# A FINE-STRUCTURAL ANALYSIS OF THE FUSION OF MYOGENIC CELLS

BRUCE H. LIPTON and IRWIN R. KONIGSBERG

From the Department of Biology, The University of Virginia, Charlottesville, Virginia 22903. Dr. Lipton's present address is the Department of Zoology, University of Texas, Austin, Texas 78712.

## ABSTRACT

The fusion of myogenic cells has been examined on the fine-structural level in muscle cell cultures of embryonic Japanese *Coturnix* quail. Cells, selected by light microscopy, were serially sectioned normal to their long axis. In this plane, oblique sections of cell membranes are rare and plasmalemmal profiles are more easily traced between adjacent cells. In seven cases, pairs of cells, apparently fixed in the process of fusion, are joined by a single cytoplasmic bridge.

Since obliquely sectioned membranes often suggest cytoplasmic confluence, tilting stage analysis was employed to resolve cell membranes in suspect cases. In contrast to such artifacts of superposition, however, the observed intercommunicating pores are contained within a pair of culs-de-sac formed by the fused membranes of both cells. These blind pouches can be traced back between the cells to the external space. The confluent regions are clearly demarcated and they are not simply areas between vesicular profiles.

The results of this analysis suggest that (*a*) at no time is there any loss of integrity of the cellular envelope, and (*b*) fusion is most probably initiated at single sites between pairs of cells, the pore enlarging, leaving first vestiges and eventually no trace of the original intervening membranes.

## INTRODUCTION

During embryonic development, single skeletal muscle cells attain lengths exceeded only by those of some peripheral nerve cells. Unlike these peripheral neurons, however, skeletal muscle fibers contain large numbers of nuclei and are, in fact, fusion products of many single myogenic cells (see Konigsberg, 1965).

Cellular fusion occurs during the normal development of at least two other cell types (Galton, 1962; Urist, 1970), but it is not a common mechanism and the polykaryocytes that are formed are not as extensive or as numerous as those which comprise skeletal muscle tissue. Although the fusion of plasmalemmata is an uncommon event, the fusion of membranous components of the cytoplasm is ubiquitous. For example, the fusion of vesicles with the cell membrane and with other vesicles, as well as the formation of vesicles from the cellular envelope or from saccular organelles, are all common phenomena. Fusion during myogenesis may be, in fact, a developmental adaptation employing mechanisms which, though present in all cells, generally play a more restricted role. While normally most cells show no tendency to form syncytia, a wide variety of cells fuse in response to infection with any of several live or attenuated viruses (Harris, 1970). Similarly, we might ask whether the intrusion of virus mobilizes those mechanisms involved in intercellular membrane fusion for activities

which ultimately lead to the fusion of one cell with another.

While the cytology of the fusion of membranous cytoplasmic organelles has been studied in detail (Palade and Bruns, 1968), surprisingly little is known about myogenic cell fusion beyond the fact that it does occur. Although the experimental evidence which excludes any other origin of the multinuclearity of muscle is compelling, the fundamental descriptions of the process of fusion remain rather unsatisfying. Time-lapse cinematography has confirmed the deduction that fusion occurs, but it does not, of course, have sufficient resolution to provide any cytological detail (Capers, 1960; Cooper and Konigsberg, 1961). In a number of fine-structural studies of myogenesis, micrographs have been presented with the suggestion that they do or might represent the fusion of myogenic cells. Several of these investigators have pointed out that the apparent cytoplasmic continuity seen in their micrographs might represent, as well, oblique sections through intact membranes. These data, therefore, remain sufficiently equivocal to have raised reasonable doubt that they do, in fact, represent cellular fusion (Mauro, Shafiq, and Milhorat, 1970, see discussion on pages 176–179).

In the present study we have attempted to resolve these doubts by examining suspected fusions only in serially sectioned material, and by employing a tilting stage to examine questionable examples of cytoplasmic continuity. Both of these procedures distinguish, in different ways, between presumed membrane discontinuities due to oblique sections and actual cytoplasmic continuity between adjacent cells.

This report is based on seven cases, serially sectioned, all of which appear to be examples of cells which were in some stage of fusion at the moment of fixation. Alternative interpretations have been considered, but none of these alternatives adequately explain all of the features of the cell pairs seen in the micrographs.

## MATERIALS AND METHODS

This investigation has been conducted on cell cultures prepared from the breast musculature of embryos of the Japanese quail (*Coturnix coturnix japonica*). Since the culture techniques have been described recently in great detail (Konigsberg, 1971), they need only be summarized here. With the use of collagenase to dissociate the tissue, cell suspensions were prepared from muscle dissected from embryos of 9 days of incubation. Suspensions were cleared of undissociated clumps by filtration, counted in a hemocytometer and inoculated at low cell densities (to preclude fusion during the ensuing 24 hr) into collagen-treated Petri plates containing growth medium. After 18–24 hr of incubation at 36.5°C under 5% $CO_2$ (in air), secondary suspensions were prepared with the use of collagenase to resuspend the cells.

Secondary cultures were then established by inoculating either 400 cells (cloning density) or 31,250 cells into 3 ml of growth medium in 5 cm Falcon Petri plates (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) (collagen treated). Growth medium consisted of Eagle's minimal essential medium supplemented with horse serum (15%), embryo extract (10%), and minimal level of penicillin, streptomycin, and fungizone.

Development in culture was monitored by phase-contrast microscopy and the cultures were fixed immediately after the appearance of the first definitive multinucleated cells. After the medium was decanted the cells were fixed for 15 min in 1.8% glutaraldehyde buffered with 0.05 M sodium cacodylate brought to pH 7.3. Sucrose was added to bring the osmolarity of the fixative to that of the culture medium. (In preliminary fixation trials it became apparent, not unexpectedly, that these cultured cells are far more sensitive to osmolarity than the tissue of origin fixed en bloc.)

After fixation the cultures were washed with several changes of cacodylate-buffered sucrose and postfixed for 20 min in phosphate-buffered $OsO_4$ (Millonig, 1962). The Petri plates were then rinsed for 3 min with 1% NaCl, dehydrated with a graded series of alcohols, and the cells were embedded in Epon 812.

After the plastic had hardened, the surface of the Petri plate was scanned with an inverted optics phase-contrast microscope, and selected areas were marked on the lower surface of the plate and photographed. A wedge-shaped slab, with the selected cells oriented at the apex, was removed, cutting through both Epon and Petri plate. The thin wedge was mounted in a flat embedding chuck, the apex was trimmed on the microtome, and serial sections were taken, cutting first through the Epon with a diamond knife in a Porter-Blum MT-2 ultramicrotome. The ribbons were picked up on uncoated 250- or 200-mesh slotted grids (type R grids: Perforated Products, Inc., Brookline, Mass.). Sections were stained for 90 sec in a saturated solution of uranyl acetate in 50% acetone, followed by 30 sec in lead citrate (Venable and Coggeshall, 1965). Sections were examined with either the Philips 200 or the Hitachi HU-11E electron microscope.

## RESULTS

During the course of this investigation criteria were evolved for selecting, by phase-contrast microscope examination of fixed, embedded cultures, configurations which were most likely to contain cells in some stage of fusion at the time of fixation. Preceding the appearance of the first multinuclear cells in culture, the individual myoblasts align to form long chains of cells. Cultures fixed at the appropriate time contain, within such chains, small syncytial elements frequently in lateral association with closely applied mononucleated cells. Myoblasts so associated, in which no distinct line of demarcation between the cells could be detected, were frequently in cytoplasmic continuity with the adjacent multinucleated cell. In practice, myoblasts were selected in which one or both tips of the cell were clearly separate (see Fig. 5 C). Although these criteria narrowed considerably the choice of material, they are by no means infallible. In our experience there is no completely reliable, light microscope criterion of early fusion.

Not infrequently, sections were observed, during the early phases of this study, in which it was not possible to resolve definite boundaries between adjacent cells. All sections in which matching discontinuities in opposing cell membranes were observed were rotated through 12° on a tilting stage and reexamined. Under this kind

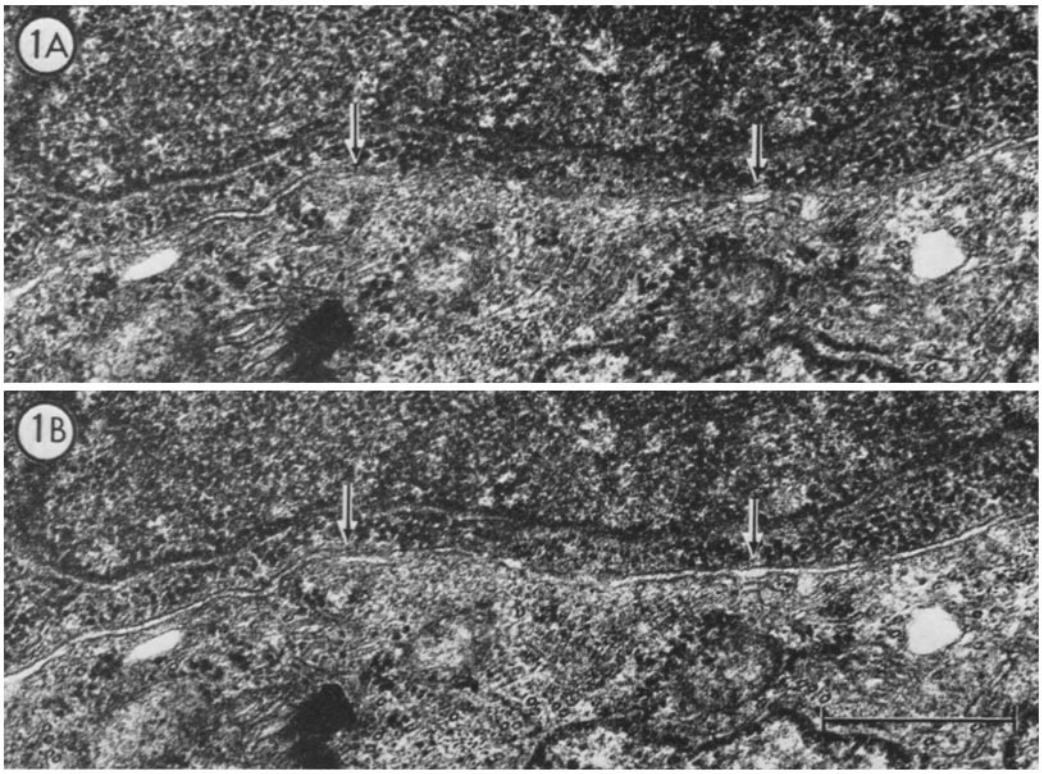



FIGURE 1 The effect of variation of the angle of incidence of the electron beam on the resolution of intervening plasmalemmata between adjacent cells. × 52,000. Scale marker equals 0.5 μ.

A. In this section the membranes separating two cells cannot be traced in the area between the arrows. The arrow on the right points to what seems to be a single, separate vesicle. Similar micrographs have been presented as illustrative of myogenic fusion.

B. The same section as in A (above) rephotographed after tilting at an angle of 12° with reference to the electron beam. What appears to be a vesicle under the right arrow in A is obviously a part of the continuous extracellular space between the cells. Intervening membranes are clearly evident in much of the area in which they appear lacking in A. Progessively steeper angles of tilt would presumably bring one or another portion of these obliquely sectioned membranes into still sharper register.

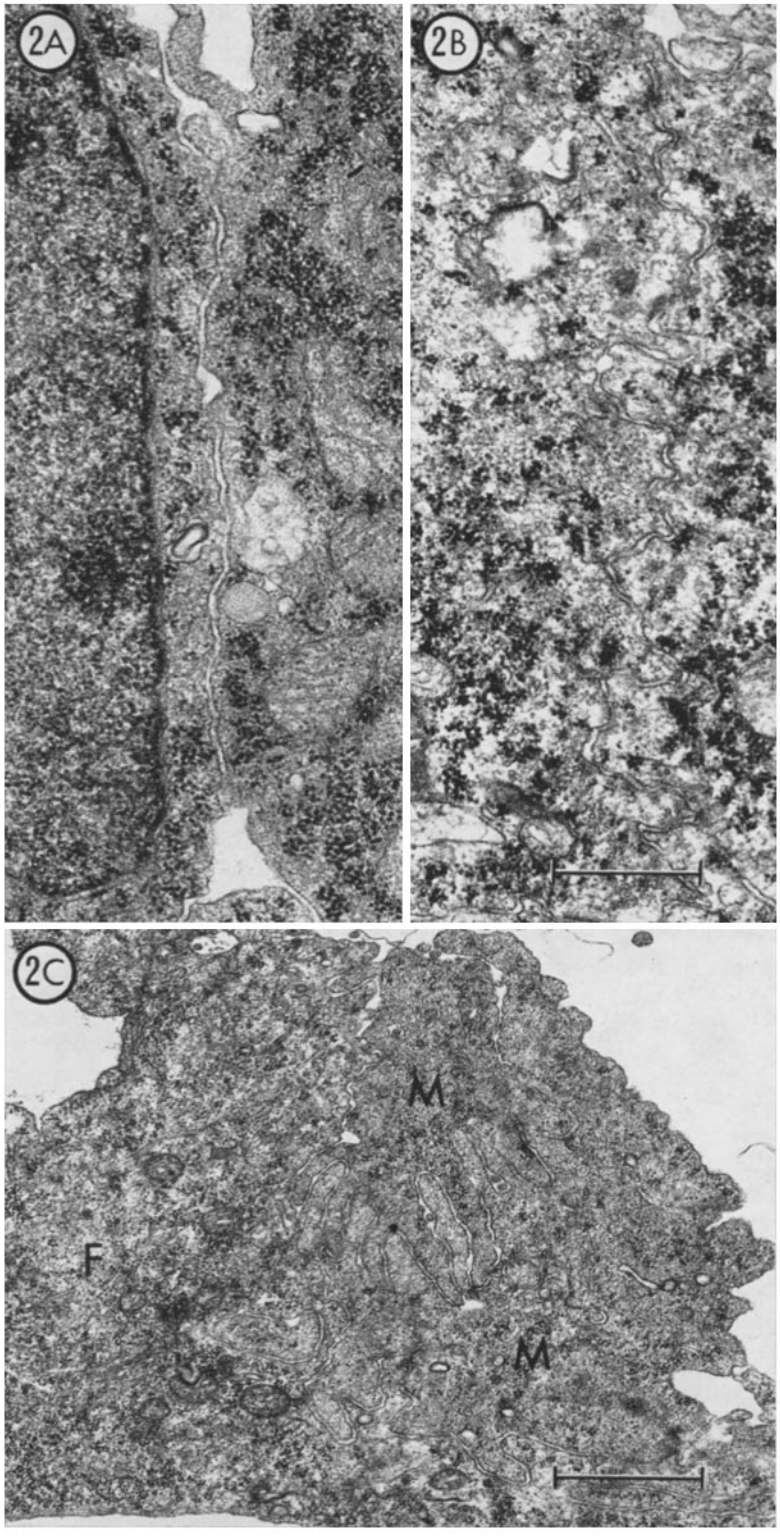



FIGURE 2 The association of opposing plasmalemmata of myogenic cells in culture. Scale marker equals 0.5 $\mu$ in A and B, and 1.0 $\mu$ in C.

A. Boundary between two myoblasts in an early culture (72 hr before multinucleated cells can be detected in culture). × 40,000.

B and C. Fixed after the appearance in culture of multinucleated cells.

B. This section shows the typical degree of interdigitation between a multinucleated cell (left side) and a mononucleated cell. × 40,000.

C. A section through two individual myoblasts (*M*) in contact with each other and with a multinucleated fiber (*F*). Notice the complexity of interdigitation between the cells and, in particular, between the myoblasts. × 20,000.

of analysis the apparent discontinuities proved, in the vast majority of sections, to be due to oblique sectioning rather than to cytoplasmic confluence (see Fig. 1).

Subsequently, we found that sectioning at right angles to the long axis of the cylindrically shaped myogenic cells (and consequently through the Petri plate) rather than tangential to their curved surfaces (that is, parallel to the surface of the plate) greatly reduces the frequency of oblique sections through the cell membranes.

In addition, in cells selected by the light microscope criteria outlined above, sections cut at right angles to the long axis reveal an extensive interdigitation of adjacent cell membranes (see Figs. 2 B and 2 C) irrespective of whether frank cytoplasmic anastamoses (described below) are observed or not. At earlier, prefusion stages, even though intercellular distances are the same (Fig. 2 A), this extensive interdigitation is never seen. The interdigitations appear, in serial section, to consist of laminar folds that are parallel to the Petri plate surface. This would explain why these folds are not evident in horizontal section and also may account for the higher frequency, in horizontal sections, of obliquely cut membranes.

All of the cells selected for examination were serially sectioned and surveyed, first, section by section at × 5000. Two micrographs selected from different levels of one serially sectioned case are reproduced in Figs. 5 and 6. One of the sections is taken from the region in which a cytoplasmic bridge joins two cells, and the other, from a more proximal (with reference to the face of the block) region, shows these two cells clearly separated from one another. The line drawing in Fig. 3 is taken from Fig. 5 A and diagrams the chief features of the region which shows complete separation of the cells. In this section (Fig. 5 A) the nuclei of three cells (as well as one large and three smaller cytoplasmic processes) can be seen. All three of the cells are distinctly separated from one another by a pair of intact cell membranes (shown also in Fig. 6 A at higher magnification). In the cytoplasm of the cell on the left, cross-sections through several bundles of ordered thick and thin myofilaments can be seen. By following this cell through successive serial sections, we were able to determine that it is, in fact, the small immature multinucleated muscle fiber in the cell group selected by phase-contrast microscopy.

The section represented in the line drawing in Fig. 4 was selected from a region approximately 1 $\mu$ farther into the block (see Fig. 5 B). At this level the nucleus of the multinucleated fiber (upper left in Fig. 5 A) can no longer be seen, being replaced in this section by a group of mitochondria. If, in this section, one traces downward the narrow extracellular space between the cell on the right and the multinucleated cell, one is led into a wide, membrane-bounded cul-de-sac (shown in Fig. 6 B at higher magnification). Continuing across the cytoplasm, in the same direction, one comes to another, narrower, cul-de-sac which limits the extracellular space on the other side of the cytoplasmic bridge and which again can be traced externally to the space immediately above the Petri plate surface (see Fig. 5 B). The cytoplasmic bridge, in this case, is present in 25–30 successive sections in the area between about the middle of the nucleus and one tip of the mononucleated cell.

The membranes in the area in which cytoplasmic continuity exists between the two cells, are clear and continuous. They can be traced down along the surface of one cell, across the bridge,

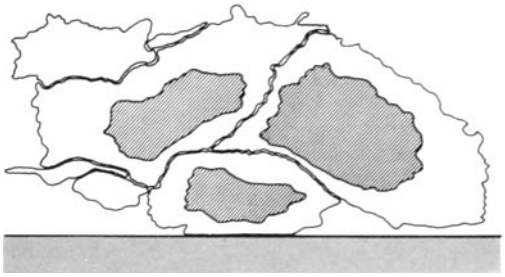


FIGURE 3 Outline drawing of the cell membranes in Fig. 5 A. Nuclei are designated by diagonal stripes.

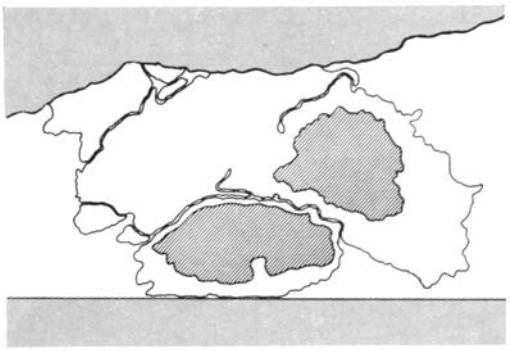


FIGURE 4 Outline drawing of the cell membranes in Fig. 5 B. Nuclei are designated by diagonal stripes.

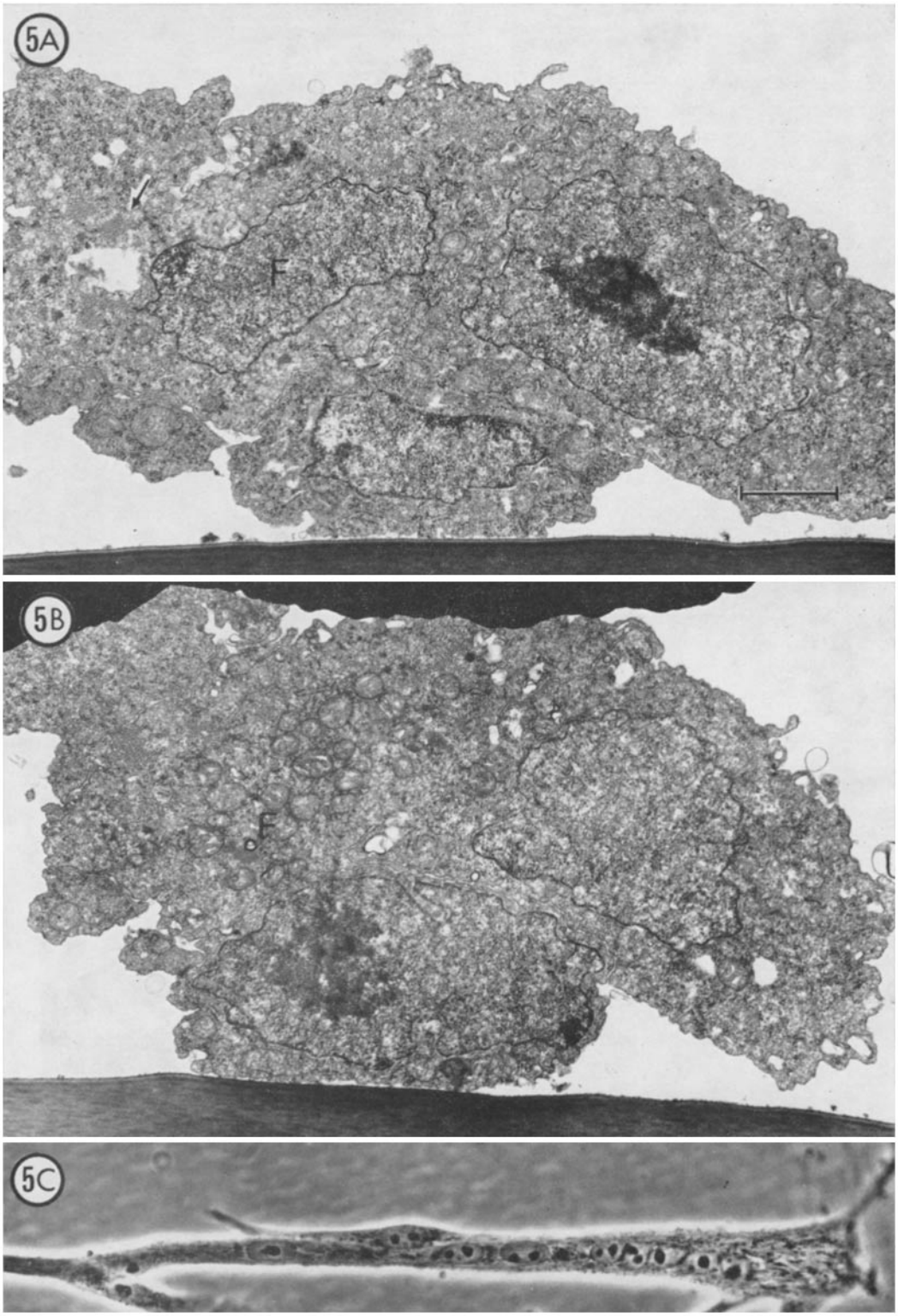



FIGURE 5 Low magnification electron micrographs (A and B) selected from two different levels of a sequence of serial sections. × 16,000. Scale marker equals 1.0 μ.

A. At this level each of the three major cells is clearly separated by a pair of cell membranes. (See Fig. 6 A also.) Nucleus of multinucleated cell, *F*. Arrow points to myofilaments.

B. In this section, farther into the block than the section above, a clearly delineated cytoplasmic bridge connects the multinucleated cell (*F*) with the upper myoblast. (See also Fig. 6 B.)

C. Phase-contrast micrograph of a single myoblast in close contact with a multinucleated cell (fixed as described, but not embedded). Similarly arranged cells were selected for serial sectioning. × 750.

and back up along the surface of the other cell, in much the same manner as the membrane investing both sperm and egg can be traced in electron micrographs of the process of fertilization (Colwin and Colwin 1963). Similar membrane configurations have also been observed in viral-induced fusion in HeLa cells (Schneeberger and Harris, 1966, their Figs. 8 and 9).

A similar region of cytoplasmic confluence from another serially sectioned case is illustrated in Fig. 7. The section in Fig. 7 A is taken from the region in which the myoblast (whose nucleus is seen in each of these sections) is completely separated from the multinucleated cell beneath it. The sections shown in Figs. 7 B and 7 C show the region containing the cytoplasmic bridge at two different levels through the cell.

One example of what appears to be an advanced stage of fusion is shown in Fig. 8. Only one end of what we assume was a separate myoblast is free (that is, unfused) for a distance of about 3 $\mu$ (Fig. 8 B). Fig. 8 A is a section cut farther into the block and shows one of the two nuclei present in the region between the two sections. The second nucleus lies in a position to the left and above the nucleus in Fig. 8 A. Elongated "vesicles" are observed in virtually every section along a plane running diagonally across these sections and roughly corresponding to a line separating the two nuclei. These "vesicles," which have a width equivalent to the space normally separating adjacent cell membranes (arrow, Fig. 8 B), appear laminar in serial section and are quite unlike any other cellular inclusion. The only other structure with which they might be confused is the smooth endoplasmic reticulum. Compare, however, the laminar inclusion in Fig. 8 A (arrow) with the Golgi saccules lying between it and the nucleus. The saccules are quite different in over-all appearance and stain less intensely.

Our interpretation of these laminar structures, on the basis of their length, width, electron opacity, and location relative to similar structures in preceding and following sections, is that they represent the vestiges of the pair of cell membranes which separated the two nuclei when they were in separate cells.

In contrast to the preceding case, the section in the micrograph shown in Fig. 9 may be an example of an extremely early stage of fusion. What appears to be a small connection between one cell and another cell can be seen two-thirds of the distance down along the region of contact between a mononucleated (*M*) cell and a multinucleated cell. Both above and below what appears to be a narrow bridge of cytoplasm, culs-de-sac similar to those observed in the previous micrographs are seen (inset). The bridge was found in only one section; neither the preceding section nor the following section showed any evidence of its presence. Since this bridge is 1200 A in diameter, it is reasonable that it would be contained in one thin section, assuming it to be circular.

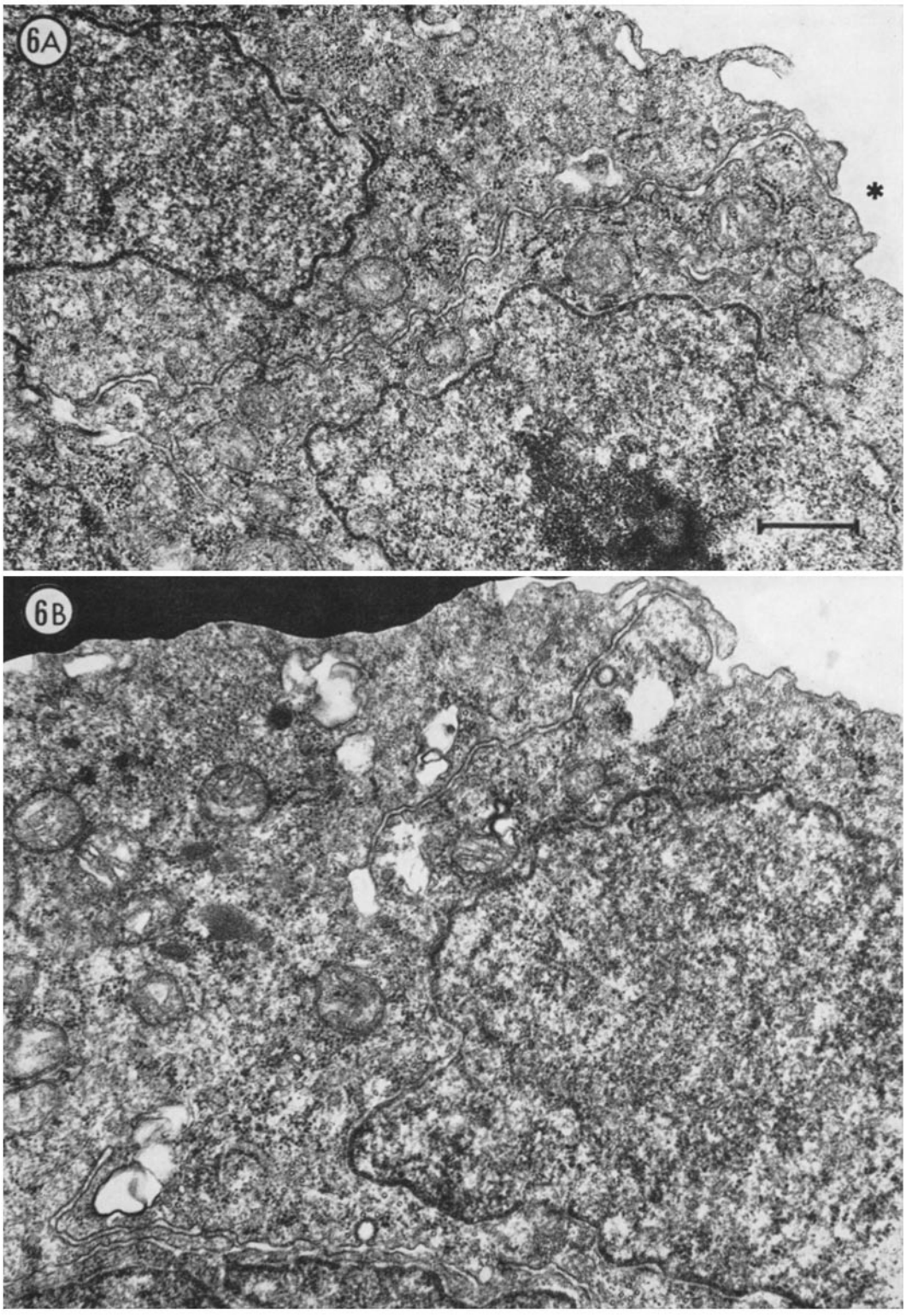
This presumed early fusion differs in one additional respect from the other six cases which we believe are examples of cytoplasmic fusion. In neither of the cells joined by the small cytoplasmic bridge can we detect the presence of any well-formed myofibrillae. Both of the cases which we consider to be late fusions contain cross-sections of thin and thick filaments arranged in the typical hexagonal pattern (Fig. 8). Such structures are also found in the four cases we have classified as "midfusion," but are restricted to the multinucleated partner only.

In all of the sections of the cells selected, one cell organelle appears with great frequency. This organelle, a fuzzy-coated vesicle (Fig. 10), is not peculiar to muscle but has been described in other cell types (Bennett, 1969; Kanaseki and Kadota,

FIGURE 6 Areas of the electron micrographs in Figure 5 shown at higher magnification. × 33,000. Scale marker equals 0.5 $\mu$.

A. This region of the section in Fig. 5 A shows the interface between the multinucleated cell (upper left) and myoblast (lower right). A pair of plasmalemmata can be traced, uninterrupted, from the space above the cells (asterisk) to the extracellular space common to these two cells and to the third cell in this section.

B. The area of Fig. 5 B, corresponding to A above, in which cytoplasmic continuity exists between the multinucleated cell and the myoblast. The most distinctive feature of the intercommunicating pore is that, in cross-section, it is bounded on both sides by a cul-de-sac formed by the fusion of the plasmalemmata of both cells. Each of these blind sacs can be traced to the exterior through the remaining extracellular space between the cells.



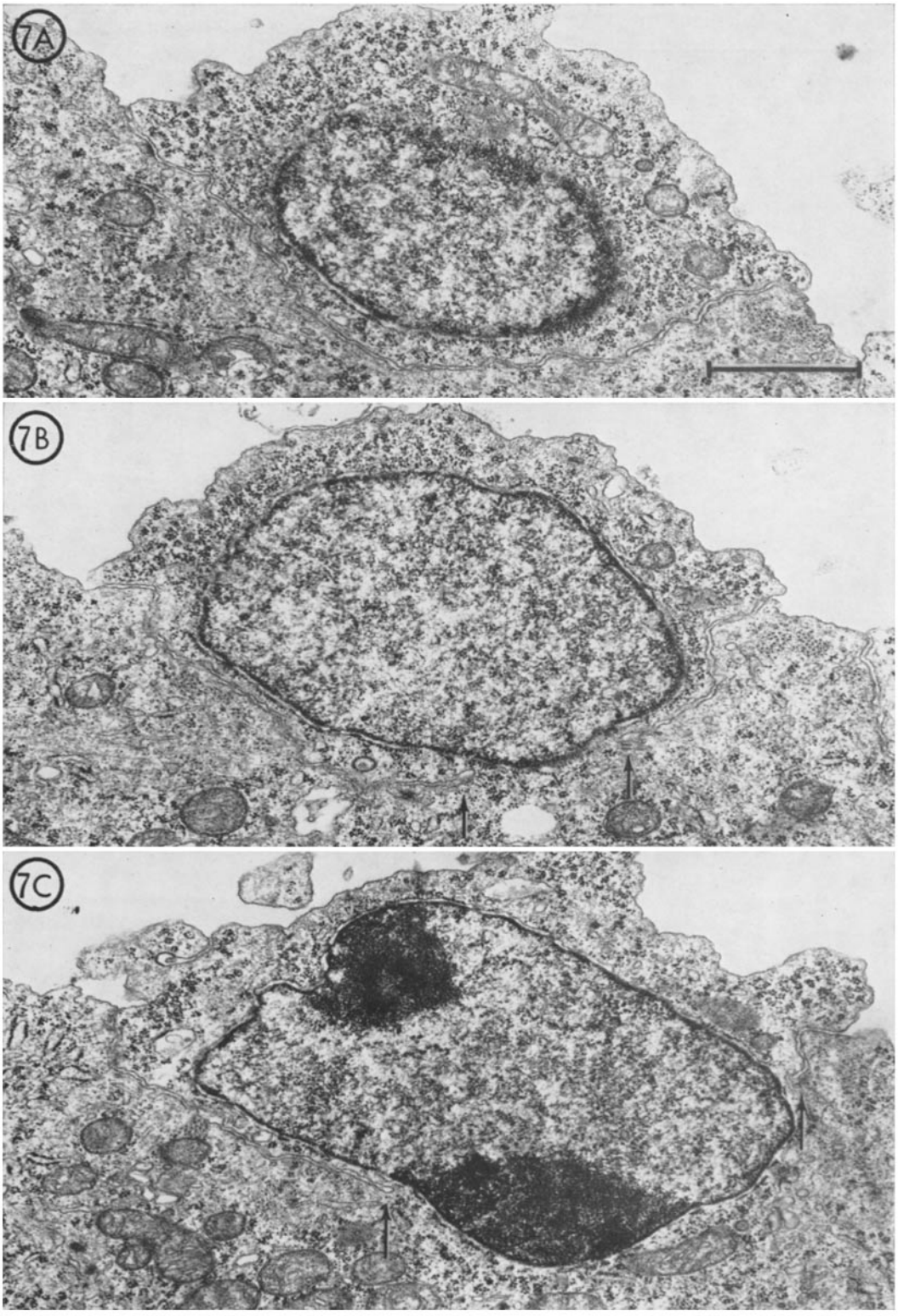



FIGURE 7 Sections through three different levels of another pair of cells, selected by the same light microscope criteria employed in the case illustrated in Figs. 5 and 6. × 23,500. Scale marker equls 1.0 μ.

A. Section in which the mononucleated cell is completely separated from the short multinucleated fiber below it.

B and C. At increasingly greater distances from the section shown in A, an intercommunicating pore of progressively larger diameter can be observed, between the cells, just beneath the nucleus of the upper cell. The arrows in both B and C mark the position of the pairs of culs-de-sac.

1969; Porter, Kenyon, and Badenhausen, 1967) and has been assumed to serve some transport function (Fawcett, 1965; Friend and Farquhar, 1967; Roth and Porter, 1964). In cultured myoblasts, most of these vesicles are associated with regions of cell-cell or cell–Petri plate interfaces. Comparing the number of vesicles touching the plasmalemma along equal lengths of cell membrane in regions of contact as opposed to regions of noncontact, we find larger numbers of vesicles per unit length in contact areas. Since the chi square value for this distribution is 13.3 ($P < 0.001$), the association of fuzzy-coated vesicles with the cell membrane in areas of contact cannot be due to chance.

Preliminary studies, using ferritin as a marker, suggest that some of these vesicles, at least, are pinocytotic. It is not unlikely, however, that a second population of similar vesicles are derived from the Golgi apparatus, from which they appear to bud (Fig. 10 B), and are transported to and fuse with the cell membrane.

These vesicles are found in all stages of either budding from, or fusing with, the cell membrane (Fig. 10), including a stage in which the inner surface of the vesicle is exposed at the surface and appears to be an integral part of the plasmalemma itself (Fig. 10 D). In some sections, by chance presumably, two such areas are apposed, on the surfaces of adjacent cells (Fig. 10 C).

These fuzzy-coated vesicles are not restricted to any particular stage of culture development. They are found in myoblasts in early as well as in late culture. They are, however, remarkably reduced in number in multinucleated muscle fibers. Similarly, the Golgi complex in well-developed multinucleated myogenic cells is also rudimentary, if present. Golgi saccules, however, like the coated vesicles, are in evidence in myoblasts at all stages in culture, showing considerable variation in complexity from cell to cell.

No surface specializations unique to muscle cells have been observed at any stage, and of the specialized junctional complexes previously described only fasciae adherentes have been observed, but rarely. Indeed, the only modification of the membrane, that is restricted exclusively to the stage at which fusion can be detected is the extensive interdigitation of the membranes of adjacent cells (Fig. 2).

## DISCUSSION

The present study is a structural analysis of the fusion of myogenic cells. The point at issue is not whether the multinuclear condition of skeletal muscle cells arises by the fusion of mononucleated myoblasts. This conclusion is already well established. It is so generally accepted, in fact, that micrographs suggesting cytoplasmic confluence between myogenic cells might appear persuasive even though similar configurations seen between liver cells would be flatly dismissed. A study of this kind, therefore, would be of questionable value without adequate controls. Two control procedures, serial sectioning and tilting stage analysis, were both employed since each has the capability of resolving different types of artifacts.

The use of such controls, in this study, validates the interpretation that the areas of apparent cytoplasmic continuity observed between myogenic cells are actually intercommunicating bridges. These bridges are distinctly different in appearance from those areas seen in micrographs published earlier in which cytoplasmic continuity is suggested by the apparent absence of plasmalemmata in regions between adjacent cells (Allbrook, 1962; Firket, 1967; Kelly and Zacks, 1969; Ross et al., 1970; Fischman, 1970; Przybylski, 1971; Shimada, 1971). On the basis of our own experience with similar sections examined on the tilting stage, we would agree with the alternative suggested by several of these investigators that such images might result from obliquely sectioned membranes between separate cells. We would suggest, in fact, that this is the most probable explanation (see Fig. 1).

The important distinctions between the cytoplasmic bridges observed in this study and the appearance of regions of oblique section are that (*a*) the areas of confluent cytoplasm observed in this study are bounded completely by membrane that is continuous with the cell membrane of both cells, and (*b*) on either side of the bridge the extracellular space can be traced to the exterior. There is thus no uncertainty at all concerning the limits of the confluent area nor are these bridges simply regions between vesicular profiles such as have been previously suggested to be involved in the process of cellular fusion (Dessouky and Hibbs, 1965; Hay, 1963; Przybylski and Blumberg, 1966). The only vesicles of similar appearance observed in this study proved to be associated with the developing T tubule system.

The identification of sites of cytoplasmic continuity between adjacent myogenic cells does not, in itself, establish that these structures represent intermediate stages in the process of fusion. After considering all of the reasonable alternatives, how-

ever, we feel that the only logically consistent interpretation is that those cells which we see united by cytoplasmic bridges *were* in the process of fusing at the moment of fixation. It is highly unlikely, for example, that the joined cells were rather in late telophase of a mitotic division. By analyzing serial sections, we have confirmed that the cytoplasmic bridges in all cases connect mononucleated cells with syncytial fibers. Since neither mitosis nor any other mode of nuclear replication occurs in multinucleated muscle fibers (reviewed in Konigsberg, 1965; see also Mintz and Baker, 1967, and Loeffler, 1970), the single nucleus could not possibly be one of two division products separated by a cytoplasmic bridge. Furthermore, the nuclei in all cases are typical interphase nuclei; the chromatin is diffuse, and one or two well-organized nucleoli are clearly evident. (Compare, for example, the nuclei in Figs. 5, 6, and 7 with the daughter nuclei of the telophase in Fig. 11.) Finally, the one distinguishing feature of cytokinesis, the presence in the furrow of cleavage filaments (Goodenough et al., 1968; Schroeder, 1968; Arnold, 1969), is completely lacking in the cytoplasmic bridges between presumed fusing cells. Such filaments are clearly evident, however, in dividing cells in muscle clones (Fig. 11).

The thesis that the cytoplasmic bridge might represent the base of a cytoplasmic process is inconsistent with the cytoplasmic inhomogeneity which exists between the multinucleated area and its postulated cell process. In the micrographs in Figs. 5, 6, and 7, actin and myosin filaments, arranged in the typical hexagonal array characteristic of myofibrils, are present only in the multinucleated component. If one assumes that the cytoplasm on the other side of the cytoplasmic bridge is a pseudopodial process, it is difficult to understand how a nucleus would be included but myofilaments excluded from such a process. In only one case were we unable to detect myofibrils in either cell (see Fig. 9). The cytoplasmic continuity in this case, however, is so narrow that the mononucleated portion could hardly be conceived of as a cell process. The exclusion of myofibrils from the mononucleated cytoplasmic area is consistent, however, with the interpretation that shortly before the time of fixation this area was a separate immature myogenic cell: a myoblast.

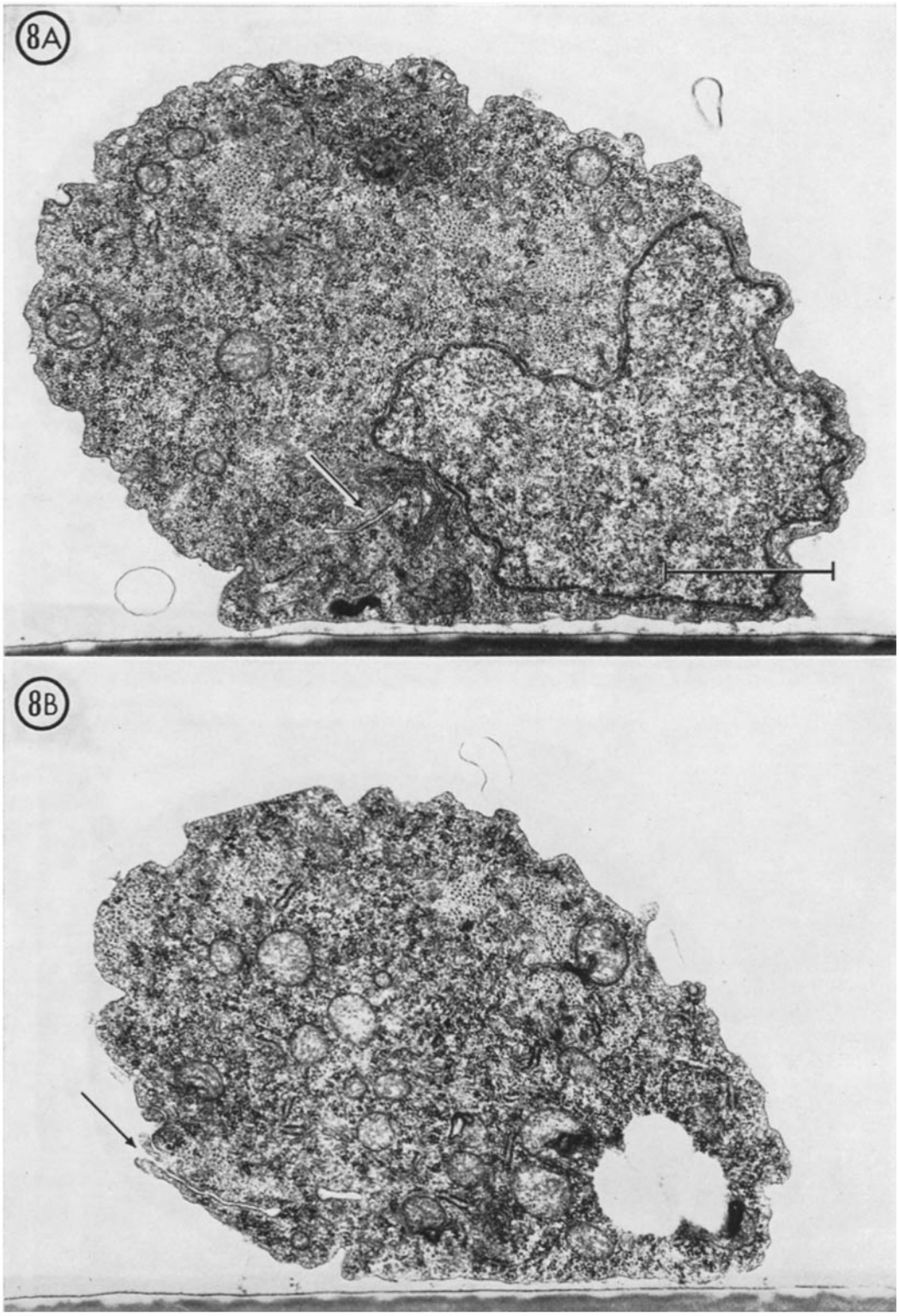
One other alternative is also excluded by the restriction of myofibrils to one cytoplasmic region only. That is, that the cytoplasmic bridges represent the tenuous interconnections between two regions of a multinuclear fiber in the process of segmentation. Multinucleated muscle fibers, in culture, sometimes appear to separate into two segments (Cooper and Konigsberg, 1961). In time-lapse cinematographic records of this phenomenon, the central region of the fiber appears to be under tension, becomes progressively attenuated, and eventually complete separation is effected. The light microscope appearance is markedly different from the configurations which we selected for electron microscopy, and it is unlikely that this cytoplasmic thinning would present the same appearance as the cytoplasmic bridges we have observed.

Examining each of the seven cases, section by section, we find only a single area of cytoplasmic continuity between any pair of cells. Although this is hardly a sufficiently large sample to evaluate statistically, it at least suggests that fusion may be initiated at a single site and may spread from there. We see in our data no reason to assume that massive regions of apposing membranes fuse simultaneously. In two of the seven cases (see Fig. 8), the area of cytoplasmic continuity is so extensive

FIGURE 8 Two sections from a series fixed apparently when fusion was close to completion. × 26,000 Scale marker equals 1.0 μ.

A. A micrograph from the middle of this group of serial sections. The arrow points to a smooth-walled laminar "vesicle" which we interpret to be a vestige of the paired membranes which originally separated two independent cells (see text). Note the similarity between the slightly swollen ends of this structure and the culs-de-sac in Figs. 5 B, 6 B, and 7 B and C. This inclusion bears little resemblance to the Golgi complex located between the upper end of this remnant and the nucleus, which is the only other smooth-walled laminar cell inclusion.

B. A micrograph of the same cell, taken from one end of the series. The arrow points to an opening, at the cell surface, of a space between opposing plasmalemmata which terminates in a blind sac. Following to the right of the blind sac in this section, one comes to another laminar vesicle similar to the one in A, but shorter and somewhat wider.



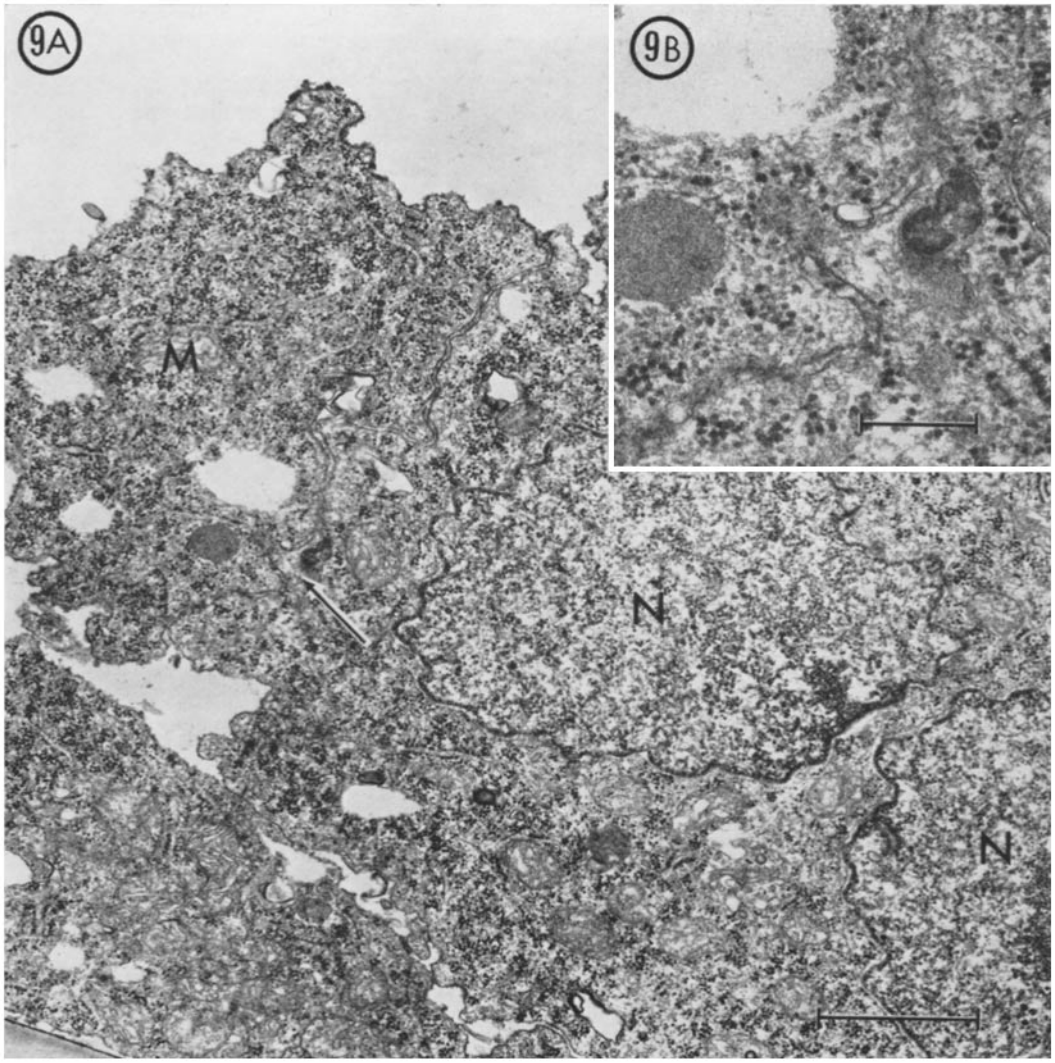



FIGURE 9 The presence of a small bridge between a multinucleated cell and a myoblast found in only one section of a serial sequence.

A. An area of the section containing the bridge at low magnification (21,000; scale marker equals 1.0 $\mu$) showing the myoblast (*M*) (the nucleus is not present at this level) and the intervening cell membranes separating it from the multinucleated cell (nuclei labeled *N*). The bridge is indicated by the arrow.

B. *Inset* shows the intercommunicating bridge at higher magnification (62,000; scale marker equals 0.25 $\mu$). The upper and lower lips of the bridge of this section also terminate in culs-de-sac.

that it could be interpreted to support either alternative mechanism. The remaining five cases, however, are compatible with the assumption that fusion is usually initiated at one site. In each case an occasional section was lost or overlapped a grid bar; however, cytoplasmic bridges of the size shown in Figs. 5 B, 6 B, and 7 B and C extend through 15–50 consecutive sections and could not possibly have been missed. We cannot exclude the possibility that small bridges of the size shown in Fig. 9 were lost.

The fact that the edge of the intercommunicating bridge joining the cells is formed by the fused membranes of both cells suggests that some process of simultaneous scission and repair of cell membranes immediately precedes cytoplasmic fusion.

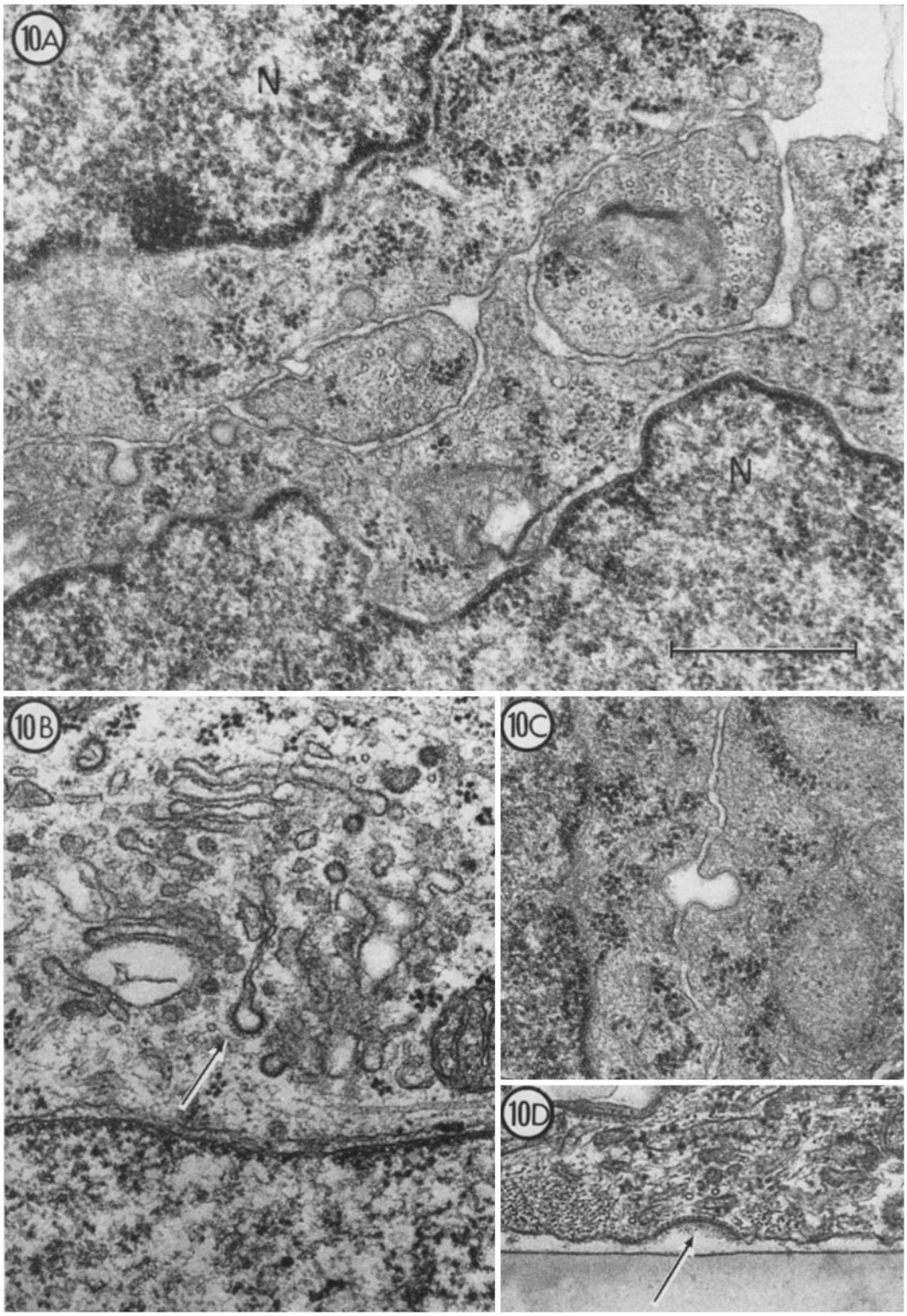



FIGURE 10 The association of fuzzy-coated vesicles with the plasmalemma of myogenic cells in areas of contact. × 57,000. Scale marker equals 0.5 μ.

A. A section of the region of contact between a very early multinucleated cell (lower nucleus, *N*) and a myoblast in which four vesicles can be seen communicating with the extracellular space.

B. Section through a portion of the Golgi apparatus showing a fuzzy-coated vesicle (arrow) which appears to be pinching off from a Golgi saccule.

C. A pair of fuzzy-coated vesicles in apposition to one another on the surface of two different cells.

D. A curved, fuzzy-coated area of the plasmalemma of approximately the same length as the circumference of a fuzzy-coated vesicle (arrow). The cell in this area is in contact with the Petri plate surface.

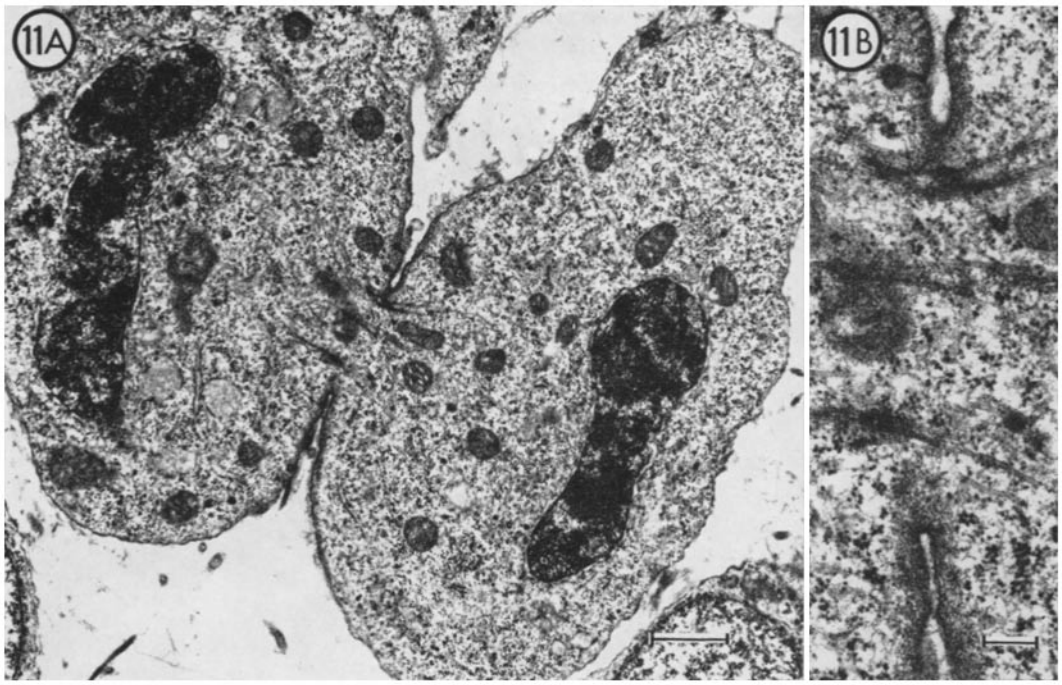



FIGURE 11 Late telophase of mitosis in a myoblast.

A. At low magnification (9500; scale marker equals 1.0 $\mu$), chromatin of the daughter nuclei as well as the remnants of the mitotic apparatus in the cleavage furrow can be seen.

B. At higher magnification (31,000; scale marker equals 0.25 $\mu$), cross- and oblique sections through the the cleavage filaments are observed in the region of high density closely applied to the membrane of the furrow.

Lucy (1970) has suggested, for example, that membrane fusion may be effected by a "change in structural organization which allows the altered membranes to participate in the fusion process and also to retain some degree of structural integrity." Lucy envisions membrane fusion to require the juxtaposition of regions of two membranes, each containing a high proportion of their lipids (or lipoproteins) arranged in globular micelles which can interdigitate and fuse to form a single entity. Unstable membrane junctions, so formed, might break down and either reestablish the original boundaries or create new ones joining two formerly separate membrane-bounded compartments. Since the micellar and bimolecular leaflet configurations are assumed to be in equilibrium, the eventual stabilization of newly formed interconnections would be predicted. Such a model would account for membrane fusion without the loss of membrane continuity at any time.

The model implies that the plasmalemma of cells which are competent to fuse is mosaic in character, in which randomly distributed patches possess a conformation facilitating fusion. Such areas very well may be below the level of resolution of our methods. In the range of images which we can discriminate, however, the fuzzy-coated vesicles, which are prevalent in areas of cell contact, represent an obvious source of structural mosaicism. Irrespective of how or where they are formed, for some finite period of time the inner surfaces of these vesicles exist as an integral part of the plasmalemma (see Fig. 10). Whether such areas play any determinative role in the fusion of myogenic cells is obviously highly speculative. These vesicles, however, must have a structural organization which facilitates fusion since they either are incorporated into the cell membrane or pinch off from the membrane. It is not unreasonable to suggest, therefore, that if two fuzzy-coated membrane areas on adjacent cells contact, they might fuse, establishing an intercommunicating pore. The massive interdigitation between myoblasts and nascent muscle fibers might serve to increase the probabil-

ity of contact between such specialized areas of the membrane.

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