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Cell-to-cell communication in the heart: structure-function correlations

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Summary. The communicating cell junctions that ensure the electrical and diffusional continuity of the intracellular space in the heart fibres can be switched from their normal conducting, or opened state, to an exceptional non-conducting, or closed state. This electrical uncoupling is observed after cell injury in the presence of Ca^{2+} ions in the extracellular fluid, after metabolic inhibition and in the presence of aliphatic alcohols (C_6 to C_9). The correlations between electrical uncoupling and gap junction morphology in the heart are briefly reviewed. A decrease of the distance between P-face particles and between the E-face pits has been found in all investigations[3, 10, 16, 18, 55], but the functional significance of this observation is not understood at present. A quantitatively very similar decrease of the average particle diameter (about −0.7 nm) has been measured in glutaraldehyde-fixed sheep Purkinje fibres[16] and in unfixed, quickly frozen rat auricles[18] that had been electrically uncoupled by three different procedures. About half of this decrease was reversible on short-term electrical recoupling (within 20 min). It is concluded that a measurable decrease of the connexon diameter correlates with electrical uncoupling.
Key words. Heart; electrical coupling; electrical uncoupling; communicating junctions; gap junctions.

From a syncytium to a coupled cell system

Up to 1954, physiologists considered the heart as a morphological and functional syncytium of fused cells building up fibres with regularly spaced nuclei but no obvious transversal cell boundaries, and with electrical properties similar to those of skeletal muscle or nerve. This continuous network accounted for the all-or-none propagation of electrical and mechanical activity from any stimulated point. This classical view was seriously questioned by Sjöstrand and Andersson's[56] electron micrographs, in which the heart fibres unequivocally appeared to be built up of single cells entirely separated by their surface membranes, abutting without fusion at the intercalated disks.

These discontinuities in the core conductor of heart fibres had to be taken into account in any explanation of electrical conduction. Silvio Weidmann, who had just showed (1952)[64] that the Purkinje fibres of ungulate hearts have cable properties similar to those of nerve[29] and skeletal muscle[32], despite their well-known cellular structure[48, 58], took up this challenge and started an investigation of the cell-to-cell conduction process in the heart. Weidmann[65] first tried a direct measurement of the cell-to-cell resistance by means of two double-barreled micro-electrodes, one on each side of an intercalated disk. This first attempt was not successful because, as is now understood, electrical transmission across the disk is so efficient that the voltage drop across the cell boundary is smaller than the error inherent in measurements by micro-electrodes. Weidmann[65, 66] then devised an entirely original approach to this problem by loading a thin myocardial bundle in a two-compartment chamber with $^{42}K^+$ over one half of its length, while the other half was continuously washed in a non-radioactive solution. The fibres in the unloaded compartment become radioactive too, and the spatial distribution of $^{42}K^+$ when diffusion equilibrium is approached corresponds to that calculated on the assumption of a cell-to-cell passage of $^{42}K^+$ across an average disk resistance of $3\,\Omega \times cm^2$. The space constant of 1.55 mm measured for the decrease of radioactivity on the unloaded side of the bundle makes improbable the alternative interpretation that loading in the washed compartment could be by way of the extracellular space only.

The same method was applied later to investigate the diffusion of several substances with different molecular weights

inside the heart fibres[30, 59, 68, 70]. These studies showed that, as in other electrically coupled cell systems[34], the cell-to-cell channels in the membranes of adjacent heart cells allow the passage not only of K^+, but also of much larger ions and molecules (see Imanaga, this Review).

These direct cell-to-cell channels account for the low myoplasmic resistivities of heart Purkinje and muscle fibres, which amount respectively to 3 times and 9 times the specific resistance of Tyrode solution[64, 67], and make it possible to retain the classical explanation of electrical propagation by local current flow. Moreover, the validity of the core conductor hypothesis for electrical transmission in the heart has been directly confirmed: the conduction that has been blocked along a heart bundle by an extracellular insulation (for instance a sucrose gap) can be reestablished by electrically shunting the two pools of solution on each side of the wall[4]. It can be recalled that such a stimulation by the action potential is impossible unless the intracellular space provides a low resistance current path.

The first evidence that the high conductance of heart junctions can decrease to values so low that the cells become virtually isolated from each other was provided by experiments on the healing-over phenomenon. Engelmann[23] had observed that the injury potential vanishes in about 15 min in frog heart and in visceral smooth muscle, in contrast to its much longer duration in the sartorius muscle (hours), and he already suggested that the injured heart or smooth muscle cells become isolated after injury. Weidmann's study of the electrical properties of heart Purkinje fibres[64] showed that their core is electrically continuous but that, unlike axons or skeletal muscle fibres, they are closed at the cut ends by high resistances (fig. 1). Micro-electrode measurements close to precisely defined injuries[11, 13] showed that the resting potential drops at the instant of injury and returns to control values within one minute. It was also demonstrated[11–13] that the presence of Ca^{2+} ions in the bathing solution is a prerequisite for this recovery of the membrane potential (fig. 2). Furthermore, continuous records of the membrane potential and input resistance close to small injuries by a laser beam demonstrated that healing-over is promoted by the rapid development (within one minute) of a new diffusion barrier that closes the leak opened up in the biological cable at the injury site, thus allowing the return of the membrane potential to normal values[13] (fig. 3). That the injury potential recorded by surface electrodes in amphibian hearts lasts for a much longer time (from 10 to 30 min[2, 20, 23, 53]) than that necessary for the development of the resistive barrier close to the injury site in sheep Purkinje fibres (1 min[13]) should be considered a consequence of the poorly defined boundaries of the mechanical injuries employed by the former authors, and the consequent persistent discharge of membrane current by partly injured cells that retain a partial electrical coupling with the uninjured tissue. The alternative explanation of a genuine difference in the time course of electrical uncoupling of amphibian and mammalian heart cells is not tenable because the duration of the injury potential after mechanical injury of sheep heart preparations is also 10–20 min (Délèze, unpublished observations).

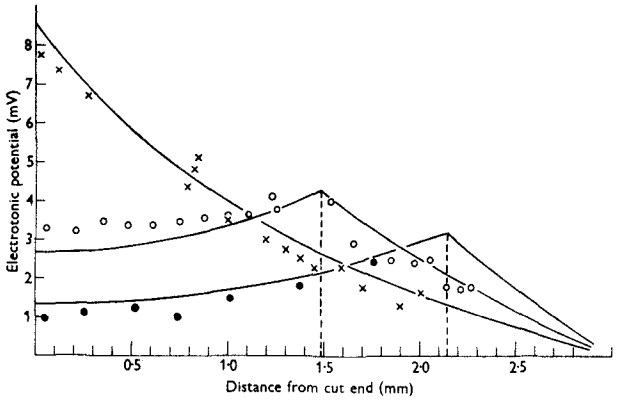


Figure 1. Spatial distribution of the electrotonic potential near the cut end of a Purkinje fibre in Tyrode solution. The polarizing electrode was set at 0.02 mm (×), 1.48 mm (○) or 2.14 mm (●). The three curves have been calculated from the equations of cable theory with the same parameters, on the assumption that the cut end of the fibre is sealed by an infinite resistance. Reproduced from Weidmann[64] with permission of The Journal of Physiology.

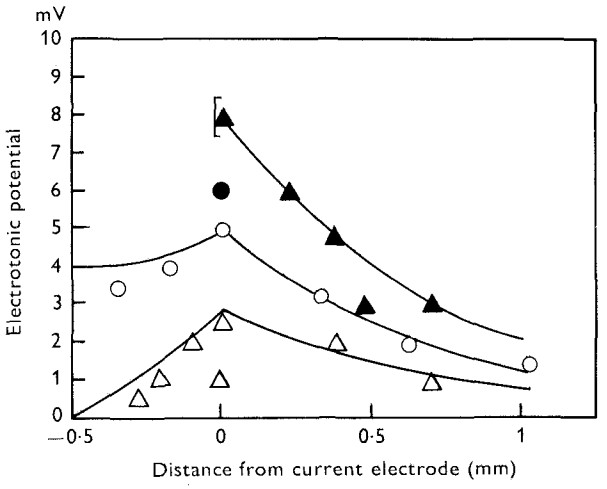


Figure 2. Spatial distribution of the electrotonic potential near the cut end of a Purkinje fibre in an isotonic solution with a cation concentration similar to that of the intracellular fluid (○; △), and with 1 mM-Ca^{2+} added (●; ▲). The polarizing electrode was set at 0.5 mm from the end. At the beginning of the experiment, the electrotonic potential was measured in the presence of Ca^{2+} ions (●) and after the solution was made Ca^{2+}-free (○). When a fresh cut was performed in the Ca^{2+}-free solution, the electrotonic potential was immediately decreased to steady lower values (△) tending towards zero at the cut end. A last series of measurements (▲) was obtained after adding 1 mM-Ca^{2+} to the solution. The three curves have been calculated from the equations of cable theory, with the same parameters chosen by trial and error to give the best overall fit, on the assumptions that the end of the fibre, initially sealed (●; ○), remains opened after the cut in the Ca^{2+}-free solution (△) and seals again after Ca^{2+} addition (▲). This fibre healed-over at the distance marked by the bar ([), which accounts for the electrotonic potential being higher than the initial value (●). Reproduced from Délèze[13] with permission of the Journal of Physiology.

By observing the diffusion of a fluorescent molecule (Procion yellow), that does not penetrate the intact cell membranes but can pass from cell to cell after it has been introduced into the cytosol, it has been possible to show that the new diffusion barrier that appears after a small injury is located at the intercalated disks[14].

Apart from healing-over, electrical uncoupling in heart seems to take place whenever the normal cell function is impaired, such as in anoxia[71], interference with ionic transport[69], or metabolic inhibition[19]. Similar effects of interference with metabolism have been described in several other electrically coupled cell systems[34], and it has been suggested that the triggering factor common to all these situations of electrical uncoupling could be a rise of the cytosolic Ca^{2+} concentration above a threshold[33, 34], though the actual intracellular Ca^{2+} monitoring during electrical uncoupling has so far been performed in two cases only, in the *Chironomus* salivary gland by means of aequorine[52] and in the heart Purkinje fibre by Ca^{2+}-sensitive micro-electrodes[10]. In both tissues, the measured threshold level for uncoupling was 5×10^{-7} M, which is close to the detection limit of the aequo-

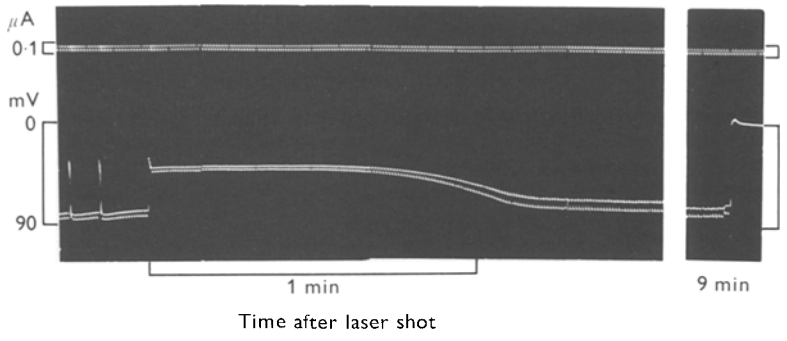


Figure 3. Rapid recovery of resting potential and input resistance, indicating development of a new diffusion barrier, in a Purkinje fibre injured by a pulse of laser light. Upper record: calibration of the square pulses of constant current fed to one intracellular micro-electrode to measure the input resistance. Bottom record: membrane potential and superimposed periodic variations (proportional to the input resistance) recorded by another micro-electrode at 120 μm from point of current application. Membrane potential and input resistance fell to about half their control values when an injury of diameter equal to that of the fibre was made at 0.55 mm from the current electrode. After recovery the input resistance settled at a higher level that fits cable theory applied to a fibre terminated by an infinite resistance at the site of injury. Reproduced from Délèze[13] with permission of the Journal of Physiology.

rine, and even more so of the micro-electrode method. A quantitative relation between the intracellular Ca^{2+} activity and the junctional conductance is clearly needed before any acceptable statement can be made concerning possible regulations of the cell-to-cell conductance by Ca^{2+} ions during the normal heart function.

The heptyl alcohol n-heptan-1-ol (heptanol), 3.5 mM in Tyrode's solution, reversibly suppresses cell-to-cell conduction in sheep Purkinje fibres[15,16] and in rat auricles[17,18], as it does in the crayfish electrical synapse[31] and in the rat pancreas and the *Xenopus* embryo[5,6]. As a rise of the intracellular Ca^{2+} concentration has been detected in squid axons in the presence of aliphatic alcohols[63], it appears possible that an increase in the cytosolic Ca^{2+} concentration triggers electrical uncoupling in this case also.

It is known that a moderate rise of the cytosolic H^+ ion concentration uncouples embryonic cells of amphibians[60] and of fish[57], but in the adult mammalian heart this effect appears to be much less important, and it has not been possible to induce electrical uncoupling by decreasing the intracellular pH over the physiological range[50].

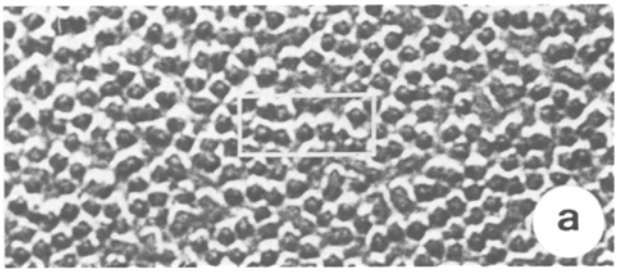
In short, the communicating cell junctions that ensure the electrical and diffusional continuity of the intracellular space in the heart fibres can be switched from a normal conducting, or opened state, to an exceptional non-conducting, or closed state. This property is shared by many permeable junctions examined so far and is generally believed to be triggered by a rise of the cytosolic Ca^{2+} or H^+ ion concentration to values which, fortunately, are not likely to be reached during normal heart function.

A morphological correlate of cell-to-cell communication: The nexus or gap junction

Dewey and Barr[21] suggested that the spots of close membrane apposition (the nexus) that they were describing in the visceral smooth muscle are regions of low electrical resistance allowing the electrotonic spread of current from cell-to-cell. Their hypothesis has been supported by the observation of transmission loss in heart after separation of the nexus membranes in hypertonic solutions[4] and, more significantly, by the persistence of electrical communication when the other types of heart cell junctions (the maculae and fasciae adherentes) are ruptured, but the nexuses are retained[22].

In thin sections of tissue blocks immersed in colloidal lanthanum hydroxide to mark the extracellular space, the nexus appears as a close apposition of the two unit membranes of the adjacent cells, separated by an electron-lucent gap 30 Å in width (the gap junction), filled with lanthanum except for a regular array of unstained subunits, 7–8.5 nm in diameter, extending from one cell membrane to the next[7,39,40,51].

X-ray diffraction by 'en face' gap junctions isolated from mammalian liver indicates that the subunits (the connexons) have the approximate shape of hollow cylinders, about 75 Å



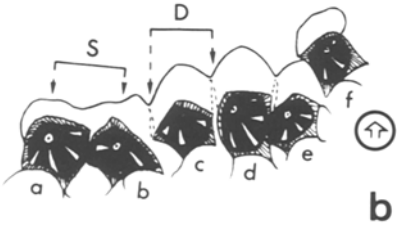


Figure 4. *a* Freeze-fractured sheep Purkinje fibre showing part of a gap junction. A central crater-like depression, which might correspond to the opening of an axial hydrophilic channel, is visible on most particles. This fibre was in a state of normal cell-to-cell electrical conduction at the beginning of fixation by glutaraldehyde. Magnification: × 300,000. *b* Enlarged drawing of 6 particles from the area within the frame on fig. 4*a*, to illustrate the selection and the procedure for measurement of P-face particle diameters. Dimensions of the particle base such as (D), perpendicular to the shadowing direction (arrow at right), are considered accurate measurements of the diameter if the edges of the particle shadow can be precisely delineated, as in particles (c) and (f). Diameters of particles with confluent bases such as (d) and (e), or with a common edge such as (a) and (b), were not measured, but their center-to-center distances, for example (S), could be. Reprinted from Délèze and Hervé[16] with permission of the Journal of Membrane Biology.

in height, with an outer diameter varying from 50 to 60 Å and a maximum inner diameter of 20 Å[8,37]. The connexons are made up of six identical protein molecules[37] with a molecular weight of 27,000 daltons in liver and heart cells[26]. The connexons set in the two adjacent membranes are firmly aligned end to end in pairs and a central hydrophilic channel has been recognized along their axis[37], but continuity across the junction could not be demonstrated, either by X-ray diffraction[8,37] or by three-dimensional reconstruction of tilted electron micrographs of isolated gap junctions[61,62]. X-ray diffraction indicates that the channel is closed at both cytoplasmic ends by an electron dense structure, probably a protein[36]. This configuration presumably corresponds to the electrically uncoupled state, because in these isolated gap junctions sucrose is excluded from the central hydrophilic part of the channel[38]. The membrane connecting elements thus detected in thin sections and in isolated gap junctions appear in metal-deposited replicas of the freeze-fractured nexus as specialized membrane associated particles which remain attached to the P-face of the freeze-cleaved membrane, while the E-face is punctated with small pits generally interpreted as the imprints of the pulled out particles[40]. The particles have a diameter of 7–8.5 nm at the base and a rounded to conic top, where a central depression, about 2 nm in diameter, which might correspond to the opening of the axial hydrophilic channel, has often been described (figs 4 and 5). The particles build up clusters that are not as a rule regularly ordered (though more or less hexagonal arrangements may be occasionally encountered) unless their average distance apart, about 10 nm, is substantially decreased. When this occurs the order increases until, when the particles begin to be confluent, the cluster becomes hexagonally ordered. Such a change has been observed in correlation with uncoupling[42,44]. The distance between the P-face particles, and their order, are also affected by the preparative process, and they have generally been found to be more dispersed in quickly frozen gap junctions than in their aldehyde fixed counterparts[3,18,49].

Gap junction alterations in uncoupled mammalian heart cells

Taking for granted that the gap junction is a low resistance pathway between electrically coupled cells – and, at least for heart tissue, there is some experimental support in favour of this hypothesis[22] – the possible transformations of this structure when the cell-to-cell electrical coupling is switched off have been examined. In mechanically injured frog hearts fixed after healing-over, the small and sparse gap junctions seen in the normal parts of the tissue do not disappear in the injured regions, nor are they grossly disrupted[2], as they are after conduction block by hypertonic solutions[22]. In a more detailed study of the effect of healing-over on the gap junctions of rabbit heart, Baldwin[3] has observed a decrease of the P-face particles distance with a frequent occurrence of hexagonally packed particle groups. This type of transformation had previously been described in the uncoupled crayfish electrical synapse[44] and mammalian liver and gastric epithelium[42], together with a decrease of the average particle's diameter and also of the width of the 30 Å gap separating the two adjacent cell membranes. Analogous structural transitions have been triggered in isolated calf lens gap junctions by raising the Ca^{2+}, Mg^{2+} or H^+ ion concentrations[43,45,46]. The decrease in the mean distance between particles observed in healed-over rabbit heart cells[3], or the correlated diminution of the distance between pits, have been confirmed in cut and healed sheep Purkinje fibres[55] and in the same cells fixed after controlled electrical uncoupling by dihydro-ouabain[10], 2-4-dinitrophenol (DNP)[10,16], heptanol[16], and return to Tyrode solution (Ca^{2+}-containing) after treatment with a hypotonic (120 m Osmoles) Ca^{2+}-free solution[16]. Despite this general agreement on a decreased distance between particles after electrical uncoupling, it must be admitted that the functional significance of this observation is by no means clear within the framework or our present knowledge of gap junction structure. It could be related to the channel closure mechanism of connexons bearing an electrostatic charge and swinging in the membrane if the charge were decreased in the closed conformation. Also, a component of the gap junction protein associated with the cytoplasmic surface of the lipid bilayer has recently been detected on electron density maps of liver gap junctions[38] and a similar, albeit larger structure, has been demonstrated in liver and heart cells by three preparative techniques for electron microscopy (thin sectioning[9], negative staining[35], and freeze-fracture combined with deep-etching[54]). This component, which has been described as flexible and labile[38], may be involved in the changes of the particle's distance from each other and order described in relation to uncoupling.

As the particles are the only cell-to-cell connecting elements recognized at present, it can be expected that a change in

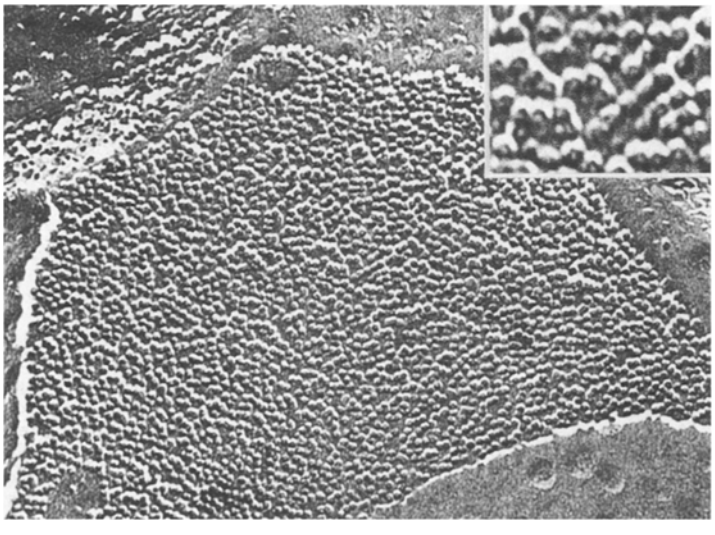


Figure 5. Freeze-fractured rat auricle quickly frozen in a state of normal cell-to-cell electrical conduction. Magnification: × 100,000. Inset: enlarged part (× 300,000) of the same junction showing a central depression on top of most particles. Reproduced from Délèze and Hervé[18] with permission of the Journal of Membrane Biology.

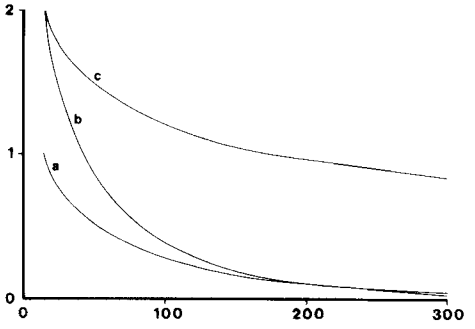


Figure 6. Comparison of the effects of a rise of either R_i or R_m in Eqn. 1 on the spread of the electrotonic potential in the rat auricle. Abscissa: distance (x) from current source (μm). Ordinate: steady-state displacement of membrane potential (V, relative units). Curve (a): control conditions (λ_a = 150 μm). Curve (b): after a rise of R_i to such a size (2.32 ×) that V is doubled at x = 15 μm, ($\lambda_{(b)}$ = 98.5 μm). Curve (c): after the same rise of V at x = 15 μm caused by an increase of R_m (164.5 × ; $\lambda_{(c)}$ = 1924 μm). Reproduced from Délèze and Hervé[18] with permission of the Journal of Membrane Biology.

their diameter with the functional state of the junction could contribute some information on possible closure mechanisms, and perhaps help in deciding between different functional schemes of the connexon, at present either a rotating and tilting set of subunits[62], and its revised version[61], or the proteic bolt seen in a presumably closing position at the cytoplasmic ends of the channel[36, 38]. The average diameter of the junctional particles was decreased after electrical uncoupling at the crayfish electrical synapse[44] and in mammalian hepatic and gastric cells[42]. There is disagreement, however, in the results obtained by different laboratories on the particles' diameters correlating with electrical uncoupling in the heart Purkinje fibres. In one study[10], a thickening of the particle was measured, from a diameter of 8.3 nm in control conditions to 9.8 after 15 min of DNP (1 mM) treatment, when the intracellular resistance had risen to more than 80 times the initial control. But after 1 h in DNP, the particles' diameters had decreased to 7.7 nm. The authors[10] suggest that the reduction in particle diameter in the DNP uncoupled crayfish electrical synapse[44] and hepatic and gastric cells[42] correspond to a structural degradation, and that an increase in particle size is more likely to correlate with the uncoupling process. In another investigation[55], the junctional particles of Purkinje cells situated at a distance less than 50 μm from a surface freshly cut either in Tyrode's solution (1.8 mM Ca^{2+}), or in an isotonic Ca^{2+}-free solution with a Na^+ and K^+ content similar to that of the intracellular fluid, showed a 10% increase in diameter within one minute after cutting in Tyrode's solution, and the particles continued to be significantly larger when the incubation period before fixation was prolonged up to 30 min. In a third study[16], in which the measurement criteria for the diameters of the particles were rigorously defined, the average particle-diameter measured in Purkinje cells with complete conduction blocks induced by three different procedures (either DNP, 1 mM; heptanol, 3.5 mM; or osmotic transition from a Ca^{2+}-free hypotonic to an isotonic Ca^{2+}-containing solution) was similarly reduced in all samples, the decrease of the pooled data amounting to about 9%. On preparations fixed after different durations of DNP treatment, the reduction in diameter appeared with a time course which paralleled the measured increase in internal resistance, reaching a steady state between 15 and 20 min when the cell-to-cell current flow became undetectable[16]. The particle diameter was never seen to increase, as described by others[10, 55], not even at the beginning of uncoupling.

One difficulty in measuring the diameter of elevated particles replicated by condensation of a directional jet of platinum and carbon is due to the frequent occurrence of particles with confluent bases or common edges, produced by the accumulation of the shadowing material on the side of the structure facing the metal source. The largest errors are of course expected in the measurement of these particles. This problem was simply avoided in the last-mentioned investigation[16], and in a succeeding one[18], by performing the diameter measurement, with a precision particle size analyser (Carl Zeiss TGZ 3), on particles with edges entirely free at the base, avoiding all those with adjacent or confluent shadows (fig. 4b). Another less obvious advantage of selecting particles with separate shadows is that the amount of metal deposit becomes independent of the distance between the particles, whereas with the tightly packed particles it decreases; thus another possible cause of error in the measurement is avoided. With these criteria, a large number of particles are not taken into the statistics, but there is no reason to expect that they should be in a different functional state.

Another difficulty in the interpretation of the gap junction alterations in relation to the functional state is linked to the uncoupling effect of the aldehyde-containing fixation fluid itself; this has been demonstrated during glutaraldehyde fixation in the crayfish electrical synapse[47] and in heart Purkinje fibres[16]. Even at a much lower concentration (2.5 mM) than that employed for tissue fixation, glutaraldehyde already increases the internal and surface resistances in Purkinje fibres[25]. Therefore the gap junction structure has also

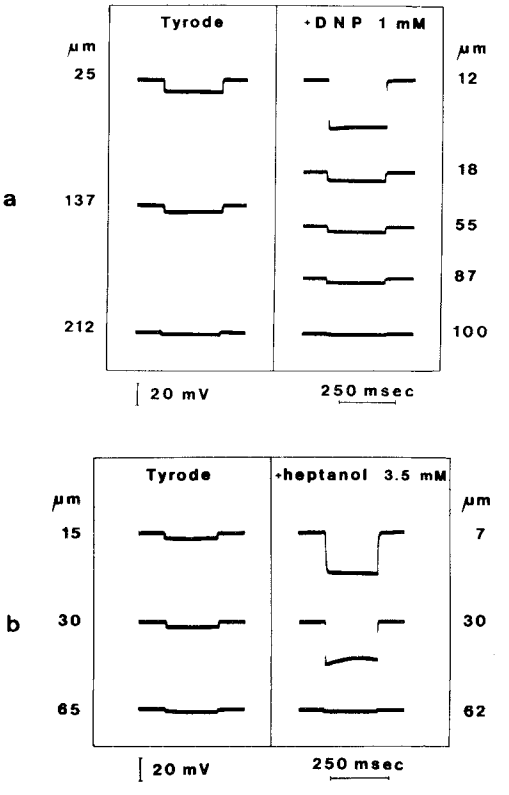


Figure 7. Membrane potentials recorded at different distances (x) from a point of current application on rat auricles in control conditions (Tyrode) and 15 min after addition of an uncoupler of cell-to-cell communication. In (*a*) (DNP) two potential steps can be recognized at 12 μm < x < 18 μm and at 87 μm < x < 100 μm, with a much less steep incline between 18 and 87 μm, which points to the appearance of transverse resistive barriers (partial electrical uncoupling). In (*b*) (heptanol) the potential close to the current electrode is high and practically uniform, but falls off between 30 and 62 μm, which indicates that current flow is restricted to one cell (complete electrical uncoupling). Reproduced from Délèze and Hervé[18] with permission of the Journal of Membrane Biology.

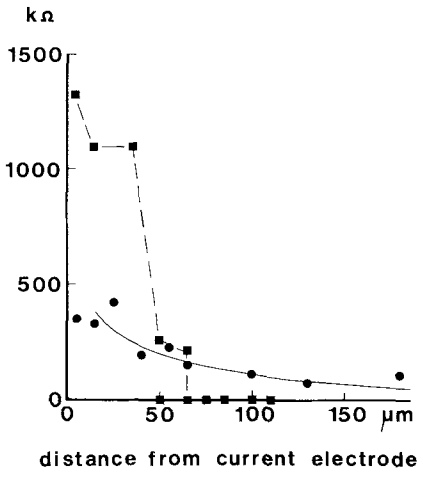


Figure 8. Variation of the steady-state membrane potential divided by current supplied, with distance from current electrode along the fibre's great axis in rat auricles with normal cell-to-cell conduction (●) and after electrical uncoupling by heptanol (■). The continuous line calculated from Eqn. 1 was fitted to the control measurements (●) by a least-square test which provided the value of λ (150 μm). The interrupted lines drawn across the average measurements (■) obtained during the steady state effect of heptanol indicate a sharp cut-off of the voltage deflection at a distance shorter than one cell length. Reproduced from Délèze and Hervé[18] with permission of the Journal of Membrane Biology.

been examined in heart preparations rapidly frozen, instead of glutaraldehyde fixed, in different functional states[17,18], and the main results obtained with this technique will now be summarized and discussed. Ultrarapid cryofixation was performed by projection of the tissue onto the polished surface of a cold metal block[27,28] by means of an apparatus (Cryoblock, Reichert-Jung, Paris) designed by Escaig[24], in which a copper block is cooled to approximately 6° K by liquid helium. The thickness of tissue that can be used for a high resolution morphological study without cryoprotectants is limited to a layer 12 to 15 μm thick[24] by the rapid growth of ice crystals further away from the cold surface where the cooling rate becomes too slow. The rat auricle was found to be convenient for this study, as the first layer of cardiac cells underneath the thin endocardium could often be frozen in a state allowing a good resolution of the gap junction (fig. 5), which was not regularly obtained with the ventricles of the same animal. Cell-to-cell conduction block was promoted by two different treatments, either DNP (1 mM in Tyrode solution) or heptanol, (3.5 mM in Tyrode). The heptanol effects are rapidly and completely reversible on washing the substance away, which allowed an investigation of the gap junction morphology of electrically recoupled cells.

The test for electrical uncoupling in this flat network of interconnected cells was based on the mathematical description of Woodbury and Crill[72] and Noble[41], which assimilates the passive electrical properties of thin cardiac tissues to those of an unbounded planar cell. In this two-dimensional model, the steady-state membrane voltage V at the distance x from an intracellular point where a current I_o is supplied is given by

$$V_x = [I_o R_i/2\pi d] K_{o\,(x/\lambda)} \qquad \text{(Eqn. 1)},$$

where R_i is the intracellular resistivity, which includes the contribution of the cell junctions in series with the cytoplasmic resistance, d is the core thickness, K_o is a modified Bessel function of the second kind[1], and $\lambda = (R_m d/2R_i)^{1/2}$ is the length constant, with R_m the resistance of one unit area of surface membrane. A theoretical curve fitted to the membrane potentials obtained at different distances from the current electrode in several auricles with normal conduction (fig. 8, circles) provided an average value of 150 μm for λ.

The junctional resistance r_j is in series and lumped with the internal resistance $r_i = 4 R_i h/\pi d^2$ of the average cell length h = 100 μm. When r_j increases moderately under the action of an uncoupling treatment, V_x will become larger at small values of x (Eqn. 1) and fall off more steeply with distance, since λ decreases as $R_i^{1/2}$ (curves a and b, fig. 6). The same rise of V at a short distance could be caused by a large increase of R_m, but this case would be easily distinguished from a rise of r_j because λ would become substantially larger (curves b and c, fig. 6). In short, V at values of x smaller than about 50 μm is a good indicator of R_i, and is nearly insensitive to changes in R_m (fig. 6).

When uncoupling proceeds further, r_j becomes large relative to r_i and the previously continuous intracellular conductor will become divided into short unit cables (one cell in length) separated by high transverse resistances located at the intercalated disks. The steady-state voltage decrement with distance will become discontinuous, with voltage steps at distances of one cell length separating much less steep potential inclines (fig. 7a, DNP).

Uncoupling by DNP or heptanol usually proceeded until the induced voltage became nearly uniform at a short distance

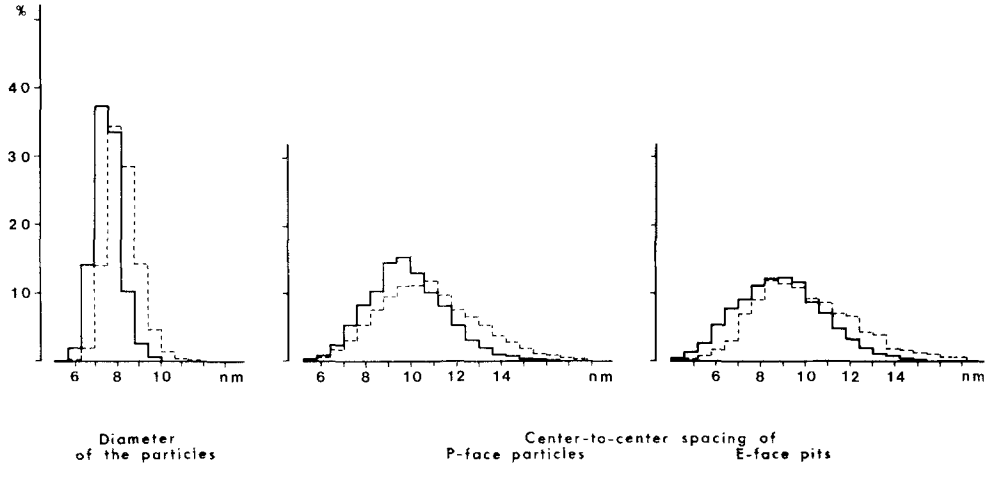


Figure 9. Size distributions of three gap junctional dimensions measured in rat auricles quickly frozen in a state of normal electrical coupling (dotted lines), and after electrical uncoupling (continuous lines). Reproduced from Délèze and Hervé[18] with permission of the Journal of Membrane Biology.

from the current electrode, instead of decreasing steeply as in the conducting auricles, and then fell off abruptly to a very low value (fig. 7b, heptanol) at some distance which, in all trials, was never found to be larger than the presumed value of the cell length (fig. 8). Since the relative position of the micro-electrodes and of the nearest transverse cell boundary cannot be recognized in the live auricle, it is expected that the electrotonic voltage of electrically uncoupled cells will be seen to vanish at the average distance $x = h/2$. This type of potential cut-off obviously indicates that current is now confined to one single cell.

The diameters of the P-face particles and their center-to-center distances, and the distances between pits, were measured in freeze-fractured replicas obtained from auricles quickly frozen in the conducting state and after electrical uncoupling by either DNP or heptanol treatment for 15 to 20 min. All three gap junctional dimensions were very similarly affected by both uncoupling treatments, the average P-face particle diameter decreasing by 8% and the distances between particles and between pits by 9%. The histograms of these measurements were shifted to the left without any major change in shape (fig. 9). Electrical recoupling after heptanol treatment promoted a return of the gap junctional dimensions towards normal values, which was about 50% complete within 20 min (uncoupling by DNP was not reversible). These results, obtained on quickly frozen rat auricles[18], entirely confirm those of a former study of glutaraldehyde fixed Purkinje fibres[16] electrically uncoupled by three different procedures, which had also showed that the diameter of the particles decreases very similarly, whatever the means employed to induce uncoupling. This change was also partly reversible as soon as electrical conduction had been re-established. It can be concluded that a decrease of the junctional particle diameter by about 0.7 nm, which might correspond to a conformational change in the connexon, correlates with the junctional channel closure on electrical uncoupling.

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Cell-to-cell coupling assayed by means of electrical measurements

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Summary. The importance of electrical measurements in the evaluation of cell-to-cell coupling in heart muscle was discussed. The presence of gap junctions in heart and smooth muscle, and the implications of this for electrical synchronization and healing-over, was emphasized. Moreover, the modulation of junctional resistance by Ca, protons and cAMP was reviewed.
Key words. Cell coupling; heart; electrical.

Nur allein der Mensch
Vermag das Unmögliche,
Er unterscheidet,
Wählet und richtet;
Er kann dem Augenblick
Dauer verleihen.
Goethe

Intercellular coupling through low resistance junctions represents a very old mechanism of cell-to-cell communication. In simple organisms such as sponges and medusae without nervous tissue, the epithelial cells receive external stimuli and convert them into electrical pulses which are conducted in all directions through low resistance junctions[31]. Evidence exists that in excitable tissues the intercellular junctions are essential for the spread of electrical activity and for electrical synchronization. It is my intention in this manuscript to review how electrical measurements have contributed to the concept that cells are in communication. In 1954, Sjöstrand and Andersson[37] demonstrated that cardiac muscle is composed of individual cells surrounded by clearly discernible membranes. As the electrical impulse propagates through cardiac muscle as though it consists of one large cell the question is, how can a cardiac myocyte initiate electrical activity in neighboring cells?

A possible explanation is the release of some transmitter