QUANTITATIVE ANALYSIS OF LOW-RESISTANCE JUNCTIONS BETWEEN CULTURED CELLS AND CORRELATION WITH GAP-JUNCTIONAL AREAS

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ABSTRACT

Electrophysiological studies of low-resistance junctions between Novikoff hepatoma cells grown in suspension cultures were carried out and correlated with gap-junctional areas per interface determined by freeze-fracture. The mean coupling coefficient between isolated cell pairs was $0.773 \pm 0.025$ (SEM) in 67G medium and $0.653 \pm 0.028$ in M67 medium; the respective means for the central pairs of four-cell chains were $0.714 \pm 0.034$ and $0.595 \pm 0.026$. Mean estimates of nonjunctional resistances for cell pairs were $3.0 \pm 0.32 \times 10^7$ ohm (67G) and $2.01 \pm 0.01 \times 10^7$ ohm (M67), and the respective estimates for specific nonjunctional resistances were $158.6 \pm 8.1$ ohm-cm$^2$ (67G) and $133.0 \pm 8.1$ ohm-cm$^2$ (M67). Mean estimates of junctional conductances were $0.409 \pm 0.058 \times 10^{-6}$ mho (67G) and $0.211 \pm 0.018 \times 10^{-6}$ mho (M67) for pairs and $0.291 \pm 0.063 \times 10^{-6}$ mho (67G) and $0.212 \pm 0.04$ mho (M67) for four-cell chains.

The mean area of gap junction per interface for separate cell populations was $0.187 \pm 0.049 \mu m^2$ and $0.269 \pm 0.054 \mu m^2$ for cells fixed in loose pellets and in suspension, respectively. When compared with the mean junctional conductance, these values gave specific junctional conductance estimates of $1.13 \times 10^2$ mho/cm$^2$ and $0.78 \times 10^2$ mho/cm$^2$, respectively. These values are higher than most previous estimates, but are consistent with the hypothesis that gap-junctional particles contain central hydrophilic channels, about 2 nm in diameter, which have cytoplasmic resistivity.

KEY WORDS electrical coupling - cell culture - hepatoma - gap junctions - freeze-fracture - junctional conductance

Low-resistance junctions have been studied by electrical methods in cultured cells, both "normal" and transformed (e.g. 5, 8, 10, 13, 21, 28). The electrical coupling mediated by these junctions has been correlated with the transfer of various small tracer molecules including fluorescent dyes (8, 13) and radiolabeled metabolites (9, 25). There is strong, though circumstantial, evidence supporting the gap junction as the structure responsible for the cell-to-cell exchange (1, 9, 13, 29).

The biological role of gap junctions is most clearly defined for excitable cells where they mediate the transfer of electrical signals. For
nonexcitable cells, the role of gap junctions may be more closely associated with their permeability to cell metabolites, nutrients, etc. (8, 17, 26, 32, 35) than with their electrical properties. Nevertheless, gap junctions are believed to transfer inorganic ions and other small molecules along the same intercytoplasmic channels (3, 19, 24). If so, the permeability of the junctions to ions might bear a direct relation to the permeability to other small molecules (3).

These arguments have provided a major impetus for studying the electrical properties of gap junctions. Two electrical properties are of particular interest. The first is gap-junctional conductance, \(^1\) which is a measure of ionic permeability. The second is specific gap-junctional conductance, which is a measure of the ionic permeability of a unit area of junction.

To measure junctional conductance between the two cells of a pair, stringent requirements must be met (2, 31). First, the cells must be in direct contact and have no indirect cellular pathways for current flow, e.g., there cannot be a third cell in contact with both cells in the pair to be tested. Second, it must be possible to pass current and to record transmembrane potential in each cell simultaneously. \(^2\) These requirements have been met by only a few systems (4, 7), and in others it has been necessary to turn to less direct approaches for estimating junctional conductance. Most indirect approaches are based on the assumption that junctional conductances and nonjunctional resistances (see fn. 1) are the same for all cells in the population (14, 33). Thus, they derive an average junctional conductance and provide no information about variations within the population.

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\(^1\) We have used junctional conductance throughout the paper in deliberate preference to junctional resistance in order to emphasize the relationship to junctional permeability (and junctional area). Strictly speaking, the electrical measurements give the conductance between the cell interiors without specifying the pathway. We assume that gap junctions, which are the only junctions seen in our system, provide the major conducting elements. Thus, junctional conductance refers to gap junctions. We have retained the use of nonjunctional resistance because this term is used so commonly outside the junction field in discussions of the electrical properties of membranes in general.

\(^2\) Even then, it is necessary to assume that the junctional channels do not open into the extracellular space.

It is even more difficult to obtain values of specific junctional conductance (11, 16, 37) because junctional areas must be measured. Most values of specific junctional conductance are derived by using either an estimate of the entire area of contact as an upper limit of junctional area (5, 11) or a mean area in conjunction with some estimate of mean junctional conductance (16).

We have made use of the simple geometry of Novikoff hepatoma cells grown in suspension culture and of somewhat less restrictive assumptions about junctional and nonjunctional homogeneity to obtain an estimate of junctional conductance and nonjunctional resistance between the two cells of each pair studied. It has then been possible to correlate the individual junctional conductances and nonjunctional resistances with other parameters of the coupled cells, e.g. cell volume and cell surface. Furthermore, we have used the estimated junctional conductances and the area of gap junction per interface to obtain an estimate of specific junctional conductance; our value is somewhat higher than that reported by most others, but is consistent with an estimate obtained for newt blastomeres (11) and with calculations based on one of the currently accepted models of gap-junction structure (3).

Some of these data have been reported in brief elsewhere (30).

MATERIALS AND METHODS

Preparations

Novikoff hepatoma cells were grown in suspension culture according to previously described methods (27). Briefly, the cultures were initiated every 2 days by taking a few tenths of a millimeter of cell suspension (at about \(10^6\) cells/ml) and diluting into 50 ml of medium, Swims 67G (Grand Island Biological Co., Grand Island, N.Y.) for earlier experiments and M67 (GIBCO) for later experiments. These media have similar compositions; the only significant difference is that 67G contains 5% pancreatic autolysate plus 5% calf serum, whereas M67 contains no autolysate and 10% calf serum. The medium was changed when pancreatic autolysate became unavailable. The cultures were shaken at 200 rpm on a gyratory shaker maintained at 37°C. The flasks were tightly sealed, so that the CO\(_2\) given off by the cells accumulated and so that, as growth proceeded, the pH dropped. Under these conditions, the cells doubled in 10-12 h and reached a saturation density of about \(2 \times 10^6\) cells/ml in 67G and about \(1-1.2 \times 10^6\) cells/ml in M67.
Throughout log growth, cells were found in all degrees of association, from singlets to large clumps. The present electrophysiological studies were made on cell pairs and four-cell chains, whereas the ultrastructural studies were made on samples of the entire population (see below).

**Experimental Arrangement**

For each experiment, 4 ml of cells in log growth (generally about 0.5–0.9 × 10^6 cells/ml) were removed and placed in a 60-mm plastic culture dish (BioQuest, BBL & Falcon Products, Cockeysville, Md.). Experiments were carried out for less than 2 h with the cells at room temperature (20°–22°C) and ambient CO₂ (thus, basic pH, about 8.0). Throughout the time period under these conditions, the cells showed no detectable changes in morphology or in the various electrical parameters measured. The cells remained rounded (cf. Fig. 2) but they adhered sufficiently strongly to the dish to permit stable penetrations with microelectrodes without additional immobilization. Microelectrodes (30–50 Mohm) were filled with 3 M KC1 by boiling under reduced pressure or according to the fiber-fill method (39). Conventional recording and current-passing methods were used (12, 13) as described in further detail in Results.

**Electron Microscopy**

Our basic procedures for electron microscopy (EM) and freeze-fracture have been described elsewhere (12). Briefly, cells at about 10⁶/ml were fixed by adding glutaraldehyde (giving a final concentration of 2.5%) to cells suspended in M67, or, as a 2.5% solution in serum-free medium (BM 42; GIBCO), to loose pellets of cells. The loose pellets, obtained after 5 min of centrifugation at 45 g, were considerably easier to process for freeze-fracture. After fixation for 2 h, the pellets were treated with 20% glycerol overnight, frozen in liquid freon, and fractured by conventional methods on a Balzers' BAF 300 (Balzers High Vacuum Corp., Santa Ana, Calif.).

For comparison with junctional conductances, we determined the area of gap junction per interface by the following procedure: (a) To ensure that we included entire interfaces in our sample, we identified membrane faces at least large enough to fill the microscope screen at × 10,000 (i.e. 57 μm² or greater). We chose this value because thin-section data (not shown) indicated that most interfaces were less than 8 μm in diameter (in fact, many faces selected by this procedure were substantially larger than 57 μm², and, thus, were likely to have included entire interfaces). (b) Faces that fulfilled criterion a and showed evidence of cell-cell apposition were defined as "interfaces." In most cases, cell-cell apposition was confirmed by the presence of a "step" exposing the P-face (inner leaflet) of one cell and the E-face (outer leaflet) of the next cell as well as a gap junction or formation plaque (12). (c) The total area of gap junction on each "interface" was obtained by adding areas of all gap junctions present. Areas of large junctions were estimated by assuming that the junctions were ellipses and by taking one-fourth the product of the major and minor axes multiplied by π. Areas of small junctions were estimated by counting the number of particles and dividing by 10⁴ particles/μm², the average density of particles in a tightly packed junction.

**RESULTS**

**Theory**

A simplified equivalent circuit for a coupled pair of cells is shown in Fig. 1 A. Cells 1 and 2 have nonjunctional resistances of r₁ and r₂, respectively, and are connected by a low-resistance junction of resistance r₃ and conductance, g₃ = 1/r₃. (The circuit applies when the cells are connected by more than one gap junction; r₃ is then the total junctional resistance.) The extracellular resistance is assumed to be negligibly low, and the shunt resistance from the junction into the intercellular space is assumed to be infinite. If all three resistances (r₁, r₂, r₃) were different, their values could only be obtained by passing current, I₁ and I₂, into cells 1 and 2, respectively, and by recording the voltage changes, V₁ and V₂, across the respective nonjunctional resistances. Experimentally, this would require two microelectrodes in each cell. Our method, however, relied on using pairs of spherical cells of essentially equal size (Fig. 2), which we assumed to have equal nonjunctional

![Figure 1](image-url) Equivalent circuits used for estimating electrical properties of junctional and nonjunctional membranes. (A) Cell pair. (B) Four-cell chain.
resistances; i.e. $r_1 = r_2 = r_m$ (assumption 1). We felt that this assumption was justified because most pairs of cells were probably daughter cells, which probably had a greater similarity of membrane properties than non-daughter cells. (When cells prelabeled with $[\text{H}]$uridine were mixed with nonlabeled cells and co-cultured for 15 h under standard conditions, most pairs of cells were either uniformly and heavily labeled or totally unlabeled. Thus, there was little aggregation of labeled and unlabeled cells. We cannot rule out the possibility, however, that the cell pairs and four-cell chains were broken off from larger clumps.) Thus, the analysis required only one current-passing and two recording microelectrodes. (The effects of errors in our assumptions are considered in the Discussion.)

The actual experimental procedure is illustrated in Figs. 2, 3, and 4. First, each cell was impaled with a microelectrode (Fig. 3). Three pulses of hyperpolarizing current ($I_1$) and three of depolarizing current were passed into cell 1, and the induced potential changes, $V_2$, were recorded in cell 2. Because we had impaled each cell with a microelectrode, it was reasonable to assume that the effective nonjunctional resistances were equal though now a bit lower than in undisturbed cells due to the damage from the impalements. However, with two electrodes we had insufficient information to calculate $r_m$ and $r_j$.

Next, another microelectrode was inserted into cell 1 and the six pulses were repeated (Fig. 4). This time, the voltage in cell 1 ($V_{1'}$) was recorded.

![Figure 2](image1)

**Figure 2** Representative photomicrographs of cell pair (a) and four-cell chain (b) Bar, 50 $\mu$m.

![Figure 3](image2)

**Figure 3** First step in typical electrophysiological experiment. (Top) Cell 1 is impaled with a microelectrode for passing current ($I_1$), and cell 2 with a microelectrode to record voltage change ($V_2$). (Bottom) Three current pulses, $I_1$ (either hyperpolarizing on left or depolarizing on right), of graded intensities, produce voltage changes, $V_2$. Calibration: vertical, 17 mV, $4 \times 10^{-9}$ A; horizontal, 90 ms.

![Figure 4](image3)

**Figure 4** Second step in experiment. (Top) Cell 1 is impaled with a second microelectrode to record voltage ($V_{1'}$). (Bottom) Three current pulses ($I_1$) are repeated, producing voltage changes in cell 1 ($V_{1''}$) and cell 2 ($V_{2''}$). Calibration: as in Fig. 3.
as well as the voltage in cell 2 ($V_2'$). We then had the requisite data, provided our assumption of equal nonjunctional resistances held. However, we had impaled cell 1 with two electrodes and cell 2 with only one; thus, it was likely that cell 1 had sustained greater damage and that $r_1$ was less than $r_2$. This effect could be compensated for, however, by assuming that the coupling coefficient, $V_2'/V_1'$, measured after impaling cell 1 with the second electrode, was the same as when cell 1 had only a single impalement, i.e., $V_2'/V_1$, which was not directly recorded (assumption 2).

We calculated nonjunctional resistances and junctional conductances from our data, from the input resistance, $r_i = V_i/I$, and from the coupling coefficient, $\alpha = V_2/V_1$, both corresponding to the condition in which each cell was impaled with one electrode. According to assumption 2, $\alpha = V_2/V_1 = V_2'/V_1'$, and $r_i$ could be obtained from,

$$r_i = V_i/I = (V_2/I)/\alpha. \tag{1}$$

In calculating $r_i$ for each pair, we used the mean value for $V_2/I$ and the mean value for $\alpha$ for each series of varied currents. For the equivalent circuit in which $r_1 = r_2 = r_m$, the input resistance is also given by,

$$r_i = \frac{r_m(r_m + r_j)}{2r_m + r_j}. \tag{2}$$

Because,

$$r_j = \left(\frac{1 - \alpha}{\alpha}\right) r_m, \tag{3}$$

then,$$
\alpha = r_j(1 + \alpha). \tag{4}$$

We obtain $r_j$ from equation (3) and $g_j$ from

$$g_j = \frac{1}{r_j}. \tag{5}$$

A similar analysis was also carried out on the internal pairs of cells of four-cell chains whose cells were all of comparable sizes. For these, we used the equivalent circuit shown in Fig. 1 B. If we assume that

$$\frac{r_2(r_1 + r_j)}{r_1 + r_2 + r_j} = \frac{r_3(r_4 + r_5)}{r_3 + r_4 + r_5} = r_{sd},$$

which is the case if, e.g., $r_1 = r_2 = r_3 = r_4 = r_m$ and $r_j = r_f$, then we can write, by analogy with the two-cell situation discussed above,

$$r_{sd} = r_2(1 + \alpha), \tag{6}$$

where $r_2$ is the input resistance of cell 2, and

$$r_{sd} = r_3 \left(\frac{1 - \alpha}{\alpha}\right), \tag{7}$$

and

$$g_{sd} = \frac{1}{r_{sd}}. \tag{8}$$

Clearly, the assumption of complete symmetry was less justifiable than for the two-cell system, and the results were correspondingly less reliable.

To obtain the necessary data, we treated cells 2 and 3 as we did cells 1 and 2 in our two-cell experiment, i.e., impaled each with a single electrode, then cell 1 with an additional electrode. We then had $V_2/I_2$ and $V_3/V_3' = \alpha$, which we assumed equal to $V_2/V_3$ for the two-electrode situation. As before,

$$r_{sd} = \frac{(V_2/I_2)/\alpha}, \tag{9}$$

and we then used equations 6 and 7 to estimate $r_{sd}$ and then equation 8 to obtain $g_{sd}$.

**Coupling Coefficient**

The most commonly used quantitative measure of electrical coupling is the coupling coefficient (2, 36). Fig. 5 shows our data on coupling coefficients for cell pairs and four-cell chains (Fig. 5). As in subsequent figures, the values from cells grown in the two different media (67G and M67) are shown separately. The general shapes of the four distributions of coupling coefficients are similar. Cell chains in both media tended to have lower coefficients than cell pairs, which would be expected even if the junctional conductances were comparable, because the effective nonjunctional resistances were lower (see Theory section above). There was also a tendency for cells in M67 to have lower coefficients (and lower junctional conductances; see below).

**Junctional Conductances**

As shown in Fig. 6, the values of $g_j$ estimated for cell pairs and for cell chains were similar and...
suggested that most pairs in our system were formed by cell division (see below), and inasmuch as cell volume generally increases as the cell progresses through the cell cycle, we anticipated cycle-dependent changes in junctions. As shown in Fig. 9, there was a slight tendency for higher conductance with large cells ($r = +0.178$) but the slope was not significant ($0.1 > P > 0.05$) (data have been plotted only for cells in M67).

As one of us has suggested previously (31), a parameter likely to be of interest in nonelectrical communication is the junctional permeability (or junctional area) per cell volume. Because junctional conductance is a measure of ionic permeability, we have calculated the ratio, junctional conductance per cell volume, and we have plotted the values from M67 cells in Fig. 10. The values are positively skewed.

**Gap-Junctional Areas**

We found that 35-50% of the interfaces identified (see Materials and Methods for definition) contained gap junctions, usually two to three per interface. The individual junctions were no-

![Figure 5](image)

**Figure 5** Distributions of coupling coefficients from four-cell chains (top) and cell pairs (bottom), for cells grown in two kinds of medium, M67 and 67G. Means ± SEM are given.

positively skewed. Again, in M67 there was a shift toward lower junctional conductances for both cell pairs and four-cell chains.

**Nonjunctional Resistances**

The values for nonjunctional resistance from cell pairs (Fig. 7) are distributed more symmetrically with a shift toward lower $r_m$ values in M67. The $r_m$ values and estimates of nonjunctional areas were used to obtain estimates of specific nonjunctional resistances (Fig. 8). (Nonjunctional areas were estimated using the radius measured from photomicrographic negatives projected at about $\times 2$ onto graph paper: $\text{Area} = 4\pi r^2$.) The specific resistances displayed a similar, though less marked, shift toward lower values in M67.

**Correlation between Junctional Conductances and Cell Volume**

It seemed useful to determine whether there was a correlation in cell pairs between junctional conductance and cell volume ($4/3 \pi r^3$, where $r = \text{radius measured as described above}$). We
FIGURE 7 Distributions of estimated nonjunctional resistances for cell pairs grown in M67 or 67G. Means ± SEM are given.

FIGURE 8 Distributions of estimated specific nonjunctional resistances from cell pairs grown in M67 or 67G. Means ± SEM are given.

FIGURE 9 Scatter diagram relating junctional conductance and cell volume for cell pairs grown in M67.

Direct estimates available for cultured cells. Furthermore, by obtaining a separate value for each pair of coupled cells, we gain information about the variation in junctional conductances (and nonjunctional resistances) as well as the relation between these values and other cell parameters such as cell volume, and, for the population, areas of gap junctions.

Our estimates of junctional conductances and nonjunctional resistances are, however, subject to certain potential errors. First, our derivation depends on two assumptions: (a) that when cell sizes are equal, the nonjunctional resistances are equal and, further, remain equal, though perhaps lower, when each cell is impaled with a single microelectrode; and (b) that the coupling coefficient is the same before and after impaling a cell with a second microelectrode. The first assumption is reasonable if, as we believe, pairs are likely to be daughter cells and, thus, more alike than non-daughter cells. This assumption cannot be tested directly, but we can determine the general direction of the errors that would be introduced if the assumption were incorrect. For a pair of cells having a coupling coefficient of 0.8, if \( r_1 = 0.5 r_2 \), the average \( r_m \) \( = (r_1 + r_2)/2 \) would be only about 14% greater than if \( r_1 = r_2 \), but the true \( g_j \) would be about 40% less. If, on the other hand, \( r_1 = 2 r_2 \), then the true mean \( r_m \) would be about 8% higher but the true \( g_j \) would be about 38% higher. Inasmuch as \( r_1 \) and \( r_2 \) had equal probabilities of being greater, there was a

DISCUSSION

Estimates of junctional conductances (or resistances) have previously been obtained from few coupled cell systems (4, 6, 7, 11, 14, 18, 33, 34), and there have been few quantitative correlations with junctional structure (16, 37). Cultured cells are usually particularly difficult to study because of their small size and complex geometry; the only values reported previously are averages obtained indirectly by assuming regularity in geometry and homogeneity in junctional conductances and nonjunctional resistances (14, 33). Our results, then, are the most...
FIGURE 10 Distributions of (a) calculated junctional conductance/cell volume and (b) cell volumes, for cells in pairs grown in M67.

FIGURE 11 Freeze-fracture view of small gap junction between cells fixed in loose pellet (see Materials and Methods). Both P-face particles and E-face pits are seen as well as the abrupt reduction in extracellular space at the level of the junction. This junction has an area of about 0.04 \( \mu \text{m}^2 \) and, thus, is similar in size to the average junction seen (\( \bar{x} = 0.08 \mu \text{m}^2 \)) \( \times \) 109,000.

net tendency to underestimate \( r_m \) with little net effect on \( g_j \). There would be a tendency to underestimate \( g_j \), however, with a smaller \( \alpha \) value and/or larger \( r_1/r_2 \).

The second assumption also is not directly testable, but again the effects of change in coupling coefficient can be estimated. The most likely change due to damage caused by the second electrode in cell 1 would be to decrease coupling (22). If the measured coupling coefficient were 0.8 and the transfer resistance (from the two-electrode measurement) were 12.3 Mohm, the estimated \( r_m = 27.7 \) Mohm \( g_j = 0.14 \times 10^{-6} \) mho. If the true coupling coefficient were 0.9 and the transfer resistance (again for the two-electrode measurement) were 12.3 as before, the true \( r_m = 26.9 \) Mohm \( g_j = 0.35 \times 10^{-6} \) mho. In other words, decreasing the coupling coefficient by inserting the second electrode in cell 1 would increase the apparent \( r_m \) slightly and decrease the apparent \( g_j \) values with a disproportionate effect on \( g_j \). Furthermore, the effect is greater the higher the coupling coefficient. Thus, inaccuracies in the two assumptions would have opposite effects on the estimated \( r_m \) values, but would lead to underestimates of \( g_j \).

A second possible problem would be the inclusion of cell pairs in late telophase joined by persistent cytoplasmic bridges or mid-pieces. The estimated \( g_j \) values for these cells would be quite high and could conceivably contribute some of the scattered high values (Fig. 6). Such a contamination cannot be ruled out, but is unlikely to be great. First, we have seen only a few mid-pieces in our extensive EM studies of these cells (13; and unpublished thin-section studies). Second, newly divided cells are likely to be appreciably smaller than cells at later stages in the cycle (20). Thus, if mid-pieces occurred frequently, we would have expected appreciable numbers of small cells with high junctional conductances (i.e., due to mid-pieces). Such was not the case, and in fact it is likely that smaller cells were selected against in our electrophysiological sample (see below).

The distributions of \( g_j \) values (Fig. 6) from cell pairs and four-cell chains were similar, both in general shape and in showing a slight shift toward lower conductances in M67 medium. This similarity gives us further confidence that our estimates are reasonable. The pronounced skewing of the curves suggests that caution should be used when comparing our mean \( g_j \) values with those obtained in other systems. Our mean values (ca 0.2–0.4 \( \times 10^{-6} \) mho) are similar to those obtained for reaggregated Fundulus embryo cells (4), Xenopus cells (34), Triturus embryo cells (11), and chick embryo cells in culture (34), but they are much lower than...
values found at septal synapses (6, 23) and giant motor synapses of crayfish (7), or in the insect salivary gland (18).

We suspect that our nonjunctional resistances \( \bar{x} = 2.01 \times 10^7 \) ohm, M67; \( 3.00 \times 10^9 \) ohm, 67G) are low because they were estimated for the condition in which each cell was impaled with a microelectrode. Furthermore, our estimates of cell surface area (but not volume) are also likely to be low, because we assumed that
the cell surfaces were smooth, whereas we know from our unpublished scanning EM pictures that the cells have numerous microvillar projections. Therefore, our values for specific nonjunctional resistance \( (\bar{r} = 133 \text{ ohm-cm}^{-2}, \text{M67}; \text{158.6 ohm-cm}^{-2}, \text{67G}) \) are likely to be quite low, perhaps by as much as an order of magnitude. Nevertheless, our values compare well with estimates from other culture systems subject to similar manipulations and errors in estimating surface area (5).

Irregularities in surface projections are likely to have much less effect on volume determinations. Thus, our junctional conductance per volume estimates are probably as reliable as the \( g_j \) values from which they were obtained. If the mean \( (\bar{x} = 1.71 \times 10^{-4} \text{ mho/um}^2 \pm 0.47 \text{ SEM}) \) is converted to junctional area per volume (using 10\(^2\) mho/cm\(^2\) as the specific junctional conductance, see below), we obtain a value of about \( 1.71 \times 10^{-4} \text{ um}^{-1} \), which is between one and two orders of magnitude below the value estimated for liver \( (6 \times 10^{-3} \text{ um}^{-1}) \), heart \( (2 \times 10^{-3} \text{ um}^{-1}) \), and smooth muscle \( (4 \times 10^{-3} \text{ um}^{-1}) \), but similar to that for baby hamster kidney (BHK) fibroblasts \( (1.3 \times 10^{-4} \text{ um}^{-1}) \) (31; and unpublished calculations).

The potential interest of these comparisons derives from the assumption (developed elsewhere; reference 31) that nonelectrical communication should be favored by high ratios. It should just be noted that the BHK fibroblasts effectively transfer nucleotides (25, 38), presumably via their gap junctions, so a low ratio does not imply absence of nonelectrical communication.

The absence of a significant positive correlation between junctional conductance and cell size possibly results from the omission of very small and very large cells from our sample (see below). (It is less likely that pairs or chains comprised of cells in grossly different stages of the cycle were studied because we were careful to choose cases where both, or all four, cells were of equal size.) These extremes are more likely to represent the early and late stages of the cell cycle and, thus, potentially the most divergent junctional sizes. From our studies of junctional formation between reaggregated cells (12; and unpublished data), we would predict that daughter cells in the first 2 h of the cycle would have, on the average, significantly smaller junctional sizes than later cells. Also, preliminary EM data suggest that cells in large clumps (greater than 30 cells) can have extremely large junctions. These large junctions may result from gradual accretion of junctional particles as the clumps are carried over during subculture.

An important goal of our study was to compare our estimated junctional conductances with our freeze-fracture data on gap-junctional area per interface to determine the likely limits on specific gap-junctional conductance. Because the electrophysiological and ultrastructural data were obtained on separate samples, a direct calculation of specific conductances for each pair of coupled cells was not possible. The samples were also different in three important senses: (a) The EM data were obtained from cells in clumps of widely varying sizes, from 2 to 100 cells (assuming indiscriminate sampling from the entire culture). The electrophysiological data, however, were obtained only from cell pairs and four-cell chains. (b) Smaller cells, less than about 12 \text{ um} in diameter, were avoided in the electrophysiological study, but were probably included in the EM study. (c) To make it possible to obtain a larger sample size and to facilitate processing of cells for freeze-fracture, the cells were loosely pelleted before fixation in the initial experiments. (The implications of these differences are considered further below). As we see in Fig. 12, the shapes of the curves of junctional conductances and junctional areas are similar, as we would expect if they were measurements on comparable populations of junctions and if the junctional areas were directly proportional to junctional conductances.

A closer comparison of the junctional area and conductance distributions, however, indicates a higher proportion of areas below 0.01 \text{ um}^2 and above 0.7 \text{ um}^2 in the EM data (from pelleted material) than expected from the conductance curves. The source of these discrepancies is unclear, but the following suggestions can be made. The disproportionately small number of lower conductances in the electrophysiological data may result from our tendency to select intermediate- to large-size cells for the electrophysiological studies (see above). A comparison (not shown) of the diameters of cells studied electrophysiologically and those present in the entire population indicates that the total population includes substantially more cells in the 11- to 13-\text{ um} range. These smaller cells are more likely to be in earlier stages of the cell cycle and, thus, to have been in contact shorter times.
resulting in smaller junctions. By omitting these cells from the population studied electrically, we potentially missed the cells with the smallest junctional conductances.

It is also likely that some of the smaller junctions were formed in the few minutes during which the cells were in the loose pellets before being fixed. The junctional areas seen in material fixed in suspension are, on average, slightly (though not significantly) larger (Fig. 12) and there is a higher proportion of interfaces with gap junctions relative to those with only formation plaques (unpublished data). Although the sample from suspension-fixed cells is smaller, the general shape of the distribution resembles that for junctional conductances, in fact somewhat better than the values from pelleted cells.

The greater percentage of areas above 0.7 \( \mu m^2 \) may reflect the possibility that cells in clumps have been in contact with neighbors longer than one cell cycle and, thus, have had time to produce larger junctions than possible for cell pairs. In this regard, it is interesting that the four-cell chains had a larger spread of conductances.

Other factors may also have contributed to the discrepancies between the EM and electrical data. The electrical data may actually have an artifactually low range as a result of errors in the assumption that the two cells in each pair have equal nonjunctional resistances (see Discussion). Furthermore, less adhesive cells were perhaps disrupted during transfer of the cells to the dish before electrophysiological study. Finally, the smallest gap junctions may have been below the limit of resolution of our electrophysiological method. (Unpublished estimates, however, suggest that we could detect junctions with as few as 10-20 particles in optimal cases.)

Thus, considering the potential complications, the ultrastructural and electrophysiological data agree remarkably well and justify using the ratio of the mean junctional conductance (0.211 \( \times 10^{-6} \) mho) to the mean junctional area per interface (0.187 \( \times 10^{-6} \) cm\(^2\) for suspension-fixed cells) to obtain an estimate of specific conductance (1.13 \( \times 10^2 \) mho/cm\(^2\) or 0.78 \( \times 10^2 \) mho/cm\(^2\), respectively).

Our estimate for specific conductance is generally higher than other estimates, most of which range from 0.1 to 1 mho/cm\(^2\) (6, 14, 16, 33, 37). In few of these cases have the correlations of structure and conductances been very precise. Many of these values are certainly low because the junctional areas used to make the calculations were high, e.g., in the tunicate heart (16), a specific conductance of 5 mho/cm\(^2\) was estimated on the basis of the area of “tight junctions”; because only a small fraction of this area was likely to have been gap junctions (just the basal “tight junctions”), the specific conductance estimate might well be low by an order of magnitude. In one study of cultured embryonic cells (33), the area of the total interface was used rather than the junctional area, again giving a value probably two or more orders of magnitude too low. In a study of cultured heart cells (14), the specific conductance was estimated with junctional areas obtained by other workers for adult heart cells. Inasmuch as the cultured cells quite possibly had smaller junctional areas, the estimated specific conductance again is probably too low. The most reliable values for adult heart are derived from morphometric analysis of thin sections that provide an average junctional area per interface (37). However, the electrical data in no case come from the same preparation as the EM data, and in some cases the data come from different species. Whether this results in an order of magnitude error is questionable, but it might account for some of the discrepancy.

Our data are consistent with the recent estimate of specific conductance provided by Ito et al. (11) from a study of Triturus blastomeres. Their estimate “of the order of 10\(^2\) mho/cm\(^2\),” was based on data from three cell pairs joined by “3-4” fine cytoplasmic processes judged to be 1 \( \mu m \) in diameter. This estimate is a lower limit for specific conductance, provided the processes were not enlarged at the point of contact and provided their cross-sectional area could be taken as the maximum possible junctional area. It should be noted that the nature of the junction in these experiments is unknown.

The value of 10\(^2\) mho/cm\(^2\) is consistent also with theoretical models of junctional structure (3) that assume that the junctional particles contain channels 1-2 nm in diameter having a resistivity comparable to that of cytoplasm.

It is interesting that the estimated conductance of the channels controlled by acetylcholine receptors in the postsynaptic membrane of the neuromuscular junction is 10\(^{-9}\) mho/particle (15), essentially the same as our estimate. Considering that our channels should have nearly three times the length (i.e. 20 vs. 7.5 nm), the
The diameter of our channels could well be 50–75% greater, resulting in channels sufficiently wide to pass molecules larger than inorganic ions.

In conclusion, our studies provide extensive quantitative data on the electrical (and related ultrastructural) properties of low-resistance junctions between Novikoff hepatoma cells grown in suspension culture. Because these cells are maintained in culture and are transformed, however, we need to be cautious in generalizing from our data to the properties of normal cells in situ. Because variations in area are common in different biological systems and even within the same system at different stages in development, we might expect comparable variations in permeability with important functional consequences.

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REFERENCES
1. Azarnia, R., W. Larsen, and W. R. Loewenstein. 1974. The membrane junctions in communicating and non-communicating cells, their hybrids and segregants. Proc. Natl. Acad. Sci. U. S. A. 71:880–884.
2. Bennett, M. V. L. 1966. Physiology of electrotonic junctions. Ann. N. Y. Acad. Sci. 137:509–539.
3. Bennett, M. V. L. 1973. Permeability and structure of electrotonic junctions and intercellular movements of tracers. In Intracellular Staining in Neurobiology. S. B. Kater and C. Nicholson, editors. Springer-Verlag New York Inc., New York 115–133.
4. Bennett, M. V. L., M. E. Sprira, and G. D. Pappas. 1972. Properties of electrotonic junctions between embryonic cells of Fundulus. Dev. Biol. 29:419–435.
5. Borek, C., S. Higashino, and W. R. Loewenstein. 1969. Intercellular communication and tissue growth. IV. Conductance of membrane junctions of normal and cancerous cells in culture. J. Membr. Biol. 1:274–293.
6. Brink, P., and L. Barr. 1977. The resistance of the septum of the median giant axon of the earthworm. J. Gen. Physiol. 69:517–536.
7. Fursphpan, E. J., and D. D. Potter. 1959. Transmission at the giant motor synapses of the crayfish. J. Physiol. (Lond.). 145:289–325.
8. Fursphpan, E. J., and D. D. Potter. 1968. Low-resistance junctions between cells in embryos and tissue culture. Curr. Top. Dev. 1:95–127.
9. Gilula, N. B., O. R. Reevs, and A. Steinebach. 1972. Metabolic coupling, ionic coupling, and cell contacts. Nature (Lond.). 235:262–265.
10. Hulser, D. F., and D. J. Webb. 1973. Relation between ionic coupling and morphology of established cells in culture. Exp. Cell Res. 80:210–222.
11. Ito, S., E. Sato, and W. R. Loewenstein. 1974. Studies on the formation of a permeable cell membrane junction. II. Evolving junctional conductance and junctional insulation. J. Membr. Biol. 19:339–355.
12. Johnson, R. G., M. Hammer, J. D. Sheridan, and J. P. Revel. 1974. Gap junction formation between reaggregated Novikoff hepatoma cells. Proc. Natl. Acad. Sci. U. S. A. 71:4536–4540.
13. Johnson, R. G., and J. D. Sheridan. 1971. Junctions between cancer cells in culture: ultrastructure and permeability. Science (Wash. D.C.). 174:717–719.
14. Jongsm, H. J., and H. E. van Rijn. 1972. Electrotonic spread of current in monolayer cultures of neonatal rat heart cells. J. Membr. Biol. 9:341–360.
15. Katz, B., and R. Miledi. 1972. The statistical nature of the acetylcholine potential and its molecular components. J. Physiol. (Lond.). 224:665–699.
16. Kriebel, M. E. 1968. Electrical characteristics of tunicate heart cell membranes and nexuses. J Gen. Physiol. 52:46–59.
17. Loewenstein, W. R. 1968. Communication through cell junctions: implications in growth control and differentiation. Dev. Biol. Suppl. 2:151–183.
18. Loewenstein, W. R., M. Nakas, and S. J. Socolar. 1967. Junctional membrane uncoupling: permeability transformations at a cell membrane junction. J. Gen. Physiol. 50:1865–1891.
19. McNutt, N. S., and R. S. Weinstein. 1970. The ultrastructure of the nexus: a correlated thin-section and freeze-cleave study. J. Cell Biol. 47:665–688.
20. Mitchison, J. M. 1971. The Biology of the Cell Cycle. Cambridge University Press, Cambridge, Eng. 128.
21. O'Lague, P., H. Dalen, H. Rubin, and C. Tobias. 1970. Electrical coupling: low resistance junctions between mitotic and interphase fibroblasts in tissue culture. Science (Wash. D.C.). 170:464–466.
22. Oliveira-Castro, G. M., and W. R. Loewenstein. 1971. Junctional membrane permeability:
effects of divalent cations. *J. Membr. Biol.* **5:**51-77.

23. Payton, B. W., M. V. L. Bennett, and G. D. Pappas. 1969. Temperature-dependence of resistance at an electrotonic synapse. *Science (Wash. D.C.)*. **165:**594-597.

24. Payton, B. W., M. V. L. Bennett, and G. D. Pappas. 1969. Permeability and structure of junctional membranes at an electrotonic synapse. *Science (Wash. D.C.)*. **166:**1641-1643.

25. Pitts, J. D. 1972. Direct interaction between animal cells. In *Cell Interactions*. L. G. Silvestri, editor. North-Holland Publishing Co., Amsterdam. 227-285.

26. Pitts, J. D., and M. E. Finbow. 1977. Junctional permeability and its consequences. In *Intercellular Communication*. W. C. DeMello, editor. Plenum Publishing Corporation, New York. 61-86.

27. Plagemann, P. G. W., and H. E. Swim. 1966. Replication of mengovirus. I. Effect on synthesis of macromolecules by host cell. *J. Bacteriol.* **91:**2317-2326.

28. Potter, D. D., E. J. Furspham, and E. S. Lennox. 1966. Connections between cells of the developing squid as revealed by electrophysiological methods. *Proc. Natl. Acad. Sci. U. S. A.* **66:**328-335.

29. Revel, J. P., A. G. Yee, and A. J. HudsPeth. 1971. Gap junctions between electrotonically coupled cells in tissue culture and in brown fat. *Proc. Natl. Acad. Sci. U. S. A.* **68:**2924-2927.

30. Sheridan, J. D. 1972. Electrical resistance of junctions between cancer cells. *J. Cell Biol.* **55:**236a-237a. (Abstr.).

31. Sheridan, J. D. 1973. Functional evaluation of low resistance junctions: influence of cell shape and size. *Am. Zool.* **13:**1119-1128.

32. Sheridan, J. D. 1974. Low resistance junctions: some functional considerations. In *The Cell Surface in Development*. A. A. Moscona, editor. John Wiley & Sons, New York. 187-206.

33. Siebenbeek van Heukelom, J., J. J. D. van der Gun, and F. J. A. Prop. 1972. Model approaches for evaluation of cell coupling in monolayers. *J. Membr. Biol.* **7:**88-110.

34. Slack, C., and A. E. Warmer. 1975. Properties of surface and junctional membranes of embryonic cells isolated from blastula states of *Xenopus laevis*. *J. Physiol. (Lond.)*. **248:**97-120.

35. Sociolar, S. J. 1973. Cell coupling in epithelia. *Exp. Eye Res.* **15:**693-698.

36. Sociolar, S. J. 1977. Appendix: the coupling coefficient as an index of junctional conductance. *J. Membr. Biol.* **34:**29-38.

37. Spira, A. W. 1971. The nexus in the intercalated disc of the canine heart: quantitative data for an estimation of its resistance. *J. Ultrastruct. Res.* **34:**409-425.

38. Subak-Sharpe, H., R. R. Bork, and J. D. Pitts. 1969. Metabolic co-operation between biochemically marked mammalian cells in tissue culture. *J. Cell Sci.* **4:**353-367.

39. Tasaki, K., Y. Tsukahara, S. Ito, I. J. Wayner, and W. Y. Yu. 1968. A simple, direct and rapid method for filling microelectrodes. *Physiol. Behav.* **3:**1009-1010.