedure we estimated that over 85% of nuclei were in multinucleated fibers by the sixth day.

Fused cultures could be maintained for an additional 2–3 days without myotube detachment.

Sequential Passaging of Myogenic Cultures

Sequential passaging of mass cultures derived from satellite cells was performed in order to determine the overall myogenic potential of the isolated cells (i.e., how long myogenic properties could be maintained through divisions in culture). Primary cultures were allowed to reach a stage of massive cell fusion (7 days from initial culturing). The cells were then subcultured and consecutive subculturings were performed every third day. Parallel cultures were allowed to develop for 2–3 additional days to determine the percentage of nuclei within myofibers. The degree of myotube formation remained high and similar to the original primary cultures during four consecutive passages. When a similar protocol was applied to muscle cultures from 10-day-old chicken embryos, a sharp reduction in myotube formation was observed in first passage cultures. We also noted that in sequentially passaged cultures of cells from young adult, formation of myotubes was observed as early as the second day. This is earlier than in the adult primary cultures.

Clonal Analysis of Percoll Isolated Cells

Clonal analysis was performed in order to assess the myogenic potential of individual cells isolated from adult muscle (i.e., whether all cells could give rise to differentiated myoblasts, or whether some of the cells were non-myogenic). In addition, we hoped to gain further understanding regarding the proliferative potential and/or differentiative stage of these cells in relation to the myogenic cell lineage (Quinn *et al.*, 1985).

In the first set of clonal analyses, Percoll-isolated cells from young adult chicken muscles were plated at a density of 100 cells per 60-mm culture dish, and fixed and stained with toluidine blue after 10 days. The number of large colonies (those with more than 16 cells) which arose was counted and the percentage of those colonies which contained myotubes was also noted. Taking into consideration plating efficiencies calculated from parallel mass cultures, we estimated that 90–92% of cells that attached following the initial 15 hr in culture generated large myogenic clones. E.g., starting with 20 cultures in a typical experiment, an average of 25 ± 6 large clones were detected per dish, of which 99% contained myotubes. Plating efficiency from parallel mass cultures was 27%; thus, 91% of attached cells gave rise to large myogenic clones. This is in contrast to results obtained with embryonic myoblasts, in which only 5–25% cells (depending on embryonic age) give rise to such large clones (Quinn *et al.*, 1984; Bonner and Hauschka, 1974).

To test the above conclusions, a second type of clonal analysis was performed in which individual cells were marked after attachment and followed for a shorter period of time in culture. These experiments were performed to rule out the possibility that small, possibly short-lived, myogenic or non-myogenic clones might have been missed in the long-term clonal analysis. Single cells were individually marked, and following 4–5 days in culture the clones descended from these cells were reacted with antibody against muscle type creatine phosphokinase (M-CPK) to identify terminally differentiated cells. Only 8.6% of the recovered clones contained less than 16 cells, and all cells in these clones were positive for M-CPK. Clones that contained less than 16 cells and were negative for M-CPK comprised 20% of the clones. These latter clones were either non-myogenic or were capable of more cell divisions which would eventually lead to large myogenic clones. The remaining 71.4% clones had more than 16 cells, were negative for M-CPK and displayed no fusion. Adding the large and small clones which were negative for M-CPK we obtained a value of 91.4%, which is identical to the estimated percentage of cells which gave rise to large myogenic colonies in the long-term clonal analysis described above. This supports the idea that most if not all M-CPK-negative clones would eventually give rise to large myogenic clones. Moreover, this study suggests that the majority of the isolated cells can undergo more than four cell divisions prior to giving rise to terminally differentiated muscle cells, and in this respect resemble the proposed myogenic stem cells (Quinn *et al.*, 1984, 1985).

Morphology of Large Clones

Cells in mass culture prior to fusion were sometimes clustered together even though they were still at low density elsewhere in the dish. We also observed that many of the clones exhibited such clustering behavior. About 50–60% of the clones contained areas with cells in a tightly opposed, sheet-like formation. In many instances, these cells were closely associated with small or large myotubes whose detection was aided by immunofluorescence staining using muscle specific antibodies (e.g., anti-M-CPK, Fig. 3). Many of these cells were actively dividing as demonstrated by the incorporation of [^{3}H]thymidine (Fig. 3). Dividing cells in other regions of these clones displayed a morphology similar to that seen in embryonic clones; e.g., M-CPK negative cells well-spaced and separated from one another (Quinn *et al.*, 1985). Other areas in the clones exhibited well-defined myotubes with minimal numbers of dividing cells (Figs. 3e–f). The clustering of mononucleated proliferating cells has not been observed by us in cultures initiated from embryonic chicken muscle. At the present,

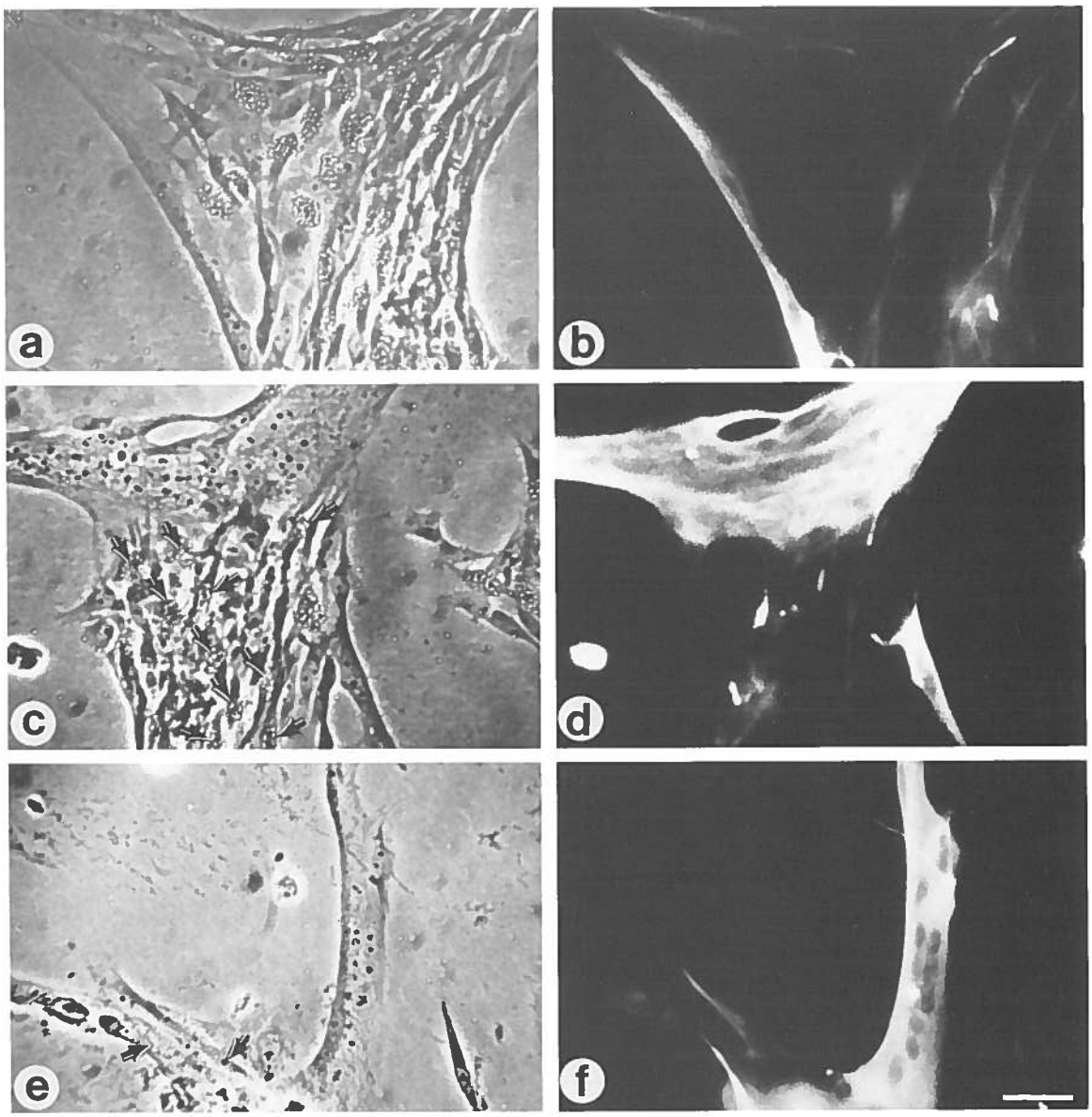

FIG. 3. Representative fields of large myogenic clones evolved from satellite cells. Cells were plated at 100 cells per 60-mm dish and clonal cultures were maintained for 10 days. During the last 2 hr in culture cells received a pulse of ^{3}[H]thymidine and were immediately fixed and processed for M-CPK detection using indirect immunofluorescence. (a, c, e) phase micrographs, ^{3}[H]thymidine incorporation is demonstrated by silver grains, arrows indicate cells with grains that did not reproduce well in the final photograph; (b, d, f) fluorescence micrographs demonstrating myotubes with or without clusters of undifferentiated, sometimes dividing cells. Bar = 20 μm.

we can only speculate on the nature of the clusters, but they may indicate different surface properties of cells from adult chicken compared to embryonic cells. Johnson *et al.* (1983) noted similar morphology in their cultures from adult chickens. Also, Yasin *et al.* (1983) observed such cell clustering in myogenic cultures from human muscles with various neuromuscular disorders.

DISCUSSION

In this study we have described a method for the direct isolation of myogenic precursor cells from the pectoralis muscle of young adult chickens. The use of Percoll density centrifugation resulted in the recovery of a cell population free of muscle fiber debris and containing less than 1% fibroblast-like cells as determined by clonal analysis. Although, in several studies on the isolation of satellite cells from normal muscle, neither difficulty in quantitation of these cells nor the presence of significant debris were described (Yasin *et al.*, 1976; Kagawa *et al.*, 1977; Jones, 1977; Cossu *et al.*, 1983, 1985), other studies have commented on this problem (Hauschka, 1974; Young *et al.*, 1978; Blau and Webster, 1981; Matsuda

et al., 1983). Debris is also apparent in photographs of satellite cell preparations taken by Johnson *et al.* (1983) and Allen *et al.* (1983, 1984) have reported attempts to reduce contamination by fiber fragments with a differential centrifugation method. Because of the elimination of cell debris by the method described here, we were able to obtain direct measurements of cell yields and plating efficiencies. An important outcome of this procedure was our ability to identify single cells during the initial culturing phase, which in turn made it possible to study the descendent clones of these single cells.

We determined that more than 90% of the clonable cells gave rise to large myogenic clones. A smaller population of myogenic cells (8–9%) produced small clones in which every cell was a terminally differentiated myoblast. These latter cells could account for those found to fuse after only a few divisions in the study of Moss and Leblond (1971). Clonal studies by Schultz and Jaryszak (1985) using 3-month-old rats have also suggested the existence of satellite cells that produce fusion capable cells after a minimal number of cell divisions. However, the criteria in the latter study for the identification of the clones as myogenic are not clear. Clonal studies performed on cells isolated from muscles of adult mice demonstrated the existence of large myogenic clones but the possible presence of small clones was not determined (Young *et al.*, 1978; Kagawa *et al.*, 1983). Our finding that over 90% of the clonable satellite cells gave rise to large myogenic clones is in striking contrast to the results obtained with myogenic precursor cells from embryonic chickens. In the embryonic cells, a majority of myogenic precursor cells undergo only one to four cell divisions and give rise to small myogenic clones with 1–16 differentiated cells (Kligman and Nameroff, 1980; Quinn and Nameroff, 1983; Quinn *et al.*, 1984). However, Quinn *et al.* (1984, 1985) described another group of embryonic myogenic cells which gave rise to large myogenic clones and had properties expected of myogenic stem cells. The latter increased in their proportion when animals of increasing embryonic ages were compared (i.e., 5 and 25% from younger and more advanced embryos, respectively).

When compared to embryonic cells in mass culture, adult myogenic cells also displayed striking differences in behavior. First, massive formation of myotubes in cultures of adult cells occurred during the fifth day in culture. This is a delay of about 36–48 hr compared to cultures of cells taken from 10- to 18-day embryos. The delay in the initiation of cell fusion could be related to the time needed for the cells to reenter the cell cycle after their quiescent period in the intact muscle or to a longer cell cycle in satellite cells. Alternatively and/or additionally, the satellite cells must produce more progeny than the embryonic cells prior to generating terminally differentiated, fusion capable cells. A second difference between satellite and embryonic mass cultures was the number of times they could be subcultured before significant reductions in myotube formation were observed. Confluent, 7-day-old primary cultures of satellite cells, which contained over 80% of the nuclei in myotubes, could be passaged four times before myotube formation was reduced. In contrast, embryonic muscle cultures displayed a marked reduction in myotube formation after the first passage.

Based on the above findings, we suggest that most of the myogenic precursor cells recovered from the adult muscle have a large proliferative potential and may be similar to the embryonic myogenic stem cells previously described (Quinn *et al.*, 1985). However, because only 30–40% of the total cells initially obtained from the adult muscle were able to grow in culture, this result may not accurately reflect the composition of the entire myogenic cell population *in vivo*. At present, we have no way of clearly identifying the myogenic precursor cells isolated from the adult muscle as the "classical" satellite cells which lie between the basement membrane and the plasma membrane of the differentiated muscle fiber (Mauro, 1961, 1979). However, in general agreement with other investigators, we have referred to the myogenic precursor cells isolated from adult muscle as satellite cells.

Several observations have led us to suggest that the contribution of fibroblasts to the satellite cell preparation is minimal. Following the initial attachment, the cultured cells appeared mostly bipolar and quite homogeneous morphologically. Overgrowth of fibroblast-like cells was observed only after five sequential subculturings. Moreover, the clonable cells that gave rise to fibroblast-like clones comprised less than 1% of the total clonable cells. This differs from the results obtained with embryonic muscle cell preparations where, even after the Percoll centrifugation step, 30% of the clonable cells gave rise to non-myogenic colonies (Yablonka-Reuveni and Nameroff, in press). Hence, in the present study, except for the Percoll centrifugation, we did not attempt further procedures for removal of possible contaminating fibroblasts. Our failure to detect clones of fibroblast-like cells is in agreement with Wright (1985). The possibility that our culture conditions do not support fibroblast attachment or growth is ruled out because we found such cells in the 20% Percoll fraction after centrifugation and were able to grow them in both mass and clonal cultures. In contrast to our study, however, various researchers have emphasized the possible contribution of fibroblasts to their satellite cell preparations and attempted to remove these cells by differential attachment for various lengths of time (Jones, 1977; Blau and Webster, 1981; Fisher *et al.*, 1983; Allen *et al.*, 1984). In extreme cases differential attachment was carried out for

16 hr (Johnson *et al.*, 1983). The procedure described here obviates the necessity for these types of procedures.

In conclusion, the data presented in this report clearly demonstrate that the method used for the isolation of chicken satellite cells gives rise to viable myogenic cells which can be maintained in mass and clonal cultures. Using this approach we demonstrated that these cells are similar in terms of proliferation and differentiation potential to myogenic stem cells from embryonic chicken. It is possible that the satellite cells from adult muscle and the embryonic myogenic stem cells are identical and originate from the same lineage. In this case the differences reported here between myogenic cells from adult and embryonic muscles in respect to higher proliferative potential of the adult cells might be explained by much higher proportions of myogenic stem cells in adult animals.

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