INTERCELLULAR CONNECTIVITY IN THE EIGHT-CELL *XENOPUS* EMBRYO

CORRELATION OF ELECTRICAL AND MORPHOLOGICAL INVESTIGATIONS

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ABSTRACT The distribution of individual intercellular electrical junctions has been examined in eight-cell *Xenopus* embryos using linear systems analysis. Morphological evidence for corresponding intercellular contacts has been sought by light microscopy and scanning electron microscopy. The electrical investigations indicated that each cell is directly coupled to each of the other seven cells by identical resistive junctions. Scanning electron microscopy of the cell surfaces of cleaved embryos revealed protrusions from the surfaces of the cells which could mediate such intercellular connections. Light microscopy of serial sections through the embryos also showed fine processes of the cell surfaces which come into contact with several other cells. The complete intercellular connectivity suggested by these results appears to be an extension of similarly close connectivity in the two- and four-cell embryos. The possible significance of this high connectivity to morphogenesis is discussed.

INTRODUCTION

The potential importance of intercellular communication in embryogenesis (see for example Loewenstein, 1966, 1967, 1968; Furshpan and Potter, 1968; Bennett and Trinkaus, 1970; Bennett et al., 1972) has led a number of investigators to examine the coupling phenomenon in amphibian embryos (Ito and Hori, 1966; Takahashi and Ito, 1968; Ito and Loewenstein, 1969; Palmer and Slack, 1970; Slack and Palmer, 1969; Sheridan, 1971). In a previous report (DiCaprio et al., 1974) we have described how linear systems theory may be used to produce electronic models which account for the electrical properties of the junctional and nonjunctional membranes in *Xenopus* embryos during the first two cleavage stages. It was concluded that electrical junctions between the cells may be modeled by simple resistive elements. A potentially significant finding was that the electrical properties of the four-cell embryo can only be explained by the existence of individual junctions linking each cell to all of the other cells in the system. This was found to be morphologically feasible by examining the embryos with the scanning electron microscope and light microscope.

Transmission electron microscopy of *Xenopus* embryos during cleavage and blastulation has demonstrated the presence of cell contacts possessing an intercellular gap of 20–30 Å (Sanders and Zalik, 1972; Singal and Sanders, 1974). These contacts are

similar to those discovered in other systems where they have been suggested to mediate electrotonic coupling (Loewenstein, 1966; Payton et al., 1969; Gilula et al., 1972; Azarnia et al., 1974).

Although it would be theoretically possible to determine the electrical parameters of more advanced cleavage stages by linear systems analysis, the number of measurements and calculations required would become formidable above the four-cell stage. However it is feasible to determine the intercellular connectivity at higher stages and this assumes some importance in view of the connectivity discovered at the four-cell stage. For the purposes of this paper we use the term "connectivity" to define the extent to which pairs of cells are directly coupled together by a junction, such that current may pass between the two cells without passing through an intermediate cell. In this paper we have elucidated the connectivity of the eight-cell *Xenopus* embryo by measuring the frequency response functions between pairs of cells. The embryos have also been examined using light microscopy and scanning electron microscopy in order to provide a morphological justification for the intercellular connections which are predicted by the electrical measurements.

MATERIALS AND METHODS

Early embryos of *Xenopus* were obtained by subcutaneous injection of gonadotrophic hormone into adult animals as described previously (DiCaprio et al., 1974). The embryos were dejellied in papain-cysteine solution (Dawid, 1965) and handled in Steinberg's physiological salt solution.

Staging

A major problem in the study of electrotonic coupling between embryonic cells is the difficulty of deciding when the membrane partition between daughter cells is complete, thereby eliminating the possibility of current flow through cytoplasmic bridges. We have previously (DiCaprio et al., 1974) described the combination of criteria by which we ensure correct staging of the embryos. For the eight-cell embryo these are:

(*a*) Commencement of the fourth cleavage: scanning electron microscopy of the apposed cell surfaces within the embryo (Sanders and Singal, 1973) shows that the membrane partitions of the first three cleavages are complete by the time that the fourth cleavage commences.

(*b*) Membrane potential: the substantial variation in membrane potential between early cleavage stages (approximately 10 mV per stage over 4–16 cells) may be used to confirm the stage (DiCaprio et al., 1974).

(*c*) Coupling ratio: a coupling ratio of unity clearly distinguishes incompletely divided cells from fully divided cells. The coupling ratio is the ratio of voltages, Vb/Va, measured in cells *a* and *b*, when a current is injected into cell *a*. The range of coupling ratios observed was 0.85 ± 0.03.

Electrical Measurements

A detailed description of the linear analysis technique has been given previously (DiCaprio et al., 1974). Band-limited white noise current is injected into one cell of an embryo via a $10^7\ \Omega$ resistance while the resultant fluctuations in the membrane potentials of the injected cell and a putatively coupled cell are recorded. The frequency response and coherence functions of the voltage ratio between the cells is then estimated by use of the fast Fourier

transform. In the present work all computations were performed with a PDP-11/40 computer using software similar to that described previously (French, 1973).

The frequency response function may be most easily understood in terms of sinusoidal voltage fluctuations. If an electrical network is linear and a sinusoidal voltage modulation is applied at one node of the network, then all of the other nodes will demonstrate a sinusoidal modulation of the same frequency. However, the amplitude and phase of each sinusoid relative to the input sinusoid will vary with the frequency of the input and the frequency response function measures this relationship. Frequency response functions are normally displayed in the form of a Bode plot, which is a plot of the relative phase shift and the logarithm of the relative amplitude (gain) against the logarithm of the frequency (D'Azzo and Houpis, 1966). The low frequency asymptotes of a Bode plot may be used to examine the purely resistive properties of the system since capacitances and inductances have infinite and zero impedance respectively under steady current conditions. The remainder of the Bode plot is determined by all of the components in the system and may be most easily understood in terms of time constants. The simplest example of a single time constant circuit is one having a single resistance and a single capacitance in series or parallel. A single time constant system will have a high frequency gain asymptote which changes by 6 dB for each doubling in frequency or octave. The phase curve will have an asymptote of $\pm 90°$ depending on the type of system. Systems having more than one time constant have more complicated Bode plots but if the time constants are coupled in a simple way then integer multiples of the 6 dB per octave and $\pm 90°$ asymptotes are found.

The coherence function is a normalized measure of the extent to which the linear frequency response function characterizes the electrical behavior of the system (Bendat and Piersol, 1966). Coherence may vary over the range 0–1, and a linear, noise-free system will have a coherence function of unity at all frequencies. Deviation below unity indicates that a signal is being observed which is not linearly correlated with the input. This may be due to nonlinearities in the system, causing frequency transpositions, or due to noise being added to the signal inside the system.

Circuit Modeling

The electrical behavior of electronic analogs of the embryonic system was predicted by use of a circuit simulation program adapted to the PDP-11/40 computer from a similar program written in BASIC (Digital Equipment Corporation Users Society no. 11–12; for a review of computer-aided circuit analysis see McCalla and Pederson, 1971). This program was able to calculate the expected frequency response functions between any two cells of a given eight-cell network.

Microscopy

Embryos intended for sectioning and examination by light microscopy were fixed overnight in the mixed aldehyde solution of Kalt and Tandler (1971) which contains 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, and 2.5% dimethyl sulphoxide. The material was dehydrated in ethanol, cleared in benzene, embedded in paraffin wax, and serially sectioned at 5 μm thickness. Sections were stained with 35% alcoholic eosin solution and micrographs were made using a Leitz Orthoplan microscope (E. Leitz, Inc., Rockleigh, N.J.) and Panatomic-X film (Eastman Kodak Co., Rochester, N. Y.).

Several techniques were used in preparing embryos for scanning electron microscopy (SEM), such that they could be manually split apart along the plane of cleavage furrows. The only procedure we have found which allowed embryos to be broken in this way was freeze drying (Sanders and Singal, 1973). The appearance of blastomeres prepared in this way was closely similar to the appearance of the living cells by light microscopy and of the corresponding regions of sectioned cells by transmission electron microscopy (Singal and Sanders, 1974). The close correlation of cell appearance after several preparative procedures has been used as a

criterion to judge the quality of preservation of tissue for SEM (Porter et al., 1973). At the low magnifications used in the present work, the freeze drying technique is considered satisfactory. Successfully split embryos were coated with a carbon and gold conducting layer and examined using a Cambridge S4 scanning electron microscope (Kent Cambridge Ltd., Willowdale, Ontario).

RESULTS

Electrical Models

In order to systematize our analysis we have defined a numbering scheme for the eight-cell embryo. Fig. 1 shows the appearance of an eight-cell embryo diagrammatically, viewed from directly above the animal pole. The four animal pole cells are smaller than the four vegetal pole cells and this size disparity has been exaggerated for pictorial clarity. The cells may be unambiguously numbered by reference to the grey crescent and we have defined the two animal pole cells which are partially covered by the grey crescent to be numbers 1 and 2, in anticlockwise rotation about the vertical axis. Numbering of the other six cells proceeds regularly. As indicated in Fig. 1, two of the diagonally opposed animal pole cells (2 and 4) are visibly apposed to each other, resulting in a separation of cells 1 and 3. This condition appears to be a continuation of the tetrahedral distortion described in the four-cell embryo (DiCaprio et al., 1974), since close apposition of the alternate pair of vegetal pole cells (5 and 7) also occurs. Although Fig. 1 shows external diagonal apposition between cells 2 and 4, we have deter-

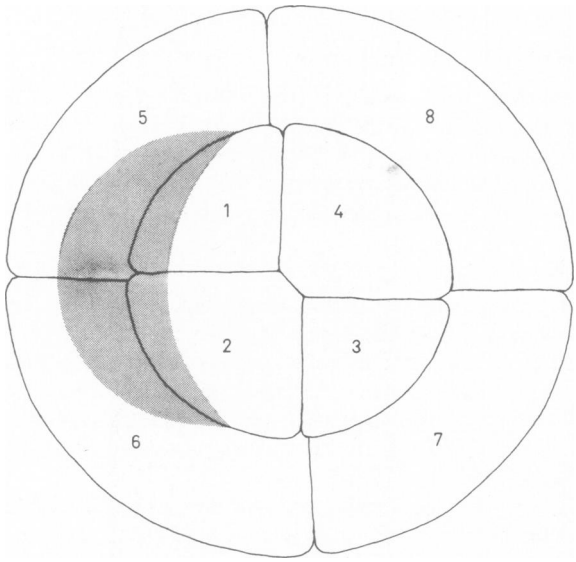


FIGURE 1 Diagrammatic view of an eight-cell embryo, viewed from the animal pole. The difference in size between the four animal pole cells (1–4) and the four vegetal pole cells (5–8) has been exaggerated. The numbering scheme is based on the grey crescent (stippled area) covering portions of the surfaces of cells 1 and 2.

mined by examining a large number of embryos that there is no preference for this contact over contact between cells 1 and 3. Consequently, contact between cells 5 and 7 randomly alternates with contact between cells 6 and 8. The external diagonal cell apposition thus appears to be unrelated to the gray crescent and is therefore unrelated to our numbering scheme.

Our approach to the problem of determining the connectivity was to propose a range of differently connected models of the embryo and to predict the expected electrical behavior of each model. Experimental measurements were then made in attempts to eliminate each model if possible. Since we were not attempting to determine the absolute membrane properties, it was necessary to assume values for the membrane resistances (R_m), membrane capacitances (C_m), and junctional resistances (R_j). These assumed values were based upon our previous determinations of the specific membrane resistances and capacitances and the junctional resistances in the two- and four-cell embryos (DiCaprio et al., 1974). The animal pole cells were all assumed to have identical surface areas. The vegetal pole cells were all assumed to have identical surface areas, larger than those of the animal pole cells. The surface areas of the cells were estimated by measuring their dimensions from scanning electron micrographs of the embryos. The actual values used were: R_m (animal pole) = 2.3 MΩ, R_m (vegetal pole) = 1.1 MΩ, C_m (animal pole) = 5×10^{-9} F, C_m (vegetal pole) = 10^{-8} F, R_j = 250 KΩ.

Seven models of possible connectivity were considered and will be referred to as models A through G. Fig. 2 shows a schematic diagram of models A and G. The cells are numbered according to the scheme of Fig. 1 and the nonjunctional components are omitted for clarity. Solid lines indicate the presence of putative junctional resistances. Model A is the simplest model that might be expected from the embryonic

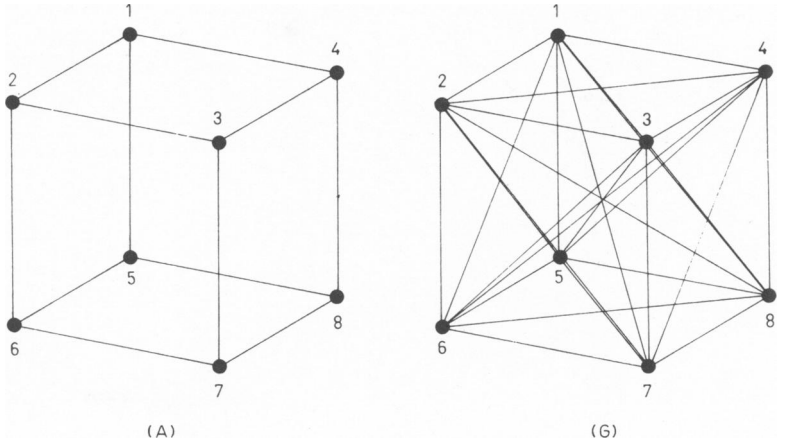


FIGURE 2 Two of the seven models (A and G) which were proposed as possibly describing the electrical properties of the eight-cell embryo. Solid lines represent junctional resistances and the nonjunctional components are omitted for clarity. Details of the connectivity of all the seven models are given in Table I.

geometry and has junctional resistances only between pairs of cells which are directly apposed along the planes of the cleavage furrows. Model G represents the other extreme of connectivity where each cell is coupled directly to every one of the other cells by an identical junctional resistance. In model G a total of 28 junctional resistances are required to pair the eight cells in all possible combinations, while in model A only 12 junctional resistances are required. As well as these two extremes five intermediate models of connectivity were considered as possible alternatives. The detailed connections involved in all of the models A through G are listed in Table I.

To simplify the analysis we injected the white noise stimulating signal into cell 1 for all measurements. Recording electrodes were then placed in any pair of cells and the frequency response and coherence functions of the voltage ratio between that pair of

TABLE I

THE CONNECTIVITY OF THE SEVEN PROPOSED MODELS FOR THE EIGHT-CELL EMBRYO

The 28 possible cell pair connections are listed vertically at the left, using the numbering scheme of Fig. 1. Asterisks indicate the presence of a particular connection in each model.

Cell pair	Models						
	A	B	C	D	E	F	G
1-2	*	*	*	*	*	*	*
1-3		*		*		*	*
1-4	*	*	*	*	*	*	*
1-5	*	*	*	*	*	*	*
1-6						*	*
1-7					*		*
1-8						*	*
2-3	*	*	*	*	*	*	*
2-4		*		*		*	*
2-5						*	*
2-6	*	*	*	*	*	*	*
2-7						*	*
2-8					*		*
3-4	*	*	*	*	*	*	*
3-5					*		*
3-6						*	*
3-7	*	*	*	*	*	*	*
3-8						*	*
4-5						*	*
4-6					*		*
4-7						*	*
4-8	*	*	*	*	*	*	*
5-6	*	*	*	*	*	*	*
5-7			*	*		*	*
5-8	*	*	*	*	*	*	*
6-7	*	*	*	*	*	*	*
6-8			*	*		*	*
7-8	*	*	*	*	*	*	*

cells were determined. If current was injected into cell 1 then no distinction could be made between the signals observed in cells 2 and 4 or between the signals observed in cells 6 and 8. The embryo is therefore electrically symmetric about the two vertical planes. The possible pairs of cells between which distinct signal transfer characteristics could be measured were thus: 1 to 2, 1 to 3, 1 to 5, 1 to 6, 1 to 7, 5 to 6 and 5 to 7. The predicted frequency response functions between each of these seven pairs, with stimulation through cell 1, were computed for each of the seven models. Experimental measurements of the frequency response functions between each of the seven pairs of cells were made and compared with the predicted results.

Preliminary examination of the predicted results indicated that accurate distinction between the models would depend upon the frequency range over which the electrical behavior could be measured. Since the predicted curves diverge with increasing frequency, it was necessary to make experimental measurements at the highest possible frequencies. This was experimentally difficult for two interacting reasons: since each cell membrane has capacitance, the injected current passed more easily through the nonjunctional cell membrane than the junctional membrane with increasing frequency. Thus it was necessary to increase the amplitude of the higher frequency components of the white noise to maintain the measurement accuracy. At the same time, the recording microelectrodes and holders, being very close to each other and having high impedances, displayed capacitative coupling. Thus high frequency current was trans-

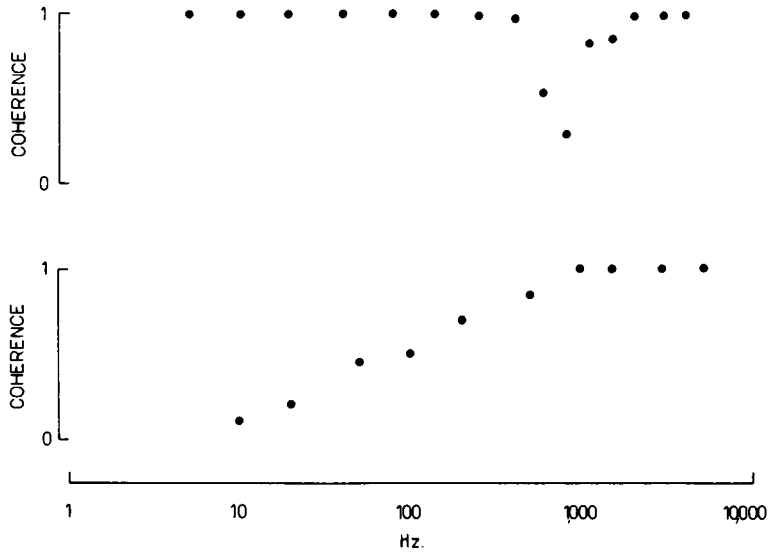


FIGURE 3 The coherence function measured between two recording electrodes while a noise signal was injected into cell 1 of an eight-cell embryo. In the upper trace the two recording electrodes were located intracellularly in two coupled cells. Similar coherence function curves were observed whichever pair of cells was impaled. In the lower trace the two electrodes have both been raised through the cell membranes so that they are resting with their tips in the bathing solution just outside of the embryo.

mitted between the electrodes without passing through the embryo. The result of this situation is illustrated in Fig. 3, which shows the coherence function between the two recording electrodes when they were inserted into embryonic cells and also when they were placed just outside the embryo. The intracellular measurement shows three distinct regions. From DC to about 500 Hz the coherence function is close to unity and is significantly reduced by removing either electrode from the embryo to the bathing solution (lower trace). A second region from about 500 to 1,000 Hz shows low coherence. A third region from 1,000 Hz upwards again shows high coherence but can also be obtained with the electrodes removed to the surrounding fluid (lower trace). We interpret results in the lowest frequency region as being an accurate measure of embryonic electrical behaviour, the second region as being due to the majority of injected power passing through the first cell membrane capacitance and the third region as being due to capacitative coupling between the electrodes and their holders. The experimental results which are presented here were all obtained from the first region.

Fig. 4 shows the frequency response functions measured between each of the seven

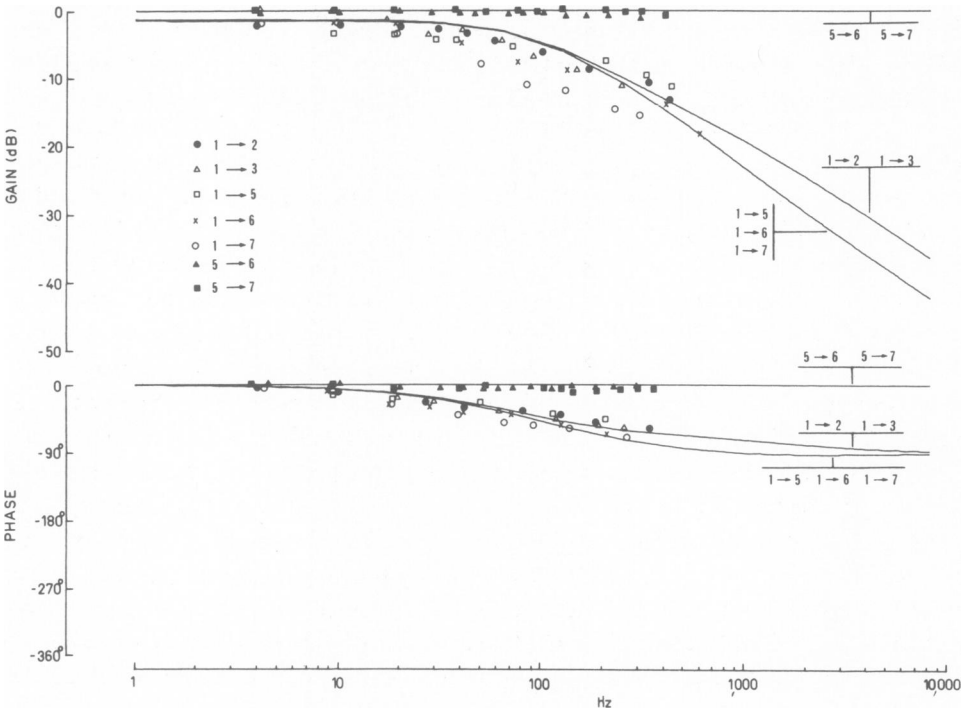


FIGURE 4 Experimentally determined frequency response functions between the seven pairs of cells. The cells are numbered according to the scheme of Fig. 1, and symbols are used to identify the individual pairs. Solid lines are theoretical predictions based upon the network of model G (Fig. 2). Each set of data was obtained from a single experiment. However, sufficient experiments were performed to ensure that each frequency response function was reproducible and representative. A total of 57 experiments was performed.

distinct pairs of cells in an eight-cell embryo. The results of one experiment are plotted for each pair. The results fall into two groups. All of the pairs containing cell 1 show a first order characteristic with a high frequency gain asymptote of −6 dB per octave and a high frequency phase asymptote of −90°. The other two pairs (5–6 and 5–7) show a zero order characteristic of 0 dB and 0° for the gain and phase asymptotes, respectively.

Fig. 5 (*a–g*) shows the predicted frequency response curves between each of the seven pairs of cells for each of the seven models. Each graph shows the predicted frequency responses for a particular cell pair. Inspection of the curves of Fig. 5 shows that not all of the results allow discrimination between possible models. Figure 5*a* shows the curves for cells 1–2 and these are sufficiently similar that no distinction between the models is possible. Fig. 5*b* shows the curves for cells 1–3 and in this case there is a distinction between models A and C, with asymptotes of −12 dB per octave and −180°, compared with the other models. Comparison with the experimental results for this pair of cells (1–3) from Fig. 4 indicates that models A and C are less probable than the others.

Similar arguments may be used for the succeeding curves. Fig. 5*c* shows the predictions for cells 1–5 and although there is more separation than in Fig. 5*a* it is not adequate to give a clear distinction between the models. Fig. 5*d* shows the predicted curves for cells 1–6 and here there is a distinction between models A, B, C, D, and E, which show second-order characteristics, and models F and G, which show first-order characteristics. Fig. 5*e* shows the predicted curves for cells 1–7 and here it is models E and G which show first-order characteristics, while B and D have second-order, and A and C, third-order characteristics. At this stage therefore the only model which is consistent with the results is G. Figs. 5*f* and 5*g* show the predicted curves for cells 5–6 and 5–7. Inspection of Fig. 4 shows that these two pairs of cells both displayed zero order curves experimentally. In Fig. 5*f* only models F and G have this characteristic. In Fig. 5*g* only models E and G show this characteristic. The conclusion is thus sustained that only model G consistently predicts the order of the frequency response function for all cell pairs. The predicted curves for all of the pairs from model G are collected together and superimposed as solid lines on the experimental curves of Fig. 4. The distinction into two classes of curves is clearly indicated, although the curves do not perfectly overlay the experimental results. However, it must be remembered that we have used estimated values for the cell membrane properties. If the model is correct in connectivity but not in the actual component values then we would expect to see such differences. These differences should not affect the asymptotes of the frequency response curves but only have the effect of shifting the curves along the frequency or gain axes.

Microscopy

In order to determine whether there was a morphological basis for the extensive intercellular coupling demanded by model G, embryos were examined by electron microscopy and light microscopy of serial sections. Scanning electron microscope examina-

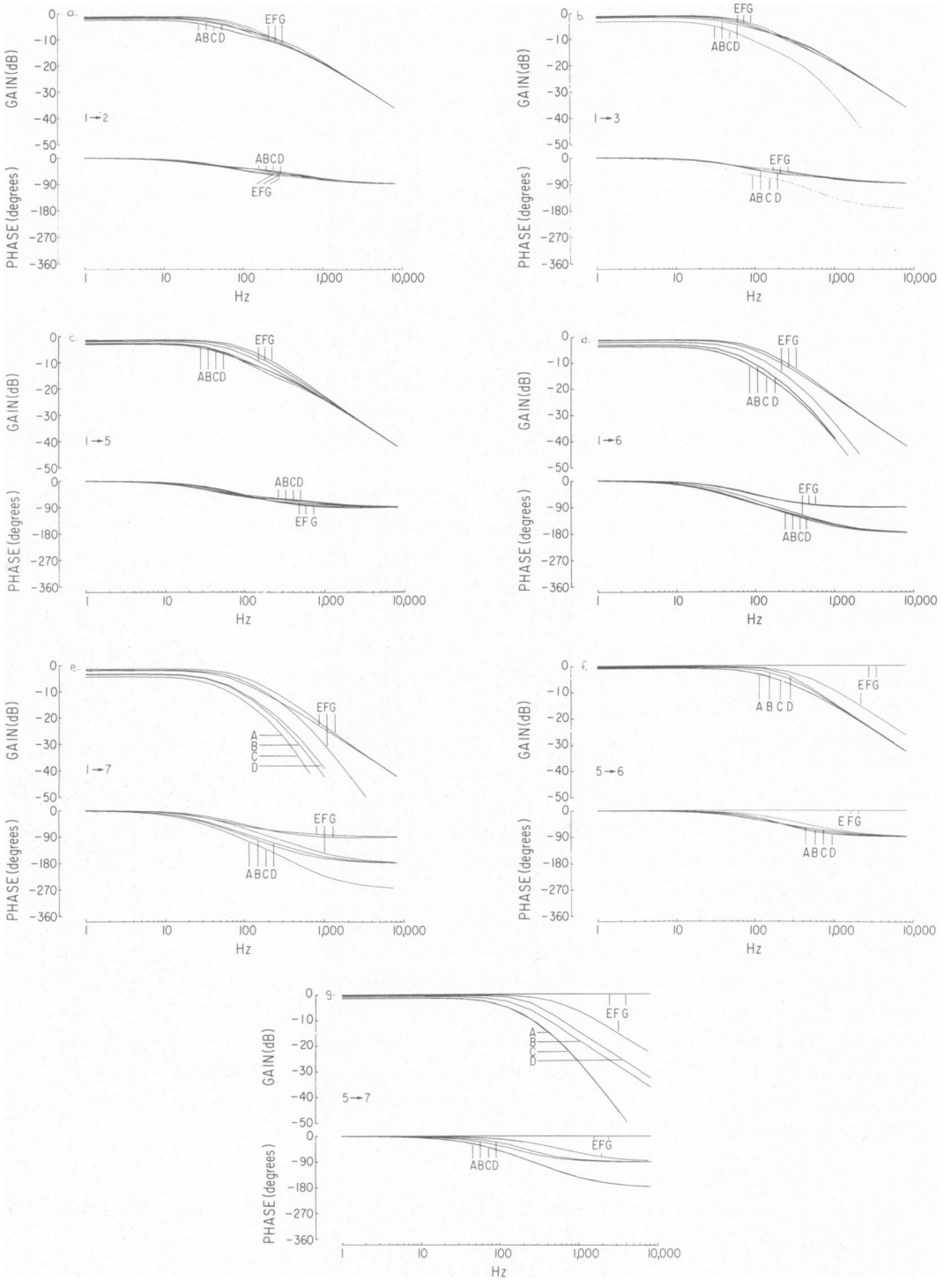
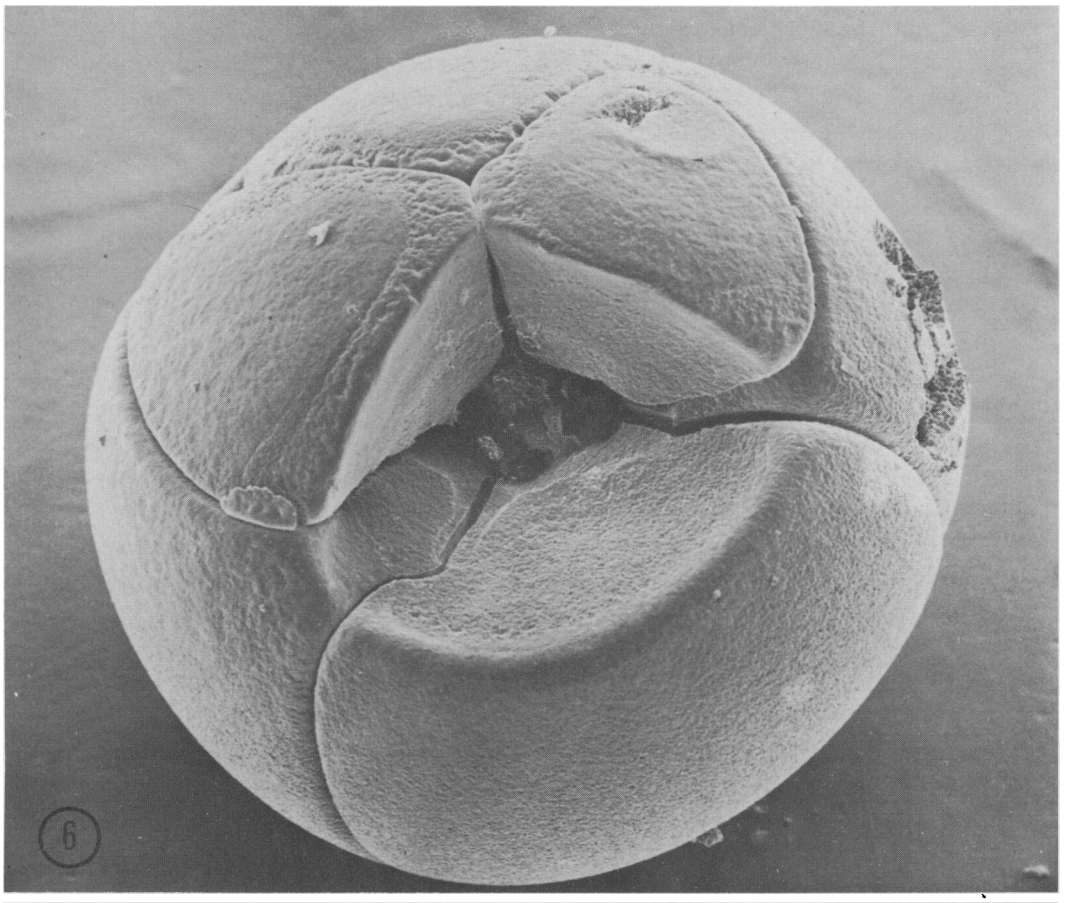


FIGURE 5 *a–g* Theoretical predictions of the frequency response functions of each of the seven pairs of cells with noise injected into cell 1. Each plot shows seven predicted results corresponding to the seven models A–G.



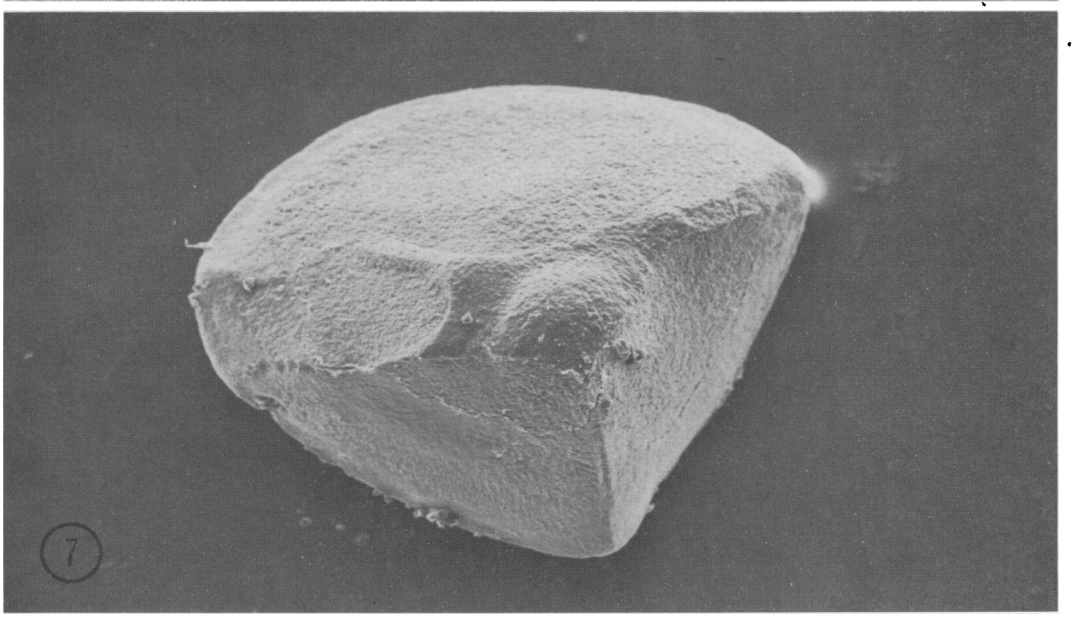


FIGURE 6 Scanning electron micrograph of an eight-cell embryo from which one cell has been removed. Five of the remaining cells possess a surface which was in direct apposition to the removed cell. ×140

FIGURE 7 Scanning electron micrograph of the cell which was removed from the embryo in Fig. 6. This cell shows faces which correspond to those of the remaining cells of the embryo. ×150

tion of an eight-cell embryo, from which one animal pole cell was removed (Fig. 6), revealed a number of direct contact faces as well as several cytoplasmic processes protruding from the area of the blastocoel cavity. If the missing cell is arbitrarily assigned the number 1, then contact faces are directly visible between the following pairs of cells: 1 to 2, 1 to 4, 1 to 5, 1 to 6, and 1 to 8. These faces are identifiable in Fig. 6 as flattened surfaces and also in Fig. 7, which shows the removed cell. The latter has faces which correspond precisely with those observed in Fig. 6. The faces seen in these figures all possess intact membrane surfaces as judged by the absence of yolk platelets when the specimen was observed at high magnification. In cases where the plasma membrane was damaged these organelles were exposed.

Fig. 8 is an enlargement of the blastocoel region of Fig. 6 and shows a number of cytoplasmic processes issuing from the surfaces lining the blastocoel. It is not possible to decide from which cells these processes originate but some appear to be in a position to make contact with the removed cell 1. It is probable that further processes have been broken during the removal of cell 1 since severed stumps are visible in Fig. 8. The finest processes observed by scanning electron microscopy were approximately 10–15 μm in diameter. Another example of the widespread occurrence of cytoplasmic processes is shown in Fig. 9, which shows cells from the vegetal pole. Here a process is insinuating itself between two other vegetal pole cells, one of which shows blunt protrusions on its surface.

Examination of serial light microscope sections (Fig. 10) provided further evidence for extensive intercellular contact. Figs. 10 B through 10 K are serial sections of an eight-cell embryo illustrating the manner in which such processes are extended and make contact with more remote cells. The finest processes observed in these sections were approximately 1 μm in diameter. By examining series of sections, many of the remote contacts required by model G have been observed.

DISCUSSION

The two extremes of connectivity illustrated in Fig. 2 have 12 and 28 intercellular connections, respectively. The number of possible models of connectivity which could be proposed between these two extremes may be obtained by adding the 16 intermediate connections, one at a time, and allowing all possible arrangements at each stage. Since this number is greater than 65,000 the consideration of all possible models is not feasible. The five intermediate models which we considered were not intended to be exclusive, but rather to represent a range of connectivities having the principal geometric groups of connections. Although all the possible models were not considered, it is important to note that the experimentally measured frequency response functions

FIGURE 8 An enlargement of the blastocoel cavity of Fig. 6. A number of processes can be observed arising from the cell surfaces lining the cavity (arrows). ×280

FIGURE 9 Scanning electron micrograph of vegetal pole cells of an eight-cell embryo showing a process from one cell (arrow) making contact with two other cells by pushing between them. The upper cell shows blunt surface projections. ×270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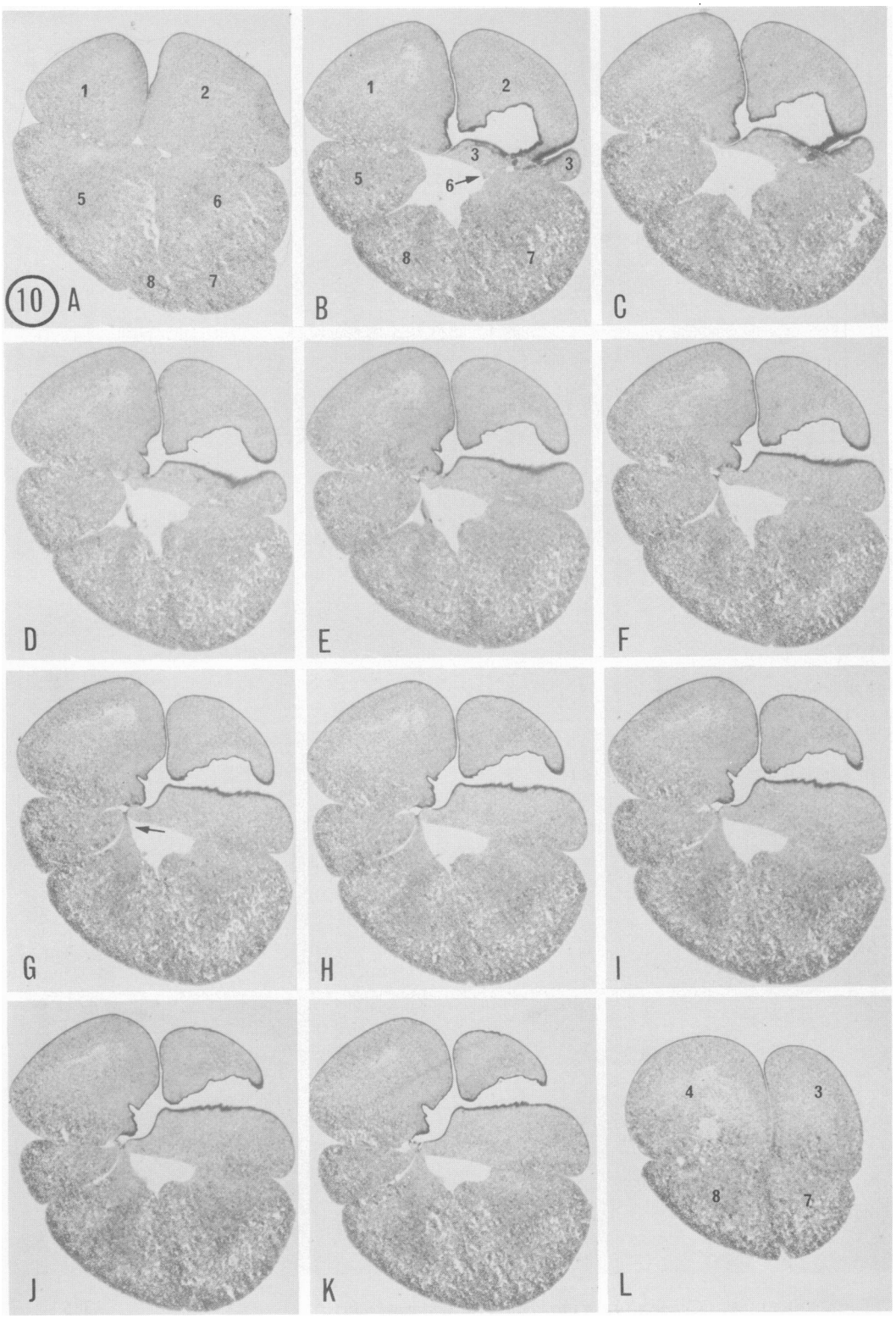
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never demonstrated second, or higher, order characteristics. If any of the intercellular connections in model G are removed, then the frequency response function between the two cells will become second order because current between them must flow through an intermediate cell. The experimental frequency response curves thus support model G and simultaneously invalidate any model of lower connectivity.

We have shown earlier (DiCaprio et al., 1974) that at the four-cell stage of development all of the cells are coupled directly to one another, and the present data indicates that this situation persists at the eight-cell stage. Whether this stage of complete connectivity is continued into still later stages is uncertain, but above a certain number of cells the situation would become morphologically infeasible due to the number of cytoplasmic processes required.

In order to unequivocally correlate the electrophysiological and morphological data it would be necessary to search the entire embryo by transmission electron microscopy for the presence of gap junctions between each cell pair, since there is increasing evidence that junctions of this type mediate electrotonic coupling (see for example Gilula et al., 1972). Although such a search has not been performed exhaustively there is evidence that contacts of the gap junction type are present at cleavage and early blastula stages (Sanders and Zalik, 1972; Singal and Sanders, 1974) and that these are the only type of intimate junction present. Distinct terminal complexes and tight junctions are not present during cleavage.

Little attention has been paid to the detailed morphological relationships of amphibian blastomeres during cleavage. Holtfreter (1947) made extensive studies on the kinetic behaviour of cells from gastrulae and later stages, describing the extension of fine processes and the considerable pseudopodal activity. He concluded that up until the neurula stage many cells were highly amoeboid and displayed rhythmical expansions and contractions. The extension and activity of similar processes has also been examined during the early morphogenesis of the sea urchin, *Psammechinus* (Gustafson, 1963) and *Fundulus* (Trinkaus, 1973). In these studies thin filopodia were observed in gastrula stages and were preceded by the appearance of blunter pseudopodia or blebs. In the present work we have found the finer processes during cleavage stages together with more blunt protrusions. It has been proposed that in the *Psammechinus* and *Fundulus* embryos the filopodia serve the purpose of attachment required for cellular locomotion. In the eight-cell *Xenopus* embryo no cellular locomotion occurs, and we therefore propose that the fine processes are extended to make remote contacts which mediate electrical coupling and may be involved in the intercellular exchange of molecules.

FIGURE 10 A–L Light micrographs of sections through an eight-cell embryo, cut in a vertical plane. Figures 10 A and 10 L are sections from near the extremities while 10 B through 10 K are serial sections from the center of the embryo. The cells are numbered according to the scheme of Fig. 1. The series illustrates the way in which cells make contact with more remote cells by means of processes. Cell 8 can be seen to have extended a process (arrow in Fig. 10 G) which has insinuated itself between processes from cells 3 and 5. ×37

The relationship of cell contacts and embryonic induction has been the center of considerable controversy (see for example Grobstein, 1961). While certain inductive interactions do not seem to require intercellular contact this may not be true for all such interactions, some of which may demand the presence of direct physical contact (Nordling et al., 1971; Tarin et al., 1973). Although the induction phenomenon has not been demonstrated in amphibian embryogenesis earlier than gastrulation, it is possible that inductive stimuli are transmitted earlier than this. The existence of extensive intercellular contact would facilitate such transmission.

It has recently been suggested (DeLaat and Bluemink, 1974) that electrical coupling in early *Xenopus* embryos may be mediated by electric current transmission through new membrane of low resistivity formed in the cleavage furrow. Such coupling would require the cavity of the furrow to be electrically isolated from the external medium, to avoid the leakage of current to ground. Slack and Warner (1973) also suggested that the early blastocoel is isolated from the bathing solution. Our ultrastructural observations (Singal and Sanders, 1974) demonstrate that sealing or tight junctions are entirely absent, and preliminary results from this laboratory indicate that the blastocoel is accessible to dyes injected into the perivitelline space and is therefore not sealed from it. This observation together with the presence of contacts of the gap junction type suggests that transmission via junctions is at least partly responsible for the intercellular spread of electric current between these cells.

The present results show complete connectivity between all of the eight cells in the embryo, and also the existence of fine cellular processes which have not been described before. As we have pointed out previously these cells could be closely electrically coupled using a lower order of connectivity by current flowing through intermediate cells (DiCaprio et al., 1974). The high order of connectivity therefore supports the view that intercellular junctions are significant in the normal development of the embryo. It seems reasonable to suppose that the fine processes mediate individual electrical connections between cells which are not directly apposed. However, the primary function of these connections may not involve electrical current flow but could allow the movement of morphogenetically important molecules directly between cells.

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REFERENCES

AZARNIA, R., W. J. LARSEN, and W. R. LOEWENSTEIN. 1974. The membrane junctions in communicating and noncommunicating cells, their hybrids and segregants. *Proc. Natl. Acad. Sci. U.S.A.* **71**:880.

BENDAT, J. S., and A. G. PIERSOL. 1966. Measurement and Analysis of Random Data. John Wiley & Sons, New York.

BENNETT, M. V. L., M. E. SPIRA, and G. D. PAPPAS. 1972. Properties of electrotonic junctions between embryonic cells of *Fundulus*. *Dev. Biol.* **29**:419.

BENNETT, M. V. L., and J. P. TRINKAUS. 1970. Electrical coupling between embryonic cells by way of extracellular space and specialized junctions. *J. Cell Biol.* **44**:592.

D'AZZO, J. J., and C. H. HOUPIS. 1966. Feedback Control System Analysis and Synthesis. McGraw-Hill, Inc., New York.

DAWID, I. B. 1965. Deoxyribonucleic acid in amphibian eggs. *J. Mol. Biol.* **12**:581.

DELAAT, S. W., and J. G. BLUEMINK. 1974. New membrane formation during cytokinesis in normal and cytochalasin B treated eggs of *Xenopus laevis*. II. Electrophysiological observations. *J. Cell Biol.* **60**:529.

DICAPRIO, R. A., A. S. FRENCH, and E. J. SANDERS. 1974. Dynamic properties of electrotonic coupling between cells of early *Xenopus* embryos. *Biophys. J.* **14**:387.

FRENCH, A. S. 1973. Automated spectral analysis of neurophysiological data using intermediate magnetic tape storage. *Comput. Programs Biomed.* **3**:45.

FURSHPAN, E. J., and D. D. POTTER. 1968. Low resistance junctions between cells in embryos and tissue culture. *Curr. Top. Dev. Biol.* **3**:95.

GILULA, N. B., O. R. REEVES, and A. STEINBACH. 1972. Metabolic coupling, ionic coupling and cell contacts. *Nature (Lond.).* **235**:262.

GROBSTEIN, C. 1961. Cell contact in relation to embryonic induction. *Exp. Cell Res. Suppl.* **8**:234.

GUSTAFSON, T. 1963. Cellular mechanisms in the morphogenesis of the sea urchin embryo. *Exp. Cell Res.* **32**:570.

HOLTFRETER, J. 1947. Observations on the migration, aggregation and phagocytosis of embryonic cells. *J. Morphol.* **80**:57.

ITO, S., and N. HORI. 1966. Electrical characteristic of *Triturus* egg cells during cleavage. *J. Gen. Physiol.* **49**:1019.

ITO, S., and W. R. LOEWENSTEIN. 1969. Ionic communication between early embryonic cells. *Dev. Biol.* **19**:228.

KALT, M. R., and B. TANDLER. 1971. A study of fixation of early amphibian embryos for electron microscopy. *J. Ultrastruct. Res.* **36**:633.

LOEWENSTEIN, W. R. 1966. Permeability of membrane junctions. *Ann. N.Y. Acad. Sci.* **137**:441.

LOEWENSTEIN, W. R. 1967. On the genesis of cellular communication. *Dev. Biol.* **15**:503.

LOEWENSTEIN, W. R. 1968. Communication through cell junctions. Implications in growth, control and differentiation. *Dev. Biol. Suppl.* **2**:151.

MCCALLA, W. J., and D. O. PEDERSON. 1971. Elements of computer-aided circuit analysis. *IEEE Trans. Circuit Theory.* **CT-18**:14.

NORDLING, S., H. NIETTINEN, J. WARTIOVAARA, and L. SAXEN. 1971. Transmission and spread of embryonic induction. I. Temporal relationships in transfilter induction of kidney tubules *in vitro*. *J. Embryol. Exp. Morphol.* **26**:231.

PALMER, J. F., and C. SLACK. 1970. Some bio-electric parameters of early embryos. *J. Embryol. Exp. Morphol.* **24**:535.

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.

PORTER, K. R., G. J. TODARO, and V. FONTE. 1973. A scanning electron microscope study of surface features of viral and spontaneous transformants of mouse Balb/3T3 cells. *J. Cell Biol.* **59**:633.

SANDERS, E. J., and P. K. SINGAL. 1973. Visualization of the outer and interblastomeric surface of early embryos of *Xenopus laevis* by scanning electron microscopy. *Micron.* **4**:156.

SANDERS, E. J., and S. E. ZALIK. 1972. The blastomere periphery of *Xenopus laevis*, with special reference to intercellular relationships. *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **171**:181.

SHERIDAN, J. D. 1971. Dye movement and low-resistance junctions between reaggregated embryonic cells. *Dev. Biol.* **26**:627.

SINGAL, P. K., and E. J. SANDERS. 1974. An ultrastructural study of the first cleavage of *Xenopus* embryos. *J. Ultrastruct. Res.* **47**:433.

SLACK, C., and J. F. PALMER. 1969. The permeability of intercellular junctions in the early embryo of *Xenopus laevis*, studied with a fluorescent tracer. *Exp. Cell Res.* **55**:416.

SLACK, C., and A. E. WARNER. 1973. Intracellular and intercellular potentials in the early amphibian embryo. *J. Physiol.* **232**:313.

TAKAHASHI, M., and S. ITO. 1968. Electrophysiological studies on membrane formation during cleavage of the amphibian egg. *Zool. Mag. (Tokyo).* **77**:307.

TARIN, D., S. TOIVONEN, and L. SAXEN. 1973. Studies on ectodermal-mesodermal relationships in neural induction. II. Intercellular contacts. *J. Anat.* **115**:147.

TRINKAUS, J. P. 1973. Surface activity and locomotion of *Fundulus* deep cells during blastula and gastrula stages. *Dev. Biol.* **30**:68.