CARDIAC GAP JUNCTION CONFIGURATION AFTER AN UNCOUPLING TREATMENT AS A FUNCTION OF TIME

KATE M. BALDWIN

From the Department of Anatomy, Howard University, Washington, D.C. 20059

ABSTRACT

Rabbit ventricle either was fixed in glutaraldehyde without injury (control) or was injured before fixation, presumably causing electrical uncoupling of the gap junctions. All tissue was then processed for freeze-fracture. Replicas of control gap junctions exhibited irregular packing of the P-face particles and E-face pits. Average center-to-center spacing of the particles was 10.5 nm. Tissue fixed 1–5 min after injury showed clumping of gap junctional particles and pits. Within the clumps, the particles and pits were hexagonally packed and the center-to-center spacing of the particles averaged 9.5 nm. In tissue fixed 15–30 min after injury, the clumps of gap junctional particles had coalesced into a homogeneous structure in most junctions. The packing of the particles and pits was hexagonal and the spacing of the particles averaged 9.5 nm. A few pieces of rabbit atrium were frozen without prior fixation or cryoprotection to try to assess the effect of glutaraldehyde fixation on gap junction structure. In this tissue the gap junctional particles were irregularly packed and their spacing averaged 10.0 nm.

KEY WORDS cardiac muscle · gap junctions · freeze-fracture · junctional particles · uncoupling

Gap junctions are generally recognized as the sites of electrical coupling between cardiac muscle cells (5, 13, 18, 21, 22). It might be expected, therefore, that the structure of cardiac gap junctions would change when the cells become electrically uncoupled. Unfortunately, gap junctions electrically uncoupled by injury appeared similar in electron microscope studies of sectioned cardiac muscle to those of uninjured control cells (2, 4). However, it has been possible, using the freeze-fracture technique, to demonstrate a change in mammalian cardiac gap junctions after an uncoupling treatment.

Peracchia and Dulhunty (25) have shown a decrease in beading periodicity and gap junction width in crayfish gap junctions uncoupled by EDTA or dinitrophenol. Freeze-fracture replicas of similarly treated junctions showed an increase in regularity of particle and pit packing and a decrease in spacing between particles and pits. Peracchia (23) also has reported similar kinds of changes in replicas of gap junctions from rat liver and stomach after uncoupling procedures. Furthermore, a decrease in particle diameter in the altered junctions has been described (23–25).

In the present study, a decrease in particle and pit spacing and an increase in regularity of particle packing is shown to occur in rabbit heart gap junctions presumed uncoupled by injury. Furthermore, it is demonstrated that shortly after uncoupling treatment, the gap junctional particles were aggregated into clumps, leaving particle-free areas within the junction. Later, these clumps coalesced into a homogeneous compact unit in most junctions.

J. CELL BIOLOGY © The Rockefeller University Press · 0021-9525/79/07/0066/10 $1.00
Volume 82 July 1979 66–75

MATERIALS AND METHODS

Adult rabbits, 3–5 kg in weight, were anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.), and the hearts were rapidly excised and placed in an oxygenated Tyrode's solution (152.5 mM Na^+, 2.7 mM K^+, 1.8 mM Ca^{++}, 148.3 mM Cl^-, 12.0 mM HCO_3^-, 0.4 mM $H_2PO_4^-$, and 5.5 mM glucose at pH 7.1). The experiments were carried out at room temperature because Weidmann (34) has reported that some ventricular trabeculae underwent progressive contracture at 37°C, but could be maintained for long periods at room temperature.

Fixed Control Tissue

The entire ventricle was cut into 2- to 3-mm slices, with the cuts made perpendicular to the interventricular septum. The largest of these slices, that from near the atrioventricular border, was placed immediately into 1.2% glutaraldehyde in 0.1 M cacodylate buffer at room temperature. The rest of the slices were used for experimental tissue (see below). After 30-min fixation, the slice of ventricle was further cut into 2- to 3-mm cubes. Each cube had the endocardium at one face, the epicardium at the opposite face, and cut surfaces at the other four faces. These cut surfaces would be expected to contain injured cells because the cuts were made before fixation (two faces) or after only a brief fixation (the other two faces). Therefore, after an additional 30- to 60-min fixation, the original cut surfaces, with their injured cells, were removed by trimming an ~0.5-mm-thick slice of tissue away from each of the original cut faces of the cubes. The endocardium and epicardium were also trimmed away so that the tissue blocks were reduced to ~1-mm cubes. The newly cut surfaces contained cells which were presumed to be uninjured because they were cut after being fixed. After a total fixation of 1.5–2.5 h, the blocks were rinsed overnight in 0.15 M cacodylate buffer at 4°C and then soaked in 25% glycerol in 0.15 M cacodylate buffer for 2 h at room temperature. The tissue blocks were placed in Balzers' specimen carriers (Balzers' Corp., Nashua, N.H.), frozen in Freon 22 and then placed in liquid nitrogen. The frozen specimens were fractured at −110°C in a vacuum of $<2 \times 10^{-6}$ torr in a Balzers' 360 M freeze-fracture apparatus equipped with an electron beam evaporation gun. Replication began within 5 s of making the last cut. In some cases, tissue was stored for several days at 4°C in either the 0.15 M buffer or the 25% glycerol solution before further processing. The results were the same for all treatments.

Unfixed Control Tissue

To assess the effects of glutaraldehyde fixation on the structure of gap junctions, a few pieces of rabbit atrium were frozen directly in Freon 22 without prior fixation or cryoprotection. To do this, a piece of atrium, ~3–4 mm across, was removed from the atrial wall. The whole piece was placed on a Balzers' specimen holder, epicardium upwards, and plunged into Freon 22. After 10 s, the specimen was placed in liquid nitrogen where it was stored until it was used. As the atrial wall is thin, averaging 0.5 mm in thickness, the frozen piece of atrial wall was shaped like a disk. This 3- to 4-mm disk had injured cells at its perimeter because these cells were cut while in Tyrode's solution. To remove these injured cells, ~1 mm of tissue was chipped away from the perimeter of the frozen disk. As a result, only the central portion remained, now a disk 1–2 mm across. This removal of injured tissue was carried out in liquid nitrogen before fracturing. The specimens then were fractured and replicated in the same manner as the fixed control tissue.

Experimental Tissue

While maintained in Tyrode's solution, pieces of ventricle from the excised hearts were trimmed into blocks somewhat <1 mm on a side. All faces of the blocks contained cut, and therefore injured, cells. The blocks were small enough to fit into the Balzers' specimen holders and no further trimming was done on them. The cut surfaces were allowed to heal-over for either 1–5 min or 15–30 min. The blocks were then fixed for 1.5–2.5 h and processed in the same manner as the controls. Fractures, and replicas, were made parallel to the upper surface of the blocks and at a depth of <100 μm from that surface. All structures replicated, therefore, were within 100 μm of a cut surface.

Measurements

The average interparticle spacings for the gap junctions were measured as follows: 61 micrographs (with a final magnification >× 200,000) of gap junctions from experimental and control tissues were shuffled so that the type of treatment was not known until measurements were recorded. The center-to-center spacing of clearly resolved adjacent particles was measured perpendicular to the angle of shadowing. Only particles that were closely adjacent were measured; obvious spaces within the junctions were avoided. Usually, 15–20 interparticle spacings were measured for a given junction, and these values were averaged. The junction was then assigned this average. In junctions where the E-face pits were clearly resolved, the center-to-center spacing of the pits was also measured. Only junctions that appeared to be oriented parallel to the surface of the replica were selected for measurements. All measurements are expressed as ±SE.

RESULTS

Fixed Control Tissue

Gap junctions from rabbit heart ranged from small spots 0.2 μm in diameter (Fig. 1 *c*) to large sheetlike areas ~2 μm in diameter (Fig. 1 *a*). The P face was occupied by a compact cluster of par-

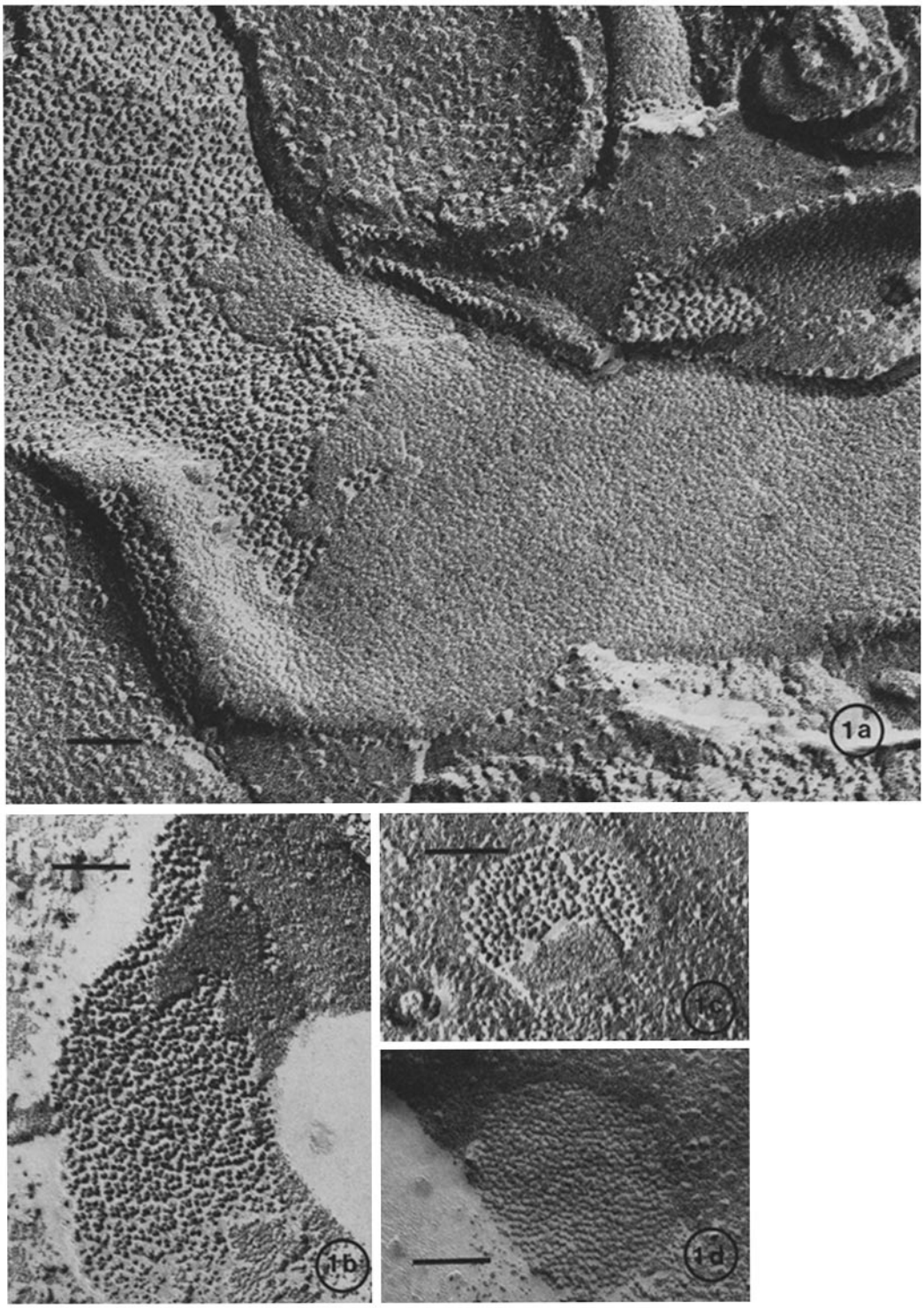

FIGURE 1 Gap junctions from fixed control tissue. Note the irregular packing of the particles on the P face and pits on the E face. Average center-to-center spacing of closely adjacent particles was 10.5 nm. Bar, 0.1 μm. × 116,000.

ticles, and the E face by corresponding pits. In the majority of junctions from this tissue (75 out of 86), the junctional particles and pits were gathered in a homogeneous array. The packing of the particles and pits was irregular, but small areas of hexagonally packed particles were sometimes seen (Fig. 1 *a–d*). The spacing between the particles was not uniform, but the average center-to-center spacing of closely adjacent, clearly resolved particles in 32 gap junctions was 10.5 ± 0.3 nm (Fig. 2). When both P-face particles and E-face pits were measured from the same junction, the spacing of the pits was always less than the spacing of the particles. Good consistent measurements of the center-to-center spacing of the pits were not obtained because of the difficulty in accurately determining the center of the pits. However, the average center-to-center spacing of the pits appeared to be ~1 nm less than that of the particles.

In 11 out of 86 junctions, the particles were somewhat clumped rather than forming a homogeneous structure. In all of these junctions, the particles and pits were hexagonally packed.

Unfixed Control Tissue

Gap junctions from unfixed, uncryoprotected tissue were similar to those of the fixed controls in that the packing of the junctional particles was predominantly irregular, but small areas of hexagonal packing were seen within the junctions (Fig. 3). The interparticle spacing was less uniform than in the fixed control tissue. The center-to-center spacing of closely adjacent P-face particles averaged 10.0 nm ± 0.3 nm for eight junctions. As in the fixed controls, the E-face pits were irregularly arranged, and their spacing was generally less than that seen for particles.

In a total of 34 junctions in this group, 26 were homogeneous structures with irregularly packed particles and pits, 6 were mostly homogeneous with irregularly packed particles (but distinct clumps of hexagonally packed particles and pits were seen within them) and 2 were entirely formed by clumped, hexagonally packed particles.

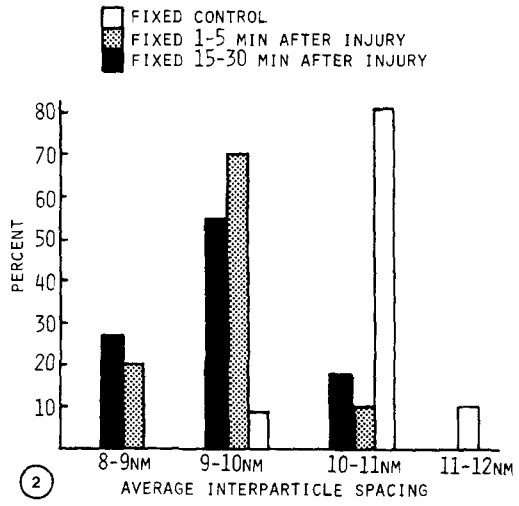

FIGURE 2 Composite histogram giving the frequency distribution of the average interparticle spacings in gap junctions expressed as percent total junctions in each group. The median for fixed control tissue (white bars) was 10.5 nm. The median for tissue fixed 1–5 min after injury (shaded bars) was 9.5 nm. The median for tissue fixed 15–30 min after injury (black bars) was 9.5 nm.

Experimental Tissue, Fixed 1–5 Min After Injury

At first appearnce, most gap junctions from tissue fixed a short time after cutting injuries appeared to be disrupted. The particles were found in small clumps with smooth particle-free areas within the junctions (Fig. 4 *a* and *b*). On close examination, however, the particles within the clumps were seen to be more tightly and regularly packed than in the control junctions. The average center-to-center spacing of closely adjacent particles in 10 junctions was 9.5 ± 0.4 nm (Fig. 2). As a rule, the particle packing was hexagonal as compared to the irregular packing seen in the controls. This difference in packing was most striking when comparing the E-face pits of the injured tissue to the control samples (Figs. 1 *a* and 4 *c*). As in the control gap junctions, the spacing of the E-face pits was less than the spacing of the particles within a given junction. The average center-to-center spacing of the E-face pits in 13 junctions was 8.2 ± 0.3 nm. Unlike that in the control junctions, the spacing of the pits was easily measured because of their very regular arrangement in rows.

The degree of particle clumping in these junctions was quite variable. In a total of 42 junctions in this group, 15 had obvious clumping (Fig. 4 *a* and *b*) while 27 had less marked clumping (Fig. 4 *c*). In no junctions did the particles form a completely homogeneous array.

Experimental Tissue, Fixed 15–30 Min After Injury

While some gap junctions from tissue fixed 15–30 min after injury were similar to those fixed 1–

FIGURE 3 Gap junction from unfixed, uncryoprotected control tissue. Here particle packing is irregular, but small areas of hexagonal packing can be seen. Average particle spacing was 10.0 nm. Ice crystals (*C*) can be seen in the cytoplasm. Bar, 0.1 μm. × 116,000.

5 min after injury (Fig. 5 *c*), most of them differed in that the particles formed a homogeneous structure with hexagonally packed particles and pits (Fig. 5 *a–b*). From measurements made on 11 junctions, the average center-to-center spacing of closely adjacent particles in these junctions was 9.5 ± 0.5 nm (Fig. 2). The center-to-center spacing of the E-face pits in nine junctions was 8.3 ± 0.2 nm.

In a total of 48 junctions in this group, 34 were homogeneous structures with hexagonally packed particles and pits (Fig. 5 *a* and *b*), 10 showed a slight degree of clumping, and in 4 the clumping was quite marked (Fig. 5 *c*).

DISCUSSION

Because it was not possible to knowingly make electrophysiological measurements on the same cells as those used for freeze-fracture studies, it was important to treat the tissue in such a way that

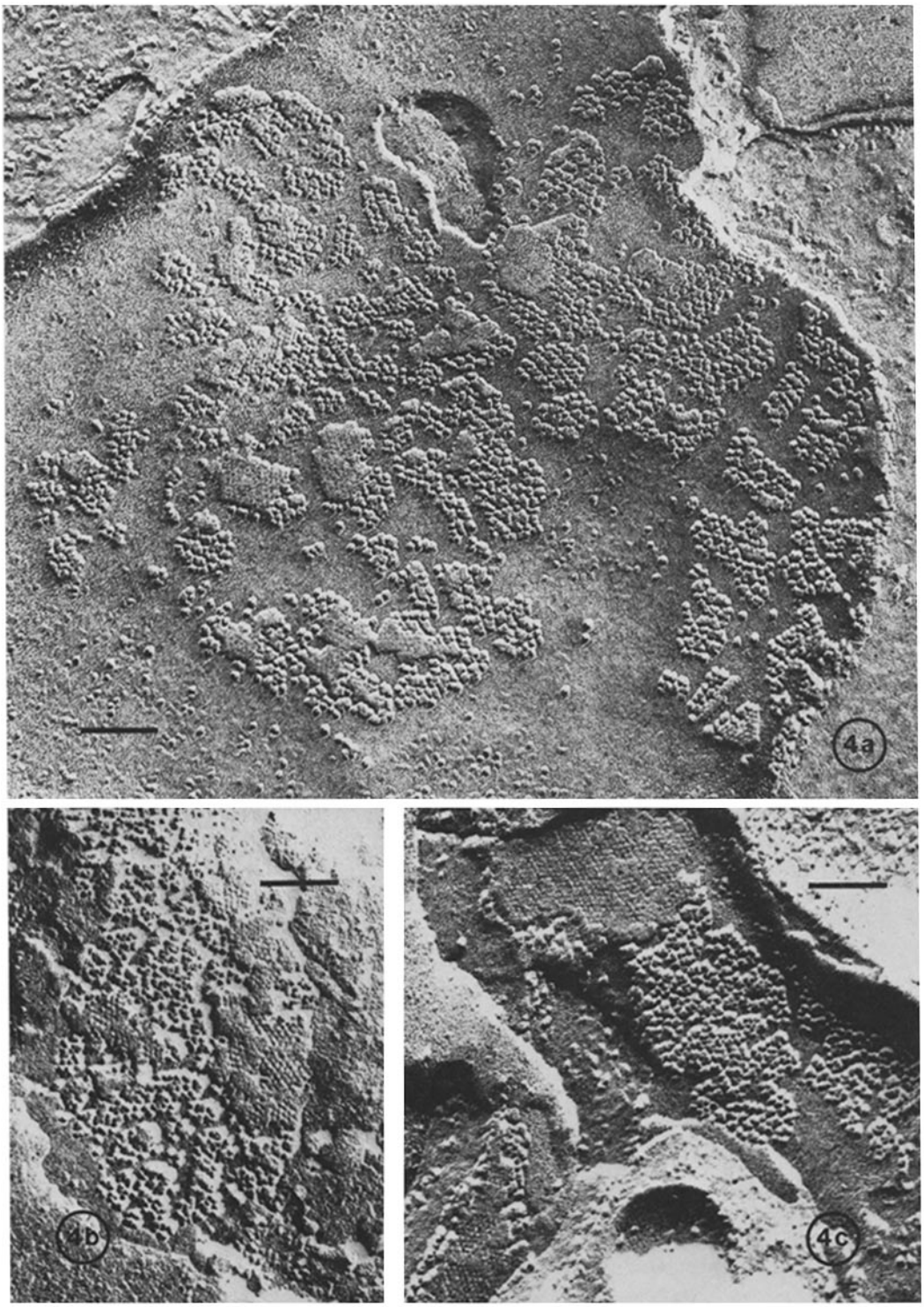

FIGURE 4 Gap junctions from tissue fixed 1–5 min after injury. Average particle spacing was 9.5 nm. Obvious clumping of junctional particles was present in some junctions from this tissue (4*a* and *b*), but the clumping was less marked in others (4*c*). Hexagonal packing was seen within the clumps. Bar, 0.1 μm. × 116,000.

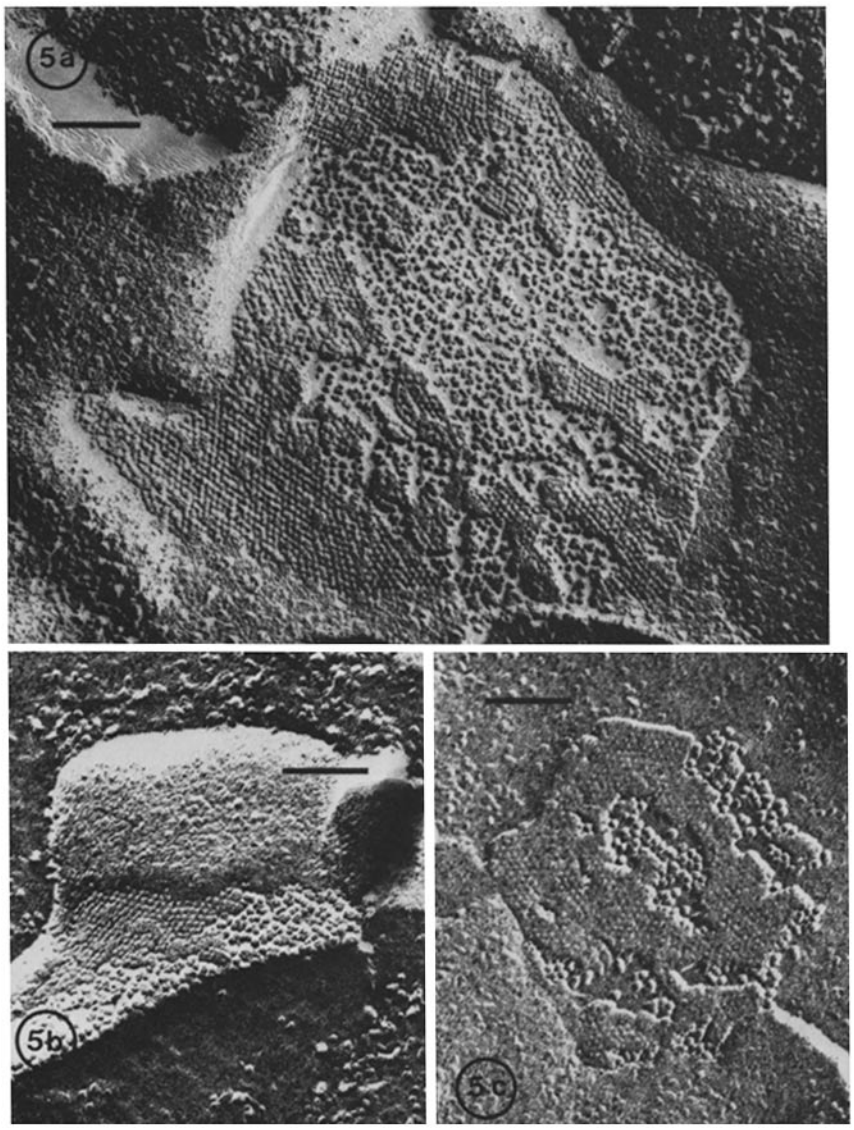

FIGURE 5 Gap junctions from tissue fixed 15–30 min after injury. The average particle spacing was 9.5 nm. Most junctions from this tissue were homogeneous (5 *a* and *b*), but a few had clumped particles (5 *c*). Note the hexagonal packing. Bar, 0.1 μm. × 116,000.

most, if not all, of the cells fractured were in the desired functional state. An attempt to do this was the basis for the methods chosen.

By a careful trimming of the blocks of control tissue during and after fixation, an attempt was made to remove all cells injured during dissection. That this attempt was largely successful is supported by two observations. First, the majority of junctions from this tissue were similar in structure to junctions from perfusion-fixed tissue (23) where none of the cells would be expected to be injured. Second, when blocks of control tissue were post-fixed, embedded, and sectioned (Baldwin, unpublished observation), most cells in them appeared uninjured and their myofibrils were in a relaxed state. A few cells, however, did appear injured because their myofibrils had undergone marked contracture. Thus, even though a few injured cells might be expected to have been present in the blocks of control tissue used for freeze-fracture,

the majority of the control junctions, those with irregularly packed particles in a homogeneous array, are presumed to have been from uninjured cells. It seems likely that those few junctions with clumped, hexagonally packed particles seen in replicas of control tissues were actually from some injured cells not removed by the trimming procedure.

As all tissue was fixed by immersion, it is possible that the cells were somewhat anoxic. However, because Peracchia (23) has shown that gap junctions uncoupled by anoxia are the closely packed, hexagonal configuration, it is reasonable to assume that any anoxia present was not so severe as to uncouple the cells.

Bennett (6) has reported that glutaraldehyde fixation caused an increase in intracellular resistance which was transient in some tissues but permanent in others. Whether the transient increase in resistance was followed by a return to the coupled state (25) or by a secondary artifactual type of uncoupling (6) is not known, but the possibility that glutaraldehyde fixation permanently alters gap-junction function must be acknowledged. In any case, junctions that are coupled at the time of fixation behave unlike those that are uncoupled at the time of fixation (3, 23, 24, 25, and this study), and we may conclude that even if glutaraldehyde does cause permanent uncoupling of gap junctions, it does so in a manner different from that seen after cellular injury.

Unfixed, uncryoprotected tissue was used in an attempt to gain information regarding the effects of glutaraldehyde fixation on the structure of gap junctions. Large cytoplasmic ice crystals could be seen in cells of the unfixed tissue (Fig. 3), but the cell membranes appeared to be intact and undamaged. The particle packing pattern in most of the junctions was irregular, similar to those from fixed control tissue. The average interparticle spacing in the unfixed control junctions, however, was not significantly different from that in either the fixed control or the fixed experimental junctions.[1] In spite of the limitations of the conventional freezing technique with unfixed, uncryoprotected tissue, we may at least conclude that the irregular packing of junctional particles in control tissue is not an artifact of glutaraldehyde fixation, because it is seen in unfixed tissues. A recent report of gap junctions in rapidly frozen, unfixed irises (28) indicates that after rapid freezing, junctional particles are irregularly arranged but widely spaced. It is possible that the appearance of the unfixed junctions in this study resulted from cell shrinkage because the particle spacing is less than that seen after rapid freezing.

Those few junctions with clumped, hexagonally packed particles seen in the unfixed control tissue may have been from cells injured (and presumably uncoupled) by the freezing and/or the dissection.

For over a century it has been known that cardiac muscle rapidly heals-over after an injury (14). This healing-over is indicated by the disappearance of the injury potential and is caused by the electrical uncoupling of the injured cells from the uninjured cells (2, 4, 9, 10). Electrical uncoupling after injury has been shown for several types of noncardiac cells also (1, 20, 27, 31). The uncoupling of intercellular junctions after injury is thought to be caused by abnormally high levels of intracellular calcium (9, 11, 20, 32). The work of Peracchia (24) on isolated gap junctions, which shows that particle packing configuration, irregular vs. hexagonal, depends on calcium concentration in the isolation medium, makes a nice connecting link between the uncoupling with calcium hypothesis and the results of this and other morphological studies on uncoupled gap junctions.

Blocks of rabbit ventricular muscle that have been cut in Tyrode's solution, allowed to heal-over, and then fixed, embedded, and sectioned have cells at their surfaces which show marked contracture (Baldwin, unpublished data). This contracture, presumably due to a high intracellular calcium concentration resulting from the injury, was not confined to the cells that were actually cut. A zone of injury 100–200 μm deep extended into the blocks from all surfaces cut in Tyrode's solution before fixation. The spread of injury to cells adjacent to those directly injured has also been reported for other types of cardiac muscle (4, 9, 10, 12). The blocks of ventricular muscle used in this study, therefore, would be expected to have had injured cells for a depth of at least 100 μm from their cut surfaces. As replicas of experimental tissue were obtained from within 100 μm of a cut (injured) surface, it is assumed that they were of injured cells. Because the intracellular calcium levels that cause contracture are similar to those that cause electrical uncoupling in cardiac muscle (35), it seems reasonable to assume that these

[1] Significance was tested at the 99% confidence level using Dunn's multiple comparison test. M. Hollander and D. A. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley & Sons, Inc., New York. 125.

injured cells are also electrically uncoupled.

The average interparticle spacings in junctions from both types of experimental tissue (9.5 nm) were significantly different from the average interparticle spacing from fixed control tissue (10.5 nm) at above the 99% confidence level using Dunn's multiple comparison test.[1]

The clumping of junctional particles in tissues fixed 1–5 min after injury varied from marked (Fig. 4*a*) to slight (Fig. 4*c*), but none of the junctions had particles in a completely homogeneous array as was often seen in tissues fixed at later times. The reason for this variability is unknown, but the difference in clumping might be explained by the different healing-over times used (1 min vs. 5 min), by variations in junctional location with respect to a cut surface, or may simply be a result of differences in individual cells in their reaction to injury. In any case, some degree of junctional particle clumping seems to be the rule for the short-term reaction of cardiac muscle cells to this type of injury.

While some junctions from tissues fixed 15–30 min after injury also had clumped particles, most of them (71%) were homogeneous structures. This would indicate that the usual pattern of reaction to injury is first a clumping of particles into hexagonally packed clusters and then a later coalescence of the clumped particles into a homogeneous, hexagonally packed unit. The presence of some junctions with clumped particles in tissues fixed 15–30 min after injury may be a result of the different healing-over times used (15 min vs. 30 min), or of variations in junctional location with respect to a cut surface, but the possibility that some junctions maintain the clumped arrangement of particles after injury cannot be ruled out. Initial clumping of junctional particles followed by coalescence into a large aggregate has recently been reported for injured, rapidly frozen, unfixed iridial gap junctions (28).

The lack of correspondence of spacing between E-face pits and P-face particles within the same junction is disturbing. It has been postulated that the particles in the membrane in one cell are lined up with the particles in the membrane of the adjacent cell and that during fracturing the pits are formed by the removal of the particles along with the P half of one of the membranes (8, 21, 22). Thus, one would expect the arrangement of the pits to coincide with the arrangement of the particles. Because the spacing of the pits was always less than the spacing of the particles, it is likely that this discrepancy is caused by some plastic deformation of either the pits, the particles, or both during fracturing rather than misalignment of the particles in one cell with respect to the particles in the other. Caspar et al. (7) have studied isolated gap junctions with negative staining and x-ray diffraction. These isolated gap junctions have been exposed to high calcium solutions and the connexon units making up these junctions are, therefore, in the form of a hexagonal lattice. The lattice constant in such junctions varies from 8.0 to 9.0 nm. Comparing this x-ray diffraction and negative-staining data to the finding in this study that hexagonally packed junctional pits are spaced an average of 8.2–8.3 nm apart would indicate that during fracturing it is the particles that undergo plastic deformation and that the pits reflect the more accurate, undeformed picture.

It is interesting to note that the "normal" structure of at least some gap junctions does not include hexagonal packing of the junctional particles as was proposed earlier (17, 21, 22, 30, 33). Furthermore, because both a decrease in particle spacing and an increase in regularity of particle packing have been seen in different tissues with a variety of uncoupling procedures (23, 25, this study), it seems likely that these effects are not unique to these tissues but may be seen whenever gap junctions are uncoupled. The clumping of particles shortly after uncoupling as reported here may also be a common feature. Such clumping was not reported by Peracchia (23) or Peracchia and Dulhunty (25), but their studies involved relatively longer uncoupling procedures and thus may have not shown that stage. Certainly, gap junctions with clumped, hexagonally packed particles are commonly reported in the literature (15, 16, 19, 26, 29), and it is possible that some of these are in fact uncoupled.

The author is grateful to Dr. S. E. Baldwin for the statistical analysis of the data and to Doctors L. V. Leak and G. M. Crosby for suggestions regarding this manuscript.

This research was supported by a grant to Howard University Medical School from the Andrew W. Mellon Foundation.

Received for publication 31 July 78, and in revised form 18 December 1978.

REFERENCES

1. ASADA, Y., and M. V. L. BENNETT. 1971. Experimental alteration of coupling resistance at an electrotonic synapse. *J. Cell Biol.* **49**:159–172.

2. BALDWIN, K. M. 1970. The fine structure and electrophysiology of heart muscle cell injury *J. Cell Biol.* **46**:455–476.
3. BALDWIN, K. M. 1974. Intercellular movement of tracers in cardiac tissue. *J. Cell Biol.* **63**(2, Pt. 2):12*a*. (Abstr).
4. BALDWIN, K. M. 1977. The fine structure of healing over in mammalian cardiac muscle. *J. Mol. Cell. Cardiol.* **9**:959–966.
5. BARR, L., M. M. DEWEY, and W. BERGER. 1965. Propagation of action potentials and the structure of the nexus in cardiac muscle. *J. Gen. Physiol.* **48**:797–823.
6. BENNETT, M. V. L. 1973. Function of electrotonic junctions in embryonic and adult tissues. *Fed. Proc.* **32**:65–75.
7. CASPAR, D. L. D., D. A. GOODENOUGH, L. MAKOWSKI, and W. C. PHILLIPS. 1971. Gap junction structures. I. Correlated electron microscopy and x-ray diffraction. *J. Cell Biol.* **74**:605–628.
8. CHALCROFT, J. P., and S. BULLIVANT. 1970. An interpretation of liver cell membrane and junction structure based on observation of freeze-fracture replicas of both sides of the fracture. *J. Cell Biol.* **47**:49–60.
9. DELEZE, J. 1970. The recovery of resting potential and input resistance in sheep heart injured by knife or laser. *J. Physiol. (Lond.).* **208**:547–562.
10. DELEZE, J. 1975. The site of healing over after a local injury in the heart. *Recent Adv. Stud. Card. Struct. Metab.* **5**:223–225.
11. DEMELLO, W. C. 1975. Effect of intracellular injection of calcium and strontium on cell communication in heart. *J. Physiol. (Lond.).* **250**:231–245.
12. DEMELLO, W. C. 1976. Influence of the sodium pump on intercellular communication in heart fibers: effect of intracellular injection of sodium ion on electrical coupling. *J. Physiol. (Lond.).* **263**:171–197.
13. DEWEY, M., W. BERGER, and L. BARR. 1969. The role of the nexus in the spread of excitation in mammalian cardiac muscle. *Anat. Rec.* **163**: 178 (Abstr.).
14. ENGELMANN, TH. W. 1877. Vergluchende Untersuchungen zur Lehre von der Muskel-und Nervenelektricitat. *Pfluegers Archiv Gesamte Physiol. Menschen Tiere.* **15**:116–148.
15. FRIEND, D. S., and N. B. GILULA. 1972. Variations in tight and gap junctions in mammalian tissues. *J. Cell Biol.* **53**:758–776.
16. GILULA, N. B. 1974. Junctions between cells. *In* Cell Communication. R. P. Cox, editor. John Wiley & Sons, Inc., New York. 1–30.
17. GOODENOUGH, D. A., and W. STOECKENIUS. 1972. The isolation of mouse hepatocyte gap junctions. *J. Cell Biol.* **54**:646–656.
18. KREIBEL, M. E. 1968. Electrical characteristics of tunicate heart cell membranes and nexuses. *J. Gen. Physiol.* **52**:46–59.
19. LARSEN, W. J. 1977. Structural diversity of gap junctions. A review. *Tissue Cell.* **9**:373–394.
20. LOEWENSTEIN, W. R., M. NAKAS, and S. J. SOCOLAR. 1967. Junctional membrane uncoupling. *J. Gen. Physiol.* **50**:1865–1891.
21. MCNUTT, N. S., and R. S. WEINSTEIN. 1970. The ultrastructure of the nexus. A correlated thin-section and freeze-cleave study. *J. Cell Biol.* **47**:666–688.
22. MCNUTT, N. S., and R. S. WEINSTEIN. 1973. Membrane ultrastructure at mammalian intercellular junctions. *Prog. Biophys. Mol. Biol.* **26**:45–102.
23. PERACCHIA, C. 1977. Gap junctions. Structural changes after uncoupling procedures. *J. Cell Biol.* **72**:628–641.
24. PERACCHIA, C. 1978. Calcium effects on gap junction structure and cell coupling. *Nature (Lond.).* **271**:669–671.
25. PERACCHIA, C., and A. F. DULHUNTY. 1976. Low resistance junctions in crayfish. Structural changes with functional uncoupling. *J. Cell Biol.* **70**:419–439.
26. PINTO DA SILVA, P., and N. B. GILULA. 1972. Gap junctions in normal and transformed fibroblasts in culture. *Exp. Cell Res.* **71**:393–401.
27. POLITOFF, A., and G. D. PAPPAS. 1972. Mechanisms of increase in coupling resistance at electrotonic synapses of the crayfish septate axon. *Anat. Rec.* **172**:384 (Abstr.).
28. RAVIOLA, E., D. A. GOODENOUGH, and G. RAVIOLA. 1978. The native structure of gap junctions rapidly frozen at 4°K. *J. Cell Biol.* **79**(2, Pt. 2):229*a*. (Abstr.).
29. REVEL, J. P., A. G. YEE, and A. J. HUDSPETH. 1971. Gap junctions between electronically coupled cells in tissue culture and in brown fat. *Proc. Natl. Acad. Sci. U.S.A.* **68**:2924–2927.
30. REVEL, J. P., P. YIP, and L. L. CHANG. 1973. Cell junctions in early chick embryo—a freeze-etch study. *Dev. Biol.* **35**:302–317.
31. ROSE, B. 1971. Intercellular communication and some structural aspects of membrane junctions in a simple cell system. *J. Membr. Biol.* **5**:1–19.
32. ROSE, B., and W. R. LOEWENSTEIN. 1976. Permeability of a cell junction and the local cytoplasmic free ionized calcium concentration: A study with aequorin. *J. Membr. Biol.* **28**:87–119.
33. STAEHELIN, L. A. 1974. Structure and function of intercellular junctions. *Int. Rev. Cytol.* **39**:191–283.
34. WEIDMANN, S. 1970. Electrical constants of trabecular muscle from mammalian heart. *J. Physiol. (Lond.).* **210**:1041–1054.
35. WEINGART, R. 1977. The actions of ouabain on intercellular coupling and conduction velocity in mammalian ventricular muscle. *J. Physiol. (Lond.).* **264**:341–365.