NEXUS OF FROG VENTRICLE

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ABSTRACT

Here we demonstrate in Rana pipiens ventricle a nexus with very unusual morphology. This tissue has been reported previously to lack nexuses. The nexus appears in thin sections of ventricle, fixed in aldehyde and OsO₄ or permanganate, as a series of punctate membrane appositions regularly alternating with regions of membrane separation. The junctional width at membrane appositions, as determined by microdensitometry and optical measurements, is 15-17 nm, and the width of the electron-translucent region between the junctional membranes is 1.8 nm. These values correspond closely to similar measurements of the more typical nexuses in frog liver. Along the nexus the mean distance between punctate appositions is 74.5 nm.

Freeze-cleave replicas of the nexuses between myocardial cells show particles 10.4 nm in diameter arranged in arrays of up to nine linked circles or partial circles on the PF-face and similar arrays of pits of shallow grooves on the EF-face. The mean diameter of the circles on both membrane fracture faces is 76.7 nm. A comparison of the thin-sectioned and freeze-cleaved nexuses demonstrates an excellent correspondence between the spacing of membrane appositions along the junction and the diameters of the freeze-cleaved circles of particles and pits or grooves.

Compelling evidence exists for the propagation of action potentials along vertebrate cardiac cells by the electrotonic spread of current across low resistance junctions. Such electrotonic spread of current has been demonstrated in Purkinje fibers (45), rat atrium (47), calf and sheep ventricle (46), and frog atrium (2, 4, 43). Correlated electrophysiological and morphological studies in guinea pig papillary muscle and frog atrial muscle (4) and rat ventricular muscle (16) have demonstrated that the nexus¹ is the intercellular junction associated with the electrotonic coupling of these cardiac cells. Additional support for the involvement of the nexus in the electrotonic coupling of cells comes from similar experiments with smooth muscle (3), the crayfish septate giant axon (28, 29), tissue culture cells (17, 19), and the fish nervous system (5-8).

In the ventricle of Rana pipiens, nexuses have been reported to be absent or not unequivocally demonstrated (38-40, 42). The failure to demonstrate nexuses in frog ventricle is difficult to reconcile with the evidence for electrotonic coupling of the term nexus to designate functional connections between cells. We believe that the term gap junction draws attention away from the physiologically important aspects of this junction.

¹ We have chosen to continue to use the term nexus for the junction described here. This usage is only a slight modification of Sherrington's (Sherrington, C. S. 1906. The Integrative Action of the Nervous System. Yale University Press; New Haven, Conn. 15-17) use of the term nexus to designate functional connections between cells.
frog cardiac cells (2, 4, 43, 44) and with the widespread occurrence of nexuses in the hearts of animals belonging to most of the major vertebrate classes (4, 20, 25, 26).

Previously, many nonmammals such as the axolotl, goldfish, turtle and chicken were also thought to lack nexuses. These species have now been shown to possess nexuses much shorter in length and much sparser in distribution than mammalian cardiac nexuses (25). The possibility existed that the frog ventricle might also possess unusual nexuses not clearly identified previously either because the junctions were labile under tissue preparative conditions (14), or because the junctions have an unusual morphology.

For this reason, we have investigated the intercellular junctions in the ventricle of Rana pikes. We report the demonstration and characterization of a nexus with very unusual morphology and present a model for the structure of this nexus.

**MATERIALS AND METHODS**

*Rana pikes* obtained from West Jersey Biological Farm, Wenonah, New Jersey were maintained at 20°C in running tap water, with forced feeding twice weekly. Males and females (sex determined by macroscopic observation) were studied during all seasons. Osmolarities were determined with an Advanced DigiMatic osmometer (Advanced Instruments, Inc., Needham Heights, Mass.).

**Dissection and Fixation**

The heart was excised from pithed frogs and placed in Ringer’s solution containing 6.5 g of NaCl, 0.14 g of KCl, 0.12 g of CaCl₂, 0.2 g of NaHCO₃, 2.0 g of glucose, and 0.01 g of NaH₂PO₄ per liter. The ventricular wall was incised from the atria to the ventricular tip, and the heart pinned open on a sheet of plastic. Some hearts were fixed immediately, some were allowed to recover from the trauma of dissection for 1 h before fixation, and others were further dissected before fixation. In the latter procedure, small 200-500-μm diameter bundles were dissected free, leaving only one end attached to the remainder of the heart including the sinus venosus; all continued to beat or were rejected. The dissected heart with isolated bundles was incubated for 1 h in Ringer’s solution and then fixed. Hearts were also fixed by injecting the fixative into the in situ ventricle of a pithed frog. These hearts were excised and immersed in fresh fixative and dissected into cubes 1 mm on a side.

Frog liver was fixed by perfusing aldehyde fixative through the aorta. Successful fixation of the liver was judged by a blanching and hardening of the liver. The fixed liver was excised, placed in fresh fixative, and dissected into cubes 1 mm on a side.

A variety of glutaraldehyde fixatives were tested. A modified Karnovsky’s fixative (21) containing 1.5% glutaraldehyde and 1.5% paraformaldehyde in 170 mosM sodium cacodylate buffer, pH 7.4 (total fixative osmolality of 831 mosM) yielded the optimum fixation and unless otherwise specified was the aldehyde fixative employed. Tissues were fixed in the aldehyde fixative for 4-5 h, rinsed overnight in 170 mosM cacodylate buffer containing 20 mg/ml sucrose (total buffer rinse osmolality of 230 mosM), and postfixed for 3 h in 2% OsO₄ in 230 mosM cacodylate buffer (pH 7.4). All fixative and buffer solutions were maintained at ±4°C, and most of them contained 0.5 mg/ml of anhydrous CaCl₂. Post-fixed tissues were rinsed with 230 mosM sodium maleate buffer (pH 5.0) and en bloc stained with 2% uranyl acetate in the same buffer, either at room temperature or at 60°C.

The permanganate fixative contained 50 ml of 1.66% sodium permanganate solution, 20 ml of a stock veronal acetate solution (2.84 g of sodium barbital and 1.15 g of anhydrous sodium acetate in 100 ml water), 0.28 g of NaCl, 0.04 g of KCl, 0.02 g of CaCl₂, and 0.02 g of MgCl₂, all diluted to a total vol of 133 ml. The pH was set to pH 7.5 with HCl. Isolated bundles were fixed for 2 h at ±4°C and rinsed repeatedly in Ringer’s solution before dehydration.

Ruthenium red (practical grade, 20% dye content, Sigma Chemical Co., St. Louis, Mo.) was employed as a tracer of the extracellular space (23, 24). Isolated bundles were fixed for 4-5 h in the aldehyde fixative, rinsed overnight at ±4°C in 230 mosM cacodylate buffer (pH 7.4) containing 10 mg/ml ruthenium red and postfixed for 3 h at room temperature in 2% OsO₄ in the same buffer containing 10 mg/ml ruthenium red. The tissue was en bloc stained as described above. All tissues prepared for thin sectioning were dehydrated in acetone (4°C) followed by propylene oxide and were embedded in Epon-Araldite plastic. Silver sections were cut on an LKB Ultrotome III (LKB Instruments, Inc., Rockville, Md.), collected on uncoated grids, and stained (with the exception of the ruthenium red-stained tissue) for 10 min with 2% uranyl acetate in 30% methanol and for 3 min in Reynolds’s lead citrate (36).

**Freeze-Fracture Studies**

Frog hearts were excised from pithed frogs, pinned open, and incubated for 1 h in Ringer’s solution. Some hearts were fixed for 1-2 h in the aldehyde fixative and bathed in 30% glycerol for 3-4 h at room temperature. Other hearts were immersed (without prior fixation) in 10% and 20% glycerol (1 h in each solution) and subsequently in 30% glycerol for 1-2 h at room temperature. Frog liver fixed by perfusion was soaked in 30% glycerol for 3-4 h at room temperature. All glycerol solutions contained 230 mosM cacodylate buffer. Small pieces of the glycerinated tissue rapidly frozen in liquid Freon 22 and liquid nitrogen were fractured and replicated at
-115°C (pressure, 5 × 10⁻⁴ Torr) on a Balzer's BAF 300 freeze-etch device equipped with a platinum-carbon electron beam gun and quartz thin-film monitor (Balzer's High Vacuum Corp., Santa Ana, Calif.). Platinum-carbon shadowing (2-nm thick film, 45° shadow angle) of the cleaved tissue was performed within 2 s of the last knife cut and was immediately followed by the deposition of a 20-nm thick carbon coat on the replica. The replicas were cleaned with Chlorox bleach, repeatedly rinsed with distilled water, and picked up on Formvar-coated 200-mesh grids. The micrographs are printed as positive images and the shadowing direction is indicated by an arrow in the lower right corner.

The freeze-cleave nomenclature proposed by Branton et al. (9) is employed to designate membrane fracture faces.

**Electron Microscopy**

All thin sections and freeze-cleave replicas were examined on a Hitachi HU-12 electron microscope at 75 kV. The electron optical magnification was calibrated with a 54,864 lines/inch replica of a diffraction grating (Ladd Research Industries, Inc., Burlington, Vt.). Photographs were taken on Kodak Contrast Lantern Slide Plates (Eastman Kodak Co., Rochester, N. Y.).

**Measurements and Statistics**

All electron micrographs utilized for both the microdensitometric measurements and the optical measurements were photographed at a magnification of 90,000.

**Microdensitometric Measurements:** The structure of the nexus at the regions of closest membrane contact was reconstructed from microdensitometric tracings recorded with a Joyce, Loebl Mark IIIc microdensitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England) at a total magnification of 4.5 million (scale: 1 mm on tracing = 0.2 nm in specimen). Microdensitometric reconstructions of frog liver nexuses were prepared for comparison. These junctional reconstructions were based on the principle that the width on a plate of a structure producing a microdensitometric peak is proportional to the width of the peak at its half-peak height, where the half-peak height is at the midpoint of a line constructed from the peak's apex perpendicular to the peak's base.

**Optical Measurements:** Electron micrographs were projected onto a screen with a Durst F-30 enlarger (Durst AG, Bolzano, Italy), and the measurements were made either directly from the projected image or from tracings of the image. The optical enlargement was calculated from the image size of a projected micrometer scale graduated in 0.1-mm units.

The total widths of the myocardial and liver nexuses were measured at a total magnification of 2,060,000 (scale: 1 mm on projected image = 0.48 nm). The intervals between the punctate appositions along the length of the myocardial nexuses and the distance of the cytoplasmic densities from the central plane of the nexus were measured at a total magnification of 817,000 (scale 1 mm on projected image = 1.2 nm). The diameters of freeze-fractured particles in heart and liver nexuses were measured perpendicular to the direction of shadowing at a total magnification of 2,140,000 (scale: 1 mm on projected image = 0.47 nm). The diameters of the freeze-fractured rings of nexal particles and pits were determined from tracings of the junctions drawn from projections at a total magnification of 2,140,000 (scale: 1 mm on projected image = 0.47 nm). Circles which best fit the midpoints of each group of circularly arrayed particles or pits were constructed on the tracings, and their diameters measured. The mean and standard deviation of each set of values was determined with a Hewlett-Packard model 9830A computer (Hewlett-Packard Co., Palo Alto, Calif.).

**RESULTS**

**Thin Sections: Aldehyde, OsO₄ and Permanganate Fixation**

Only two types of intercellular junctions were observed in thin sections of frog ventricle fixed either in aldehyde and OsO₄ or in sodium permanganate. The first type, the fascia adherens, has previously been described in the frog heart (1, 4, 38, 42). The second type of intercellular junction, the nexus, was often found near the fascia adherens. This unusual nexus appears at low magnification as a specialized region of plasma membrane apposition similar to nexuses of other tissues but lacking the precise parallel arrangement of the juxtaposed plasma membranes (13, 14, 26). An unusual cytoplasmic density lies along each junctional membrane.

At higher magnification, the myocardial nexus frequently appears as two to seven punctate regions of membrane apposition regularly alternating with regions in which the junctional membranes diverge 5-15 nm apart (Figs. 1b, d, f, 2a, c, and d). At the points of membrane apposition, the aldehyde- and OsO₄-fixed nexus usually appears septalaminar with a 2.0-nm electron-translucent region between the junctional membranes (Fig. 1b, d, and f), and the permanganate-fixed nexus appears pentalaminar (Fig. 2a, c, and d). The mean periodic distance between the regions of membrane apposition along the length of the nexus is 74.5 nm (Fig. 3b). Figures 1b, d, and f illustrate the types of junctional regions measured.

The junction may also appear as a short septalaminar nexus in aldehyde- and OsO₄-fixed ventricle (Fig. 4a) and as a short pentalaminar nexus in...
Figure 1 Comparison of the appearance of the aldehyde- and OsO₄-fixed myocardial nexuses in thin sections and in freeze-cleave replicas. All micrographs at × 240,000. (a) Freeze-cleave showing arrays of shallow pits and grooves of the EF face arranged in circles. The arrows point to small clusters of pits adjacent to the circles. The second arrow from the top points to a region in which one of the shallow grooves appears to consist of a line of pits. (b, d, and f) Thin-section images showing regions of membrane apposition alternating with regions of wider membrane separation. Arrows point to clear septalaminar membrane appositions. Note the appearance of the diffuse cytoplasmic densities along the junction. (c and e) Freeze-cleavage showing arrays of 10.4-nm particles on the PF face arranged in partial circles and adjacent clusters.
Figure 2. Comparison of the appearance of the permanganate-fixed myocardial nexuses in thin section and freeze-fractured images to illustrate the correspondence between the diameters of the freeze-fractured rings of particles and the distance between membrane apposition points along the junction. All micrographs at × 340,000. (a, c, and d) Thin-section images of a permanganate-fixed nexus illustrating membrane appositions alternating with regions of wider membrane separation. (b and e) Freeze-cleavage showing arrays of nexal particles arranged in circles on PF face. Arrows point to larger, irregularly shaped particles.
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Width of the Frog Myocardial Nexus

Figure 5 illustrates the microdensitometric method used to reconstruct nexuses. The purpose of comparing myocardial and liver nexuses was to determine whether the two junctions were similar in width and structure (Figs. 1, 2, and 4e). The results of these measurements are presented in Table I.

The mean total widths of the aldehyde- and OsO₄-fixed nexuses and the permanganate-fixed nexuses in frog ventricle from microdensitometric measurements, 15.8 nm and 16.6 nm, respectively, are very similar to the width of liver nexuses, 16.6 nm. Direct measurements of the projected images gave an average width of 16.0 nm.

The width of the less electron-dense region (gap) between the junctional membranes of the aldehyde- and OsO₄-fixed myocardial nexus at the membrane apposition points is 1.8 nm, compared between membrane appositions along the length of the thin-sectioned nexus (B). The mean diameter of the rings of particles was 76.7 SD 9.1 (n = 57), of the rings on the EF face 77.0 S.D. 6.3 nm (n = 46), and the mean distance between membrane appositions was 74.5 SD 9.1 nm (n = 57).
Figure 4  (a) A short septalaminar nexus in a thin section of an aldehyde- and OsO₄-fixed ventricle. × 340,000. (b) Freeze-fracture showing a small isolated cluster of particles on the PF face of the myocardial nexus. × 340,000. (c) A short pentalaminar nexus in a thin section of a permanganate-fixed ventricle. × 340,000. (d) Thin section of ruthenium red-filled myocardial nexus. × 340,000. (e and f) Appearance of liver nexus in thin section and on the PF face of freeze-fracture replica. Fig. 4e, × 190,000. Fig. 4f, × 150,000.
FIGURE 5 The method used to reconstruct the structure of the myocardial and liver nexuses is illustrated for the reconstruction of cytoplasmic lamella 1 (C1) and extracellular lamella 1 (E1). The construction for C1 has four steps: (i) The baseline of peak 1 (line aB) was constructed from point (a), the base of the trough between peaks 1 and 2, to point B, the point at which the curve of peak 1 first reveals roughness due to cytoplasmic densities along the membrane. (ii) Line DC was constructed through the apex of peak 1 (point D) perpendicular to the extension BC of line aB. (iii) The midpoint of line DC was determined. A line normal to CD at E establishes F, the half-peak height of peak 1. (iv) The distance between lines G' and F' is the width of peak 1 at its half-peak height. The width of C1 in the junction is determined by dividing the distance between lines G' and F' by the total specimen magnification.

Cytoplasmic lamella 2 (C2) is reconstructed and measured similarly. The method for reconstructing extracellular lamella 1 (E1) is analogous to that for C1, starting with the construction of the baseline peak 2 (line ab) from point (a), the base of the trough between peaks 1 and 2, to point b. The remainder of the reconstruction steps for E1 are identical to those for C1. For convenience, the corresponding lines in the reconstruction of E1 compared to that of C1 are labeled with the same letters, but lower case. Extracellular lamella 2 (E2) is reconstructed identically. The widths of the lipid bilayer lamellae (L1, L2) of each junctional membrane and the junctional intermembrane region (S) follow from the determination of the cytoplasmic and extracellular lamellae. The total widths of nonjunctional sarcolemma were determined from microdensitometric reconstructions in which the cytoplasmic and extracellular lamellae were reconstructed by the procedure described for C1.

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Ruthenium Red

When ruthenium red was added to the post-aldehyde fixation buffer rinse and the OsO4 fixative, it filled the entire extracellular space of the ventricular bundles with an amorphous, electron-dense precipitate. The precipitate was also present within the confines of the myocardial nexuses where it delineated the usual morphology of these junctions (Fig. 4d). The electron-dense precipitate filled the entire intrajunctional space of the nexus including the narrow 1.8-nm electron-transparent region between the junctional membranes at the regions of membrane apposition (Fig. 4d). This demonstrates that the intrajunctional spaces, where the junctional membranes diverge and are 5-15 nm apart, are not sealed from the extracellular space (at least not postfixation).

Freeze-Cleavage

Freeze-cleavage of fixed and unfixed frog ventricle exposes extensive membrane fracture faces of the cardiac muscle cells. The fascia adherens, as expected from previous reports in other animals (26, 27), is not clearly identifiable on the membrane fracture faces. The nexus appears on the PF face as a very distinctive array of particles of...
uniform diameter arranged in up to nine linked or partial circles (Figs. 1c, e, 2b, and e). Frequently, small clusters of closely packed particles of a diameter similar to that of the particles in the circles are attached to the circles (Figs. 1c, e, and 2e), and rarely such clusters of closely packed particles appear alone on the fracture face (Fig. 4b).

The complete or nearly complete circles of particles are of uniform size with a mean circle diameter of 76.7 nm (Fig. 3a) and contain 20 particles. In general, only small nexuses consist entirely of closed circles of particles (Fig. 2b), while the larger nexuses consist of many linked partial circles, i.e. short arcs and C-shaped rows of particles (Figs. 1c, e, and 2e).

The surface enclosed by the circles or partial circles of particles appears very smooth and particle-free (Figs. 1c, e, 2b, and e), although occasionally one to two particles appear near the center of the circle (Fig. 2e). A particle-free zone usually surrounds the entire array of nexal particles (Figs. 1c, e, and 2e).

The majority of the nexal particles are very uniform in size and shape, but there is a small percentage of larger, more irregularly shaped particles (Fig. 2b and e) which may represent fusion of two or more particles. The distribution of particle diameters for the myocardial nexus is illustrated in Fig. 6 and is compared to the distribution of nexal particle diameters for the frog liver nexus. The mean diameter of the particles of the myocardial nexus is 10.4 nm compared to 10.3 nm (Fig. 6) for the liver nexus. If the larger irregular particles are included, the mean diameter of the particles in the myocardial nexus is 10.8 nm. The particles in the freeze-fractured myocardial nexus are thus very similar in diameter to the particles of the frog liver nexus.

The nexus appears on the EF-face as arrays of pits or grooves which are arranged in up to 9, or more, linked circles (Figs. 1a, 7a, b) often possessing small clusters of associated pits (Fig. 1a) corresponding to the small clusters of tightly packed particles attached to the circles on the PF face. The pits or grooves are unusually shallow and are best revealed in junctions shadowed at a very low angle with respect to the fracture face (Fig. 1a) such that the surface is seen in high relief. In such nexuses, the surface area enclosed by the circles of pits or grooves often appears to bulge out. The mean diameter of the circles of pits 77.3 nm (Fig. 3c) closely corresponds to the mean diameter of the circles of particles on the PF face.

Although the frog heart nexus does not normally cross-fracture to reveal both the nexal particles and pits or grooves on adjacent PF and EF faces, two examples of this type are shown in Fig. 7a and b. In Fig. 7a, several rings are present on the EF face, and at the transition from the EF face to the PF face the nexal particles are revealed. In Figure 7b, a single ring on the EF face is present and a small portion of the EF face has been fractured away to reveal the adjacent nexal particles on the PF face. In both of these examples, the particles appear to almost protrude to the surface of the EF face. Junctions of this type are rare because the transition of the cleavage plane from the PF face of one junctional membrane to the EF face of the other normally occurs at the border of the nexus of the frog heart, leaving the EF face abutting the side of the nexus (Fig. 7c-e).

Similar arrays of particles and pits or grooves arranged in circles were present in the freeze-cleave replicas of unfixed frog ventricle, thus demonstrating that the unusual arrangement of the nexal particles is not a result of fixation.

| TABLE I |
|-----------------------------------------------|
| Microdensitometric Measurements of the Frog Myocardial and Liver Nexuses |
| Frog cardiac nexus (aldehyde and OsO₄ fixation) | Frog liver nexus (aldehyde and OsO₄ fixation) | Frog cardiac nexus (permanganate fixation) |
| Mean ± SD (n) | Mean ± SD (n) | Mean ± SD (n) |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Total junction width | 15.8 ± 1.0 (25) | 16.6 ± 0.9 (25) | 16.6 ± 0.9 (11) |
| Cytoplasmic lamella 1 (C₁) | 2.9 ± 0.4 (25) | 3.2 ± 0.3 (25) | 3.5 ± 0.4 (11) |
| Lipid bilayer lamella 1 (L₁) | 2.3 ± 0.5 (25) | 2.1 ± 0.2 (25) | 2.8 ± 0.4 (11) |
| Extracellular lamella 1 (E₁) | 2.2 ± 0.3 (19) | 2.2 ± 0.3 (24) | 3.9 ± 0.8 (11) |
| Junctional gap (S) | 1.8 ± 0.2 (19) | 2.0 ± 0.2 (24) | 2.9 ± 0.5 (11) |
| Extracellular lamella 2 (E₂) | 2.0 ± 0.2 (19) | 2.1 ± 0.3 (24) | 2.8 ± 0.4 (11) |
| Lipid bilayer lamella 2 (L₂) | 2.4 ± 0.4 (25) | 2.2 ± 0.3 (24) | 3.4 ± 0.5 (11) |
| Cytoplasmic lamella 2 (C₂) | 2.8 ± 0.4 (25) | 3.0 ± 0.4 (25) | 3.4 ± 0.5 (11) |
| Nonjunctional membrane width | 7.8 ± 1.0 (11) | 8.1 ± 0.5 (7) | 8.1 ± 0.5 (11) |
FIGURE 6 Histograms comparing the distributions of the diameters of particles of the freeze-fractured myocardial nexus (A) and liver nexus (B). The mean diameter of the myocardial nexal particle was 10.4 SD 1.1 nm (n = 537) and the mean diameter of the liver nexal particle was 10.3 SD 1.0 nm (n = 524).

DISCUSSION

Using thin-sectioning and freeze-cleavage techniques, we have demonstrated an unusual nexus in frog ventricle, a tissue previously reported to lack nexuses (38–40). The demonstration of this nexus provides a morphological basis for the spread of current electrotonically in this tissue (44) and is consistent with the widespread occurrence of nexuses in other vertebrate hearts (4, 20, 25, 26) and with the concept that nexuses are involved in the electrotonic coupling of cells (3–8, 13, 14, 16, 17, 28, 29).

Several lines of evidence described here support the view that this junction is a nexus. The width of the junction at the regions of membrane apposition in thin sections (Table I) is similar to the width of the frog liver nexus and corresponds well to the range of widths (15.0–18.0 nm) published for other nexuses (6, 10, 15, 18, 26). The width of the electron-lucent region (gap) between the junctional membranes of the uranyl acetate-en block-stained myocardial nexus at the points of closest membrane apposition is 1.8 nm, compared to 2.0 nm for the frog liver nexus, and again corresponds well to the published values for other nexuses (10, 18, 26, 35). In the freeze-fracture replicas, at regions where both the PF face of one junctional membrane and the EF face of the other are revealed at the particle or pit array the extracellular space is greatly reduced. In favorable cases, the cleavage plane through the junction clearly reveals the 10.4-nm diameter particles on the PF face of one junctional membrane contiguous with the pits or grooves on the EF face of the other (Fig. 7 a and b). This appearance is characteristic of freeze-fractured nexuses (11) and is consistent with current models of nexal structure (11, 26). Finally, although the diameter of the circularly arrayed particles (10.4 nm) is larger than the diameters (7–9 nm) usually reported for other freeze-fractured nexal particles (12, 26, 27, 34, 37), it corresponds well to the diameter of freeze-fractured particles of the frog liver nexus under our conditions.

The best evidence that the junctions in thin sections correspond to the nexus demonstrated by freeze-fracture is provided by the close similarity in the mean distance between the nexal-like membrane appositions along the length of the junction in thin sections and the diameter of the rings of particles and pits in the freeze-fractured nexus.

Model of the Frog Myocardial Nexus

Figure 8 presents a model for the frog ventricular nexus. In this model, the nexal subunits of each junctional membrane are arranged in arrays of linked circles. Each nexal subunit in one junctional membrane and the corresponding subunit in the other junctional membrane abut along the central plane between the junctional membranes. In common with other models of nexal structure (26), each nexal subunit is depicted as possessing a 1.0–2.0 nm diameter central channel, such that the abutment of corresponding nexal subunits in each junctional membrane forms a single channel from the cytoplasm of one cell to that of the other cell. McNutt and Weinstein (26, 27) and Staehelin (41) have reviewed the evidence for such intercellular transnexal channels. In the model, the junctional membranes are held in apposition in the
Figure 7 Freeze-cleavage showing myocardial nexuses at the transition of the cleavage plane from the PF face to the EF face. (a and b) Two predominantly EF face nexuses with adjacent 10.4-nm nexal particles on the PF face. Note that the particles appear to almost protrude to the surface of the EF face. × 160,000. (c, d, and e) Nexuses on the PF face illustrating the narrowing of the extracellular space (E) at the nexus. Note the perturbation of the EF face by the underlying nexal particles at the arrows in c and e. × 100,000.
regions where the nexal subunits abut but are free to bulge outwards in a domelike fashion from each other at the center of the circles of nexal subunits where nexal subunits are lacking.

If cut in cross section, the junction as indicated in the model would appear as a periodic series of membrane apposition points (points A, B, and D) separated by regions in which the membranes diverge. This appearance is similar to that of the myocardial nexuses in thin sections.

Since the freeze-cleavage replicas indicate that the circles of nexal particles are not always complete, an example of this is illustrated at point C in the model. If a transverse section through the model passes through one of these incomplete circles at the point where the circle is incomplete (point C), the junctional membranes will approach each other at this point because of the adjacent nexal subunits in the circle but will not come into apposition because there is no subunit at point C to hold them together. In the thinnestioned nexus in Fig. 1f, the double arrow points to a region which is probably of this nature.

Additionally, the model illustrates that the slightly longer regions of membrane apposition present in some of the nexuses in thin sections may appear if the plane of the transverse section through the junction lies tangential to one of the circles of nexal subunits (point D), or if it passes through a cluster of tightly packed nexal subunits attached adjacent to the circles (point A). The model explains the difference between the frog myocardial nexus and most other vertebrate nexuses seen in thin sections.

At present, it is unclear why the frog myocardial nexus has this unusual structure compared to other nexuses. Decker and Friend (12) have suggested that nexuses consisting of strands of particles may represent stages in the development of vertebrate nexuses. This is unlikely in the frog ventricle, since the unusual nexuses are the predominant form present in the adult heart. The recent demonstration of unusual nexuses between the lacis and mesangial cells of the kidney (33), between vertebrate photoreceptor cells (34), in crayfish ganglia (30, 31, 32), in the ciliary epithelium of the albino eye (22), and in mammalian arteries and veins (37) makes it probable that nexuses with unusual morphology may be more common than previously recognized.

Addendum
Since submission of this manuscript, results similar to those described here have been reported for frog atrial fibers (F. Mazet and J. Cartaud, 1976. Freeze fracture studies of frog atrial fibres. J. Cell. Sci. 22:427-434).

We wish to thank David E. Colflesh for his superb technical assistance and Vivian Schotter for her generous help in preparing the manuscript. In addition we would like to especially thank Dr. N. B. Gilula for his invaluable assistance in teaching us the freeze-fracture technique and in advising us on interpretation of the images.

The investigation was supported by a grant from the National Institutes of Health GM 20628. Dr. Brink was a recipient of National Institutes of Health postdoctoral fellowship GM 05231.

 Portions of this work were reported in the Abstracts of the 30th Annual Meeting of The Society of General Physiologists 1976.

Received for publication 17 November 1976, and in revised form 21 January 1977.

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\[\text{FIGURE 8} \quad \text{A model for the frog myocardial nexus. The small circles represent the nexal subunits bridging the junctional membranes. The black dot at the center of each circle represents a 1.0-2.0-nm pore through the nexal subunit. Points A, B, and D are membrane appositions of the junctional membranes. Point C is at an incomplete circle of nexal subunits.}\]
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