FORMATION AND GROWTH OF GAP JUNCTIONS IN MOUSE MYOCARDIUM DURING ONTOGENESIS: A FREEZE-CLEEVE STUDY

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SUMMARY

The freeze-cleeve technique demonstrates the presence of gap junctions at early stages of mouse cardiac muscle ontogenesis. The formation and growth of these junctions were studied at 4 stages of development: 10, 14, 18 days post-coitum (dpc) and at the adult stage. The diverse aspects of the gap junctions are interpreted as different steps in their formation. The first indication of this formation seems to be the presence of linear arrays of 9-nm particles on PF faces. At one end of these arrays a small aggregate of particles appears which acts as nucleation site and grows by incorporating individual gap particles and/or linear arrays. Nexuses with arms and/or central particle-free zones would represent intermediate steps in the formation of junctions. The largest nexuses could be formed by fusion of smaller ones and/or by accretion of gap particles. Analysis of the size distribution of gap junctions shows their growth during their development. At 10 dpc the surface area (S) of nexuses ranges from 0.1 to 3 x 10^{-2} \mu m^2, at 14 dpc from 0.1 to 15 x 10^{-2} \mu m^2, at 18 dpc from 0.1 to 26 x 10^{-2} \mu m^2, and at the adult stage from 0.1 to 54 x 10^{-2} \mu m^2. The percentage of large nexuses (S > 0.5 x 10^{-2} \mu m^2) steadily increases from 10 dpc to the adult stage.

Fixation by glutaraldehyde before glycerol infiltration does not induce any modification in the size distribution of adult heart gap junctions.

INTRODUCTION

Ultrathin sections, negative staining, diffraction analysis, and freeze-etch replicas have demonstrated the basic structure of gap junctions or nexuses (Revel & Karnovsky, 1967; Chalcroft & Bullivant, 1970; McNutt & Weinstein, 1970, 1973; Steere & Sommer, 1972; Benedetti, Dunia & Bloemendal, 1974; Gilula, 1974a; Amsterdam, Joseph, Lieberman & Lindner, 1976). Biochemical studies on isolated gap junctions have provided some data on their composition (Evans & Gurd, 1972; Goodenough & Stoeckenius, 1972; Goodenough, 1974; Gilula, 1974b; Benedetti et al. 1976). These junctions have been observed in both adult and embryonic tissues (McNutt, 1970; Friend & Gilula, 1972; Benedetti et al. 1974; McNutt & Weinstein, 1973; Revel,
Yip & Chang, 1973; Satir & Gilula, 1973) and also between both normal and transformed cultured cells (Revel et al. 1971; Johnson & Sheridan, 1971; Pinto da Silva & Gilula, 1972; Gilula, Reeves & Steinbach, 1972; Johnson, Hammer, Sheridan & Revel, 1974). Present between both non-excitable and excitable cells, they are probably the most frequent junctions in animal tissues. Although it is now generally accepted that they establish ionic and metabolic coupling between cells (Furshpan & Potter, 1968; Pitts, 1971; Revel, Yee & Hudspeth, 1971; Dehaan & Sachs, 1972; Gilula et al. 1972; Bennett, 1973; Cox, Krauss, Balis & Dancis, 1974; Johnson et al. 1974; Sheridan, 1974) definitive proof of this hypothesis is still lacking. However, low-resistance pathways and metabolic cooperation always correlate with the presence of gap junctions.

The formation of nexuses has been studied in vitro and in situ using the freeze-cleave technique. Yee (1972) and Johnson et al. (1974) have reported their morphogenesis, respectively, between hepatocytes in the regenerating rat liver, and reaggregated Novikoff hepatoma cells, while Benedetti et al. (1974, 1976), Decker & Friend (1974), and Albertini, Fawcett & Old (1975) have described the process of their formation and growth, respectively, in the differentiating eye lens epithelium, the neural groove during amphibian neurulation and the ovarian follicle. Decker (1976) has demonstrated the thyroxine regulation of the differentiation of gap junctions between ependymoglial cells of Rana pipiens.

Although the ultrastructure and the physiology of the gap junctions in the adult heart (Dewey & Barr, 1964; Barr, Dewey & Berger, 1965; Dreifus, Girardier & Forssmann, 1966; Revel & Karnovsky, 1967; Muir, 1967; McNutt, 1970; McNutt & Weinstein, 1970; Mazet & Cartaud, 1976) as well as their distribution and extent (Spira, 1971a; Mobley & Page, 1972; Matter, 1973; Page & McAllister, 1973; Arluk & Rhodin, 1974; Hirakow & Gotoh, 1976) have provided the subject of numerous studies, relatively little is known about gap junctions in the embryonic and developing heart. However, a few nexuses have been observed in the embryonic cardiac muscles of chick, mouse and rat (Muir, 1965; Pager & De Ceccaty, 1969; Polinger & McNutt, 1971; Nanot, 1971; Spira, 1971b; Challice & Viragh, 1973; Hirakow & Gotoh, 1976) and in human foetal heart (McNutt, 1970a; Dehaan & Sachs, 1972). Gap junctions have also been demonstrated between myocardial cells in culture (Dehaan & Hirakow, 1972; Dehaan & Sachs, 1972; Masson-Pevet, Jongsm & Bruijne, 1976). From studies of pulse-rate synchronization in pairs of isolated cardiac cells (Dehaan & Hirakow, 1972; Dehaan & Sachs, 1972), it has been found that synchrony is attained in only a few minutes, suggesting that the electrical coupling and formation of gap junctions are rapidly established.

The present report concerns a freeze-cleave study of the formation and growth of gap junctions in the mouse myocardium during ontogenesis. The importance of this structure during tissue differentiation has been emphasized (Loewenstein, 1973) and its particular role in cardiac muscle has been reviewed (Dehaan & Sachs, 1972). Reports of part of this study have been published previously (Gros & Challice, 1976a, b).
Fig. 1. 10 dpc embryonic heart. The cleavage plane has broken through 2 myocardial cells, C₁ and C₂, revealing the intracellular organelles and the plasma membrane fracture faces P₁ and E₁ of the cell C₁. The P faces are studded with numerous intramembranous particles, whereas the E faces show only a few particles. Arrows indicate closely packed arrays of particles which represent the aspect of the P fracture faces of gap junctions. es, extracellular space; g, Golgi apparatus; m, mitochondria; mf, myofibrils. × 22 400.
MATERIALS AND METHODS

Freeze-cleaving

Mice were mated overnight and the fertilized females were selected in the morning on the basis of the presence of the vaginal plug. Mating occurs mostly during the night, generally around 2 a.m. (Rugh, 1968). The embryonic hearts were used between 9 and 10 a.m. The 6 to 8 h that elapsed between the probable time of mating and the following morning were not included in the recorded age of the embryos. 10, 12, 14 and 18 dpc embryonic hearts were obtained from pregnant mice; adult hearts from 3-month-old female mice. The ventricles were dissected in physiological salt solution (130 mM NaCl, 2.7 mM KCl, 2.2 mM CaCl₂, 1 mM Na₂HPO₄, 11.3 mM NaHCO₃, 0.25 mM MgCl₂, 11.1 mM glucose) and small pieces immersed for 20 min sequentially in 10, 20 and 30 % glycerol in physiological salt solution, at 4 °C. Adult hearts were also fixed for 30 min, at 4 °C, in 5 % glutaraldehyde solution in 0.1 M cacodylate buffer at pH 7.4, before being infiltrated with glycerol in physiological salt solution. The specimens were mounted on gold disks and frozen in Freon 22 cooled with liquid nitrogen. Freeze-fracturing at ~100 °C, occasionally etching (from 1 to 2 mm), and platinum carbon shadowing were carried out using a Balzers freeze-etch apparatus according to Moor & Mühlethaler (1963). Cleaned replicas were examined with a Hitachi HU 11 Cs or Siemens Elmiskop 1a electron microscope.

Surface measurements

Electron microscopes were calibrated with a germanium-shadowed carbon replica (54,864 lines in.²). Measurements of the surfaces of the gap junctions were all performed from micrographs of magnification 90,000 x using graph paper. Surface measurements were carried out on 3 hearts at each stage studied.

RESULTS

Formation of gap junctions

Freeze-cleaving splits the plasma membrane, producing 2 fracture faces (Branton, 1966): P face directed toward the exterior of the cell and E face directed toward the cytoplasm (Branton et al. 1975). The fracture faces P and E of the plasma membranes of embryonic and adult myocardial cells are studded with intramembranous particles distributed at random, but in both cases the P faces show from 5 to 6 times more particles than E faces (Fig. 1).

At 10 dpc, the earliest stage which has been studied, gap junctions are rare and in

Fig. 2. 10 dpc embryonic heart. Arrows indicate 2 linear arrays of particles on the P face of a cell 1 (C₁). Note the paucity of intramembranous particles around the linear arrays. E₂, E fracture face of the cell 3 (C₃); P₂, P fracture face of the cell 2; mf, myofibrils. × 45,000.

Fig. 3. 10 dpc embryonic heart. Formation plaque with exceptionally long linear arrays of 9-nm particles and aggregates (arrows). The extracellular space (es) is quite reduced in the formation plaque and widens on each side. Sometimes the particles in the linear arrays seem to be coupled in pairs (black-headed arrows). White-headed arrows indicate 10-11-nm particles. × 120,000.

Fig. 4. 10 dpc embryonic heart. E fracture face with linear array of pits demonstrating the gap-like nature of the linear arrays of particles of the P faces. × 99,000.

Fig. 5. 10 dpc embryonic heart. P (1) and E (2, 3) fracture faces of small gap junctions. Hexagonal arrays of pits (circled) clearly appear on the EF face. × 120,000.
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certain replicas no intercellular junctions or even any indication of their formation have been found. This does not prove that nexuses are not present in such hearts, but it does indicate that if present they are rare. In other replicas small gap junctions and what we believe to be images of their formation can be observed. The first indication of the formation of nexuses seems to be the presence of linear arrays of 9-nm particles (usually from 6 to 20 particles, sometimes more) on the P faces, in certain areas of the plasma membrane which are generally characterized by the relative lack of intramembranous particles of smaller and more commonly observed sizes (Figs. 2, 3). These particular regions were called 'formation plaques' by Johnson et al. (1974). Aggregates of 2 or 3 rows of linear arrays and loosely organized clusters of particles are also present in such regions (Fig. 3) where the intercellular space between the apposed membranes is greatly reduced (Fig. 3). On the E faces, alignments of small depressions which would correspond to the linear arrays of particles of the P faces are observed (Fig. 4). In the neighbourhood of the linear arrays some 10-11-nm particles can be seen (Fig. 3). Less frequently we can observe small hexagonal arrays of particles with a 9-nm centre-to-centre spacing, with the corresponding fracture face E showing similar arrays of 3.5-5-nm depressions (Fig. 5). These structures are characteristic of gap junctions. Sometimes, these early nexuses have straight or curved 'arms' formed by one or two rows of particles (Figs. 6, 8). These arms are part of the gap junctions as verified by the complementary EF face pits (Fig. 7).

Some larger nexuses possess, within their central region, one or two circular particle-free zones, or zones containing only a few particles (Figs. 9, 10). At this stage (10 dpc) we observed 93 junctions, of which 10 were in the form of linear arrays and 83 were macular gap junctions. 84% of these macular nexuses displayed arms or central particle-free zones, or both.

The above description applies to both 12 and 14 dpc hearts with only slight modifications. At 12 dpc (Figs. 11, 12) both linear and hexagonal arrays of particles are

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Fig. 6. 10 dpc embryonic heart. Gap junction formed mainly by closely packed particles but also by loosely packed particles (arrow). Arrowhead indicates a linear array forming an arm. Note the small aggregates of 9-nm particles in the left lower part and in the neighbourhood of the main nexus the presence of 10-11-nm granules (small arrowheads). x 90 000.

Fig. 7. 10 dpc embryonic heart. E fracture face of a gap junction with an arm (arrow). E face replicas reveal the complementary image of the arms demonstrating their gap-like structure. x 120 000.

Fig. 8. 10 dpc embryonic heart. In the right part of the micrograph a stretched gap junction with 3 arms (arrowheads). The rows of particles indicated by the arrow are perhaps joining the closely packed particles. x 90 000.

Fig. 9. 10 dpc embryonic heart. P fracture face of a hexagonally arrayed gap junction with 2 central particle-free zones. x 130 000.

Fig. 10. 10 dpc embryonic heart. E fracture face of a gap junction with 2 pit-free zones (arrows) demonstrating that the presence of particle-free zones on the PF face is not due to the pulling out of gap particles during the fracture process. x 132 000.

Fig. 11. 12 dpc embryonic heart. Linear arrays of particles (arrowheads) and a gap junction with a particle-free zone and a long branched arm (arrow). x 48 000.
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observed and both are more common than at 10 dpc. 25.2% of the macular gap junctions had arms and/or central particle-free zones. In 163 junctions observed we counted 55 single linear arrays of particles. By 14 dpc (Fig. 13) the linear arrays of particles have become rarer, whereas the hexagonal arrays are now relatively numerous; 9.3% of the macular gap junctions (215) displayed arms or central particle-free zones or both.

At 18 dpc (Fig. 14) and at the adult stage (Fig. 15) the rows of particles are no longer seen; only macular gap junctions are present, a significant number having attained a large size. Some micrographs suggest that the large nexuses, for these 2 latter stages, may have been formed by the fusion of smaller junctions (Fig. 14) and/or by the accretion of 10-11-nm individual particles which span the membrane (gap particles) (Fig. 15). As the formation of nexuses proceeds the formation plaques are encroached upon by the common 7.5-nm intramembranous particles and only around the relatively large gap junctions does a particle-free halo persist. The above observations are summarized in Table 1.

Size distribution of gap junctions during the development

Single linear arrays of particles (or 'monolinear' gap junctions) were not taken into account in the calculation of size distribution because of the difficulty in measuring their surface areas. When present they represent a small percentage of the total area of gap junctions. The central particle-free zones present in certain nexuses were excluded from the surface measurements. The smallest macular junctions measured were composed of from 8 to 10 closely packed particles.

Fig. 16 shows the distribution of the area $S$ of the gap junctions through 4 stages examined. At 10 dpc the area of the gap junctions observed ($n = 83$) ranges from 0.1 to $3 \times 10^{-2} \mu m^2$; at 14 dpc ($n = 215$) from 0.1 to $15 \times 10^{-2} \mu m^2$; at 18 dpc ($n = 158$) from 0.1 to $26.3 \times 10^{-2} \mu m^2$ and at the adult stage ($n = 92$) from 0.1 to $54 \times 10^{-2} \mu m^2$.

Fig. 12. 12 dpc embryonic heart. Zone rich in linear arrays of particles (arrowheads). Note also 2 small gap junctions with arms (arrows). $\times 48,000$.

Fig. 13. 14 dpc embryonic heart. Fracture through a gap junction-containing zone. Large gap junctions are indicated by arrows and a small aggregate of particles by an arrowhead. Note in the right upper part a junction with a central particle-free region.

Fig. 14. 18 dpc embryonic heart. Convergence in a small region of numerous gap junctions of different sizes. Note in the neighbourhood of nexuses 10-11-nm particles (arrowheads). In such a region, the largest junctions could be formed by the fusion of smaller ones or by addition of gap particles. $\times 120,000$.

Fig. 15. Adult heart. Part of a large gap junction from glutaraldehyde-fixed preparation. Dashed line delimits the $P$ fracture face from the $E$ fracture face. A small part of the $E$ face of the junction, with a hexagonal array of pits, can be seen (large black arrow). Note in close proximity to the nexus, the presence of 10-11-nm particles on the $P$ face (small black arrows) and pits on the $E$ face (small white arrow). The peripheral particles seem to leave deeper pits on the $E$ face than the usual gap junction particles: compare the pits indicated by the white arrows and these of the pitted lattice (large black arrow). Such a micrograph could suggest the incorporation of the 10-11-nm particles in the neighbouring gap junction. $\times 210,000$. 
An arbitrary division was made at the size of \(0.5 \times 10^{-2} \mu m^2\) to define 2 classes (class I and class II) of gap junctions, this number representing the median area of the overall number \((N = 548)\) of nexuses observed. At 10 dpc the percentage of gap junctions of class I \((S \leq 0.5 \times 10^{-2} \mu m^2)\) is 67\%; at 14 dpc, 57\%; at 18 dpc, 44\% and at the adult stage, 31\%. The histogram in Fig. 17 depicts these measurements.

All the above studies were performed on unfixed specimens treated with glycerol before freezing. To detect a possible action of glycerol on the size of the gap junctions,

Table 1. Summary of the observations about the diversity of the gap junctions during the heart development

<table>
<thead>
<tr>
<th></th>
<th>10 dpc</th>
<th>12 dpc</th>
<th>14 dpc</th>
<th>18 dpc</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear arrays of particles observed</td>
<td>10</td>
<td>55</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macular gap junctions observed</td>
<td>83</td>
<td>108</td>
<td>215</td>
<td>158</td>
<td>92</td>
</tr>
<tr>
<td>Macular gap junctions with 'arms' or central particle-free zone/or both</td>
<td>+ (8.4%)</td>
<td>+ (25.2%)</td>
<td>+ (9.3%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Small gap junctions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Large gap junctions</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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</table>

Fig. 16. Histograms showing size distribution of gap junctions observed in 10, 14, 18 dpc and adult mouse hearts (A, B, C, D, respectively). Abscissa, area, \(S\), of gap junctions. Ordinate, percentage of ratios no. of gap junctions in class intervals/total no. (\(n\)) of gap junctions observed at each stage: for A, B, C, D, \(n = 83, 215, 158,\) and 92, respectively. Three hearts used for each stage, unfixed specimens.
3 adult hearts were fixed before being infiltrated with the glycerol solutions (see Materials and methods). In the specimens treated in this way (Fig. 15) the ultrastructure of the gap junctions in the replicas is identical with that of unfixed specimens. Their size distribution after glutaraldehyde fixation is given in Fig. 18. The areas of the nexuses observed ($n = 125$) range from $0.1 \times 10^{-2}$ to $32.4 \times 10^{-2} \mu m^2$ and $34\%$ of these junctions have an area $S \leq 0.5 \times 10^{-2} \mu m^2$.

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**Fig. 17.** Histogram illustrating the percentages of class I (□, $S \leq 0.5 \times 10^{-2} \mu m^2$) and class II (□, $S > 0.5 \times 10^{-2} \mu m^2$) gap junctions in relation to their total number ($n$) observed at each stage.

**Fig. 18.** Histogram showing size distribution of gap junctions in 3 adult mouse hearts. Specimens fixed with glutaraldehyde before being infiltrated with glycerol, $n = 125$. Axes as in Fig. 16.
DISCUSSION

**Embryonic myocardium and gap junctions**

Muir (1965) observed a few nexuses in ultrathin sections of the 14 dpc rat embryonic myocardium, as did Pager (1968) and Pager & de Ceccatty (1969). Spira (1971b) found close oppositions (4-nm gap) between chicken myocytes, whereas the freeze-cleave studies of Polinger & McNutt (1971) demonstrated the presence of small nexuses. Gap junctions have also been observed in human foetal myocardium (McNutt, 1970; Dehaan & Sachs, 1972). In the literature the earliest stage at which gap junctions have thus far been reported in mouse heart is 13 dpc (Nanot, 1971; Challice & Viragh, 1973). The present freeze-cleave study unquestionably demonstrates the existence of gap junctions as early as 10 dpc in the developing cardiac muscle. The small size of these junctions and the limited membrane area that they occupy explain why it has been so difficult to detect them in sectioned material. Since the first myocardial contractions appear in the tubular heart of 8-dpc embryos (Rugh, 1968) gap junctions might be expected to exist at this time if they represent the sites of electric coupling between cardioblasts.

**Formation of gap junctions**

Pinto da Silva & Gilula (1972) observed different gap particle arrays, especially small macular and linear rows and they speculated that such different arrays may represent aspects of the gap junction differentiation. Yee (1972), Decker & Friend (1974), Benedetti *et al.* (1974), Johnson *et al.* (1974), and Decker (1976) from studies of freeze-cleave replicas of regenerating or differentiating tissues, all reached the same conclusion. Fujisawa, Morioka, Nakamura & Watanabe (1976) suggested that such a structural diversity may reflect the existence of functionally different gap junctions.

The ontogenetic sequence found in the present study suggests that the arrays of particles and depressions, respectively, observed on the P and E fracture faces during cardiac muscle development represent stages of gap junction formation and growth. The following observations are pertinent: the presence of isolated 10-11-nm particles in the formation plaques; the presence of linear arrangements of gap particles at the earliest stages (10, 12 and 14 dpc) and then, later their absence (18 dpc and adult) (Table 1); the general increase in size of the gap junctions during heart development (Figs. 16, 17).

The mode of development of nexuses suggested by the present study is similar to that proposed by other authors (Benedetti *et al.* 1974; Decker & Friend, 1974; Johnson *et al.* 1974; Decker, 1976). In the formation plaques linear arrays of gap particles of various lengths appear. At one end of these rows, small aggregates develop either by a coiling of the linear arrays, or by the accretion of other gap particles. Such aggregates of particles act as nucleation sites which grow by incorporation of individual particles, on other linear arrays, or both. The possibility that small aggregates develop alone, without going through the linear-array stage cannot be eliminated, but the linear arrangements appear to be characteristic of differentiating junctions. They have been observed in the neural groove of amphibian neurulae (Decker & Friend,
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1974), between differentiating lens fibres (Benedetti et al. 1974), between differentiating ependymogial cells (Decker, 1976), in the neural retina of chicken (Fujisawa et al. 1976), and in the cardiac muscle of amphibian larvae (Mazet, 1977). The greatest number of linear arrays were found at the 12 dpc stage, which suggests that the twelfth and thirteenth days are a particularly active period for gap junction differentiation. Such linear arrays have not been observed in the 18 dpc and adult hearts.

Nexuses with arms and/or central particle-free zones could represent intermediate steps in the formation of the junctions. The free end of a junction arm could bend until it touches the ‘body’ of the junction, forming a ring enclosing a particle-free zone. The particle-free zones formed would then become filled with gap particles by accumulation. Larger nexuses would then be formed by the fusion of smaller ones and/or by accretion of 10-11-nm particles. In the later stages (18 dpc and adult) these 10-11-nm particles have been demonstrated to be gap particles. They have also been observed by most authors studying the formation of gap junctions and were interpreted as precursors of the 9-nm subunits of the gap junctions (Johnson et al. 1974; Decker & Friend, 1974; Decker, 1976). With this hypothesis it is interesting to note the decrease in size of the gap particles. Could this change be attributed to the interaction of particles during their packing? The reasons for this change in size are still unclear. In the earlier stages studied 10-11-nm particles are also present in the PF faces, but they are never very numerous and we have not been able to detect their corresponding depressions in the EF faces, which is probably due to the difficulty in discovering and observing such small isolated pits.

Such a process of formation of the gap junctions presupposes, at least, 2 conditions: translational mobility of the junctional particles, and of their linear arrays in the plasma membrane; and a mutual affinity between the gap particles. These factors have been discussed by Decker & Friend (1974) on the basis of the fluid mosaic membrane model proposed by Singer & Nicolson (1972).

Size distribution of the gap junctions

Figs. 16 and 17 show a general increase in size of the gap junctions with increasing age, although a significant number (31%) of junctions of class I ($S < 0.5 \times 10^{-2} \mu m^2$) remain in the adult heart. Such a dynamic process is a feature characteristic of a population growing in size and it supports the present hypothesis of gap junction formation and growth in cardiac muscle. In the 18 dpc and adult cardiac muscles analysed, roughly 10% of the largest nexuses ($S > 0.5 \times 10^{-2} \mu m^2$) were incomplete in the replicas, which suggest a slight underestimate of their size distribution. The existence of a virtually continuous spread of size in the adult myocardium suggests that even there nexuses continue to develop. However, at this stage no linear arrays of particles (supposed to be the first manifestations of junction differentiation) were seen. Observations of linear arrays at the earlier stages are not rare, but they are not that frequent: roughly 2 linear arrays for 10 macular nexuses observed (see Table 1). Thus, in the adult heart, the differentiation of new gap junctions appears to have reached a minimum, making the linear arrays rare, or absent.

Several papers allow us to compare the size of the gap junctions in the mouse adult
heart (0.1 x 10^-2 μm² to 0.54 μm²) to that of gap junctions in other kinds of tissues and to ascertain that they are similar (Revel et al. 1971; Friend & Gilula, 1972; Albertini & Anderson, 1974a, b; Fry, Devine & Burnstock, 1977).

Fixation by glutaraldehyde before glycerol infiltration does not induce any modification in the size distribution of adult heart gap junctions. Replicas of nexuses of fixed and unfixed specimens show identical particle arrays, as reported by Breathnach, Gross, Martin & Stolinski (1976). McNutt & Weinstein (1970, 1973) had noticed after infiltration of unfixed preparations of adult mouse heart by 40% glycerol solutions, that the particle arrays tended to become looser. In both cases in the present study, both closely and loosely packed arrays were seen.

The present study shows that gap junctions form and increase in size during development of the myocardium. With the hypothesis that gap junctions are the sites of ionic and metabolic coupling between cells, such a development necessarily influences cellular physiology and in particular the degree of intercellular communication. Attempts to quantify the junctional membrane surface area involved in gap junctions, during development and at the adult stage, are under investigation.

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