J. Membrane Biol. 13, 61–78 (1973)


# Cell Communication Induced by Lysolecithin

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Received 14 July 1972; revised 23 April 1973

*Summary.* Incubation of the free living soil amoeba *Acanthamoeba castellanii* with lysolecithins results in aggregation of the cells into multicellular masses. The cells in these masses form close junctions and are electrically coupled.

Our data support the hypothesis that specialized membrane regions like gap and/or tight junctions constitute a site of electrical coupling between cells.

The possible role of lysophospholipids within the phenomenon of cell communication is discussed.

The integrative regulation of organs and tissues in higher animals is achieved by way of two channels of communication, the nervous and the circulatory systems. Since cells within a tissue are mutually interconnected, a third communicative system may be present, if information can be transported selectively through special pathways directly from cell to cell (Loewenstein, 1966, 1968; Furshpan & Potter, 1968).

Electrical and electrochemical properties of cells and their membranes can be measured by injecting a current via a microelectrode into one cell and simultaneously recording by means of a second microelectrode the resulting voltage change in the same cell or another one. In this way the phenomenon of electrical cell coupling has been demonstrated in many cell systems.

Specific junctional structures like desmosomes, tight junctions, intermediate junctions (Farquhar & Palade, 1963), focal tight junctions (Trelstad, Hay & Revel, 1967), septate junctions (Wood, 1959; Wiener, Spiro & Loewenstein, 1964; Bullivant & Loewenstein, 1968; Gilula, Branton & Satir, 1970) and gap junctions (Benedetti & Emmelot, 1965; Robertson, 1966; Revel & Karnovsky, 1967), which seem to exist in these cell systems could be important with respect to the phenomenon of electrical cell coupling.

The question of the communicative role of these specific junctional structures and how they are formed was at the basis of our experiments.

We looked for a simple unicellular system to which we could add an inductor to form a multicellular mass, after which we could use the microelectrode technique to examine whether the chosen inductor was able to form communicative contacts. We decided for a number of reasons to study the possibility of electrical coupling in *Acanthamoeba* cells that were induced to adhesion by lysolecithin.

First, Spies, Elbers and Linnemans (1972) observed the induction of cell adhesion by lysolecithins in *Entamoeba invadens* and *Acanthamoeba castellanii*. Electron-microscopic analysis of the adhering cells revealed that the contact areas in *Entamoeba* resemble intermediate junctions. *Acanthamoeba castellanii* cell clumps show two different kinds of contact areas. Some regions greatly resemble the tight junctions found by Farquhar and Palade (1963) in the junctional complex of the distal convolution of rat kidneys and in epithelial cells of a rat thyroid follicle. The same junctions have been found in cultured cell lines of fibroblasts (Flaxman, Revel & Hay, 1969; Gilula, Reeves & Steinbach, 1972), in the embryonic chick heart (Trelstad *et al.*, 1967) and in cell cultures (Sotello & Llinas, 1972). Other regions resemble the gap junctions which are present in the mouse heart and liver (Goodenough & Revel, 1970), cells of *Chironomous* salivary glands (Rose, 1971; Berger & Uhrik, 1972), Novikoff hepatoma cells (Johnson & Sheridan, 1971) and fibroblasts in culture (Gilula *et al.*, 1972; Pinto da Silva & Gilula, 1972). Figs. 1, 2, 3 and 4, illustrating these contacts, are taken from the paper of Spies *et al.*, 1972.

A second important criterion for the choice of the above-mentioned cell system was that the inductor has a physiological character and that it can be synthesized under normal conditions in many cells.

As we assume that cell communication would be effective if the junctional membrane is more permeable to electrolytes and nonelectrolytes than the nonjunctional membrane, the inductor has to be able to influence the membrane permeability. Cell membranes contain proteins and lipids. Both have an amphiphilic character which makes an efficient coupling between the protein and the lipid possible. Knowledge concerning the significance of proteins in relation to the membrane permeability is poor compared with that of the lipids. Many experiments on cells and model systems – liposomes, bilayer, monolayer – have indicated that the permeability of the cell membrane is determined to a great extent by the kind of lipids present (van Deenen, 1971). These experiments also yielded information about the influence of many other substances on the permeability of the membrane.

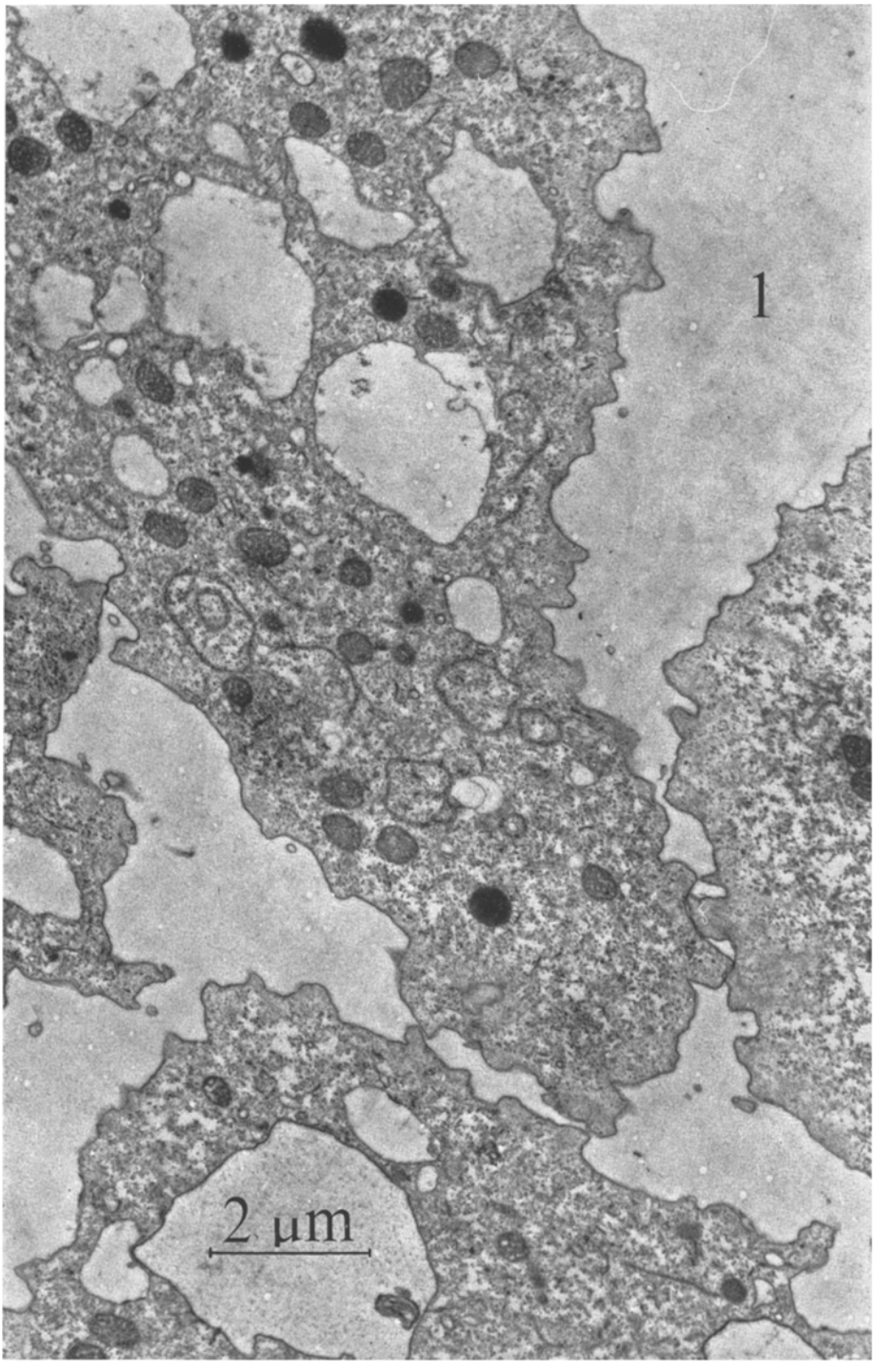


Fig. 1. *A. castellanii* treated with egg lysolecithin. Four cells contacting each other can be seen. 11,000 ×

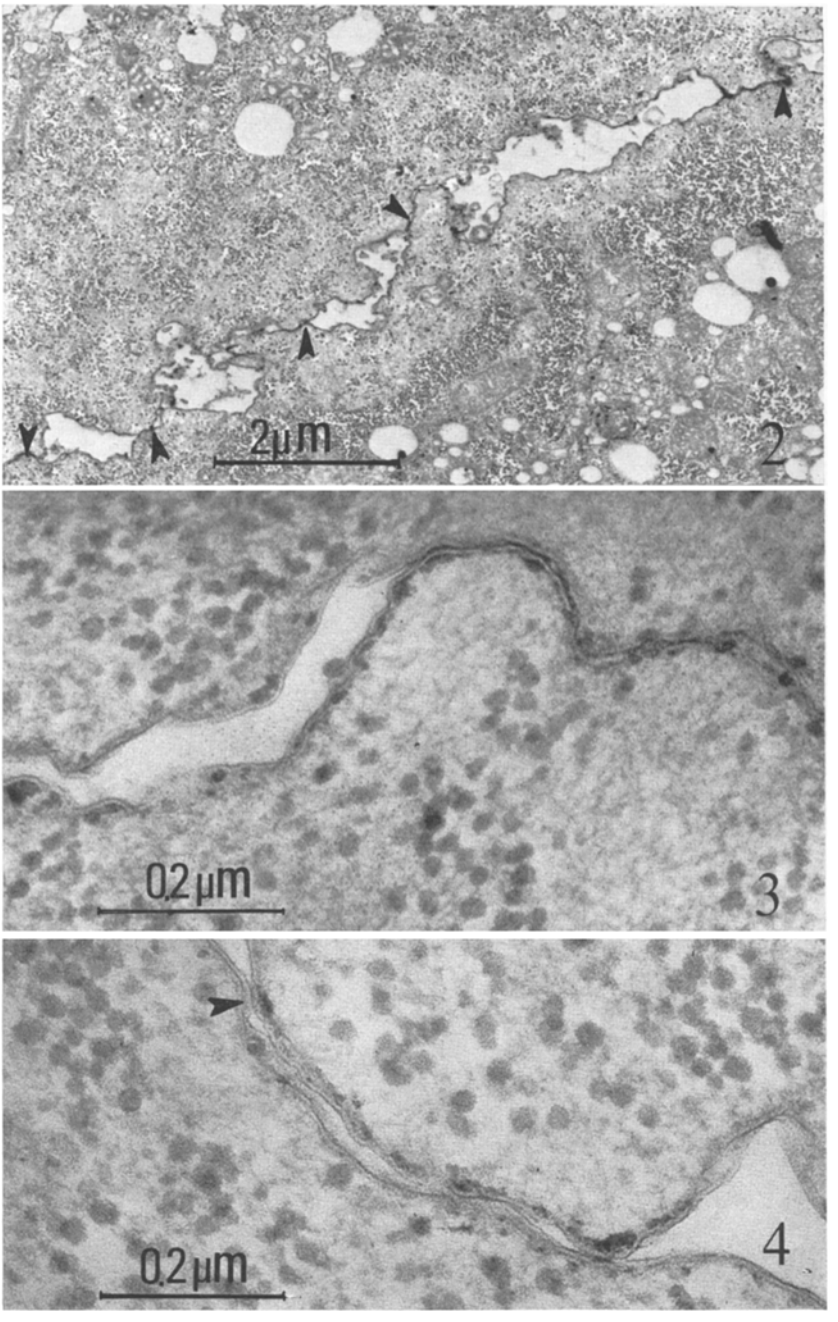


Fig. 2. *A. castellanii* treated with egg lysolecithin. Five junctionlike areas of contact are visible (arrows). 14,000 ×

Fig. 3. *A. castellanii* treated with egg lysolecithin. Two membranes are forming a contact area with the character of a gap junction. 145,000 ×

Fig. 4. *A. castellanii* treated with egg lysolecithin. The fusion line of the "focal tight junction" has the same thickness as one of the outer leaflets of the cell membrane (arrow). 145,000 ×

Thus, it appears that lysolecithin can drastically decrease the resistance of a bimolecular lipid layer (van Zutphen, 1970) and that it is able to increase the permeability of liposomes and erythrocytes (Reman, 1971). To explain this increase in permeability, Reman (1971) proposes a thinning of the bilayer, whereas the formation of micellar units is proposed by several other authors (Haydon & Taylor, 1963; Bangham & Horne, 1964; Gent, Gregson, Gammack & Raper, 1964; Lucy, 1964, 1968; Branton, 1966; Saunders, 1966; Chapman, 1968).

Spies *et al.* (1972) used egg lysolecithin, stearoyllysolecithin and dilauroyllecithin as the inducing agents. In this study palmitoyllysolecithin, 1-palmitoyl-*sn*-glycero-3-phosphorylcholine (hereafter referred to as 16:0-lyso-PC), was the inductor. It was assessed by light microscopy that 16:0-lyso-PC caused exactly the same type of adhesion as the other lysolecithins. This is not surprising because of the close similarity of the stearoyl- and palmitoyllysolecithin molecules, which only slightly differ in the length of their paraffine chains.

## Materials and Methods

### *Cell Cultures*

Amoeboid cells of *A. castellanii* were cultured and experimentally induced to adhesion as described by Spies *et al.* (1972): The organisms were harvested from the growth medium by centrifugation at $600 \times g$ for 1 min and washed 3 times in minimal medium by resuspension and centrifugation. The minimal medium was a 67-mM sodium phosphate buffer of pH 5.5 to which 83 mM D(+)-glucose and 0.5 mM $CaCl_2$ had been added.

A pellet of about $25 \times 10^6$ washed cells was resuspended in a test medium containing 1,500 μg 16:0-lyso-PC dispersed on a vortex-mixer in 1 ml minimal medium. These final suspensions were used for electrophysiological measurements. Control experiments were performed with suspensions that did not contain 16:0-lyso-PC.

Some typical experiments were carried out with 7- to 8-day-old aerated cultures of *A. castellanii*, without adding 16:0-lyso-PC to the washed cells.

### *Experimental Procedure*

Electrophysiological procedures were applied to determine electrotonic spread within a multicellular mass of aggregated *A. castellanii* cells. For the experimental set-up *see* Fig. 5.

The microelectrodes used for the experiments were glass micropipettes filled with 3 M KCl and having a d-c resistance of 20 to 40 MΩ and a tip-potential in the bathing fluid of about 2 mV. By impaling a given cell it is possible to estimate roughly the cell membrane resistance, by measuring the input-resistance, i.e., the seeming increase of the electrode resistance due to the impalement. This measurement was carried out by

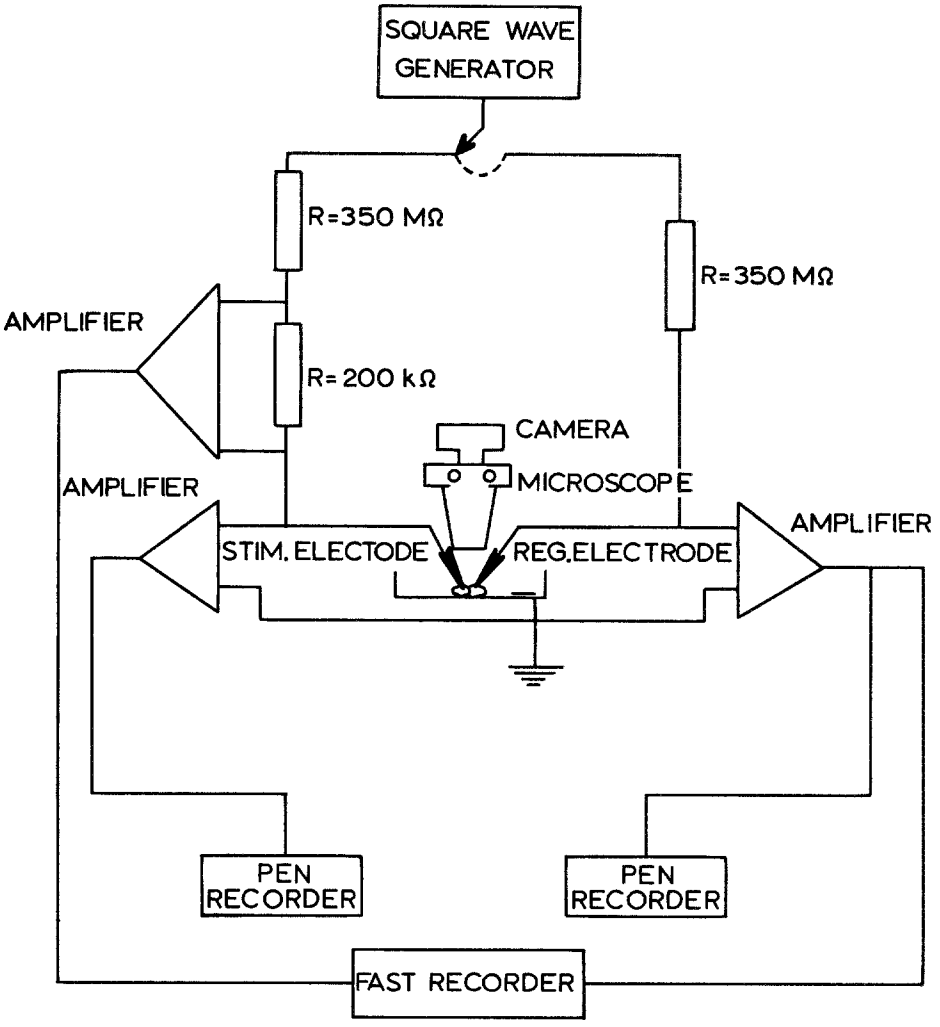


Fig. 5. Simplified diagram of the experimental set-up

supplying a 10-Hz symmetrical square-wave current with an amplitude of $10^{-9}$ amps to the micropipette which results in a voltage drop across the electrode. A 10-Hz symmetrical square-wave with an amplitude of about $10^{-8}$ amps was injected into one cell by way of the stimulating electrode to measure the electrical coupling (for convenience all measuring results were normalized to a stimulation current of $10^{-9}$ amps). The injected current was measured as a voltage drop across a resistor between the square-wave generator and the stimulating electrode and recorded on a fast recorder (bandwidth DC-3 kHz). The second electrode recorded the voltage changes in the same cell or another cell caused by the current injection. These changes were fed into a high input-impedance amplifier. For accurate measurement of the fast voltage changes due to the current injection, the output of this amplifier was also fed into the fast recorder. Two pen-recorders (bandwidth DC-0.5 Hz) recorded the intracellular potential.

## Test of a Model

We confined ourselves to measurements of electrotonic spread in aggregates consisting of only two cells. This enables one to make a simple model (*see* Figs. 6 and 7). Suppose as a hypothesis, that the two amoeba come close together and that no interaction between the adjacent membrane areas or any other structural changes take place. A theoretical value for the electrical cell communication in that case can be computed on the basis of size of contact areas. If this theoretical value is clearly not in agreement with measured values, the hypothesis mentioned has to be rejected in favor of a hypothesis that structural changes do take place.

The amount of communication predicted by the theoretical model depends on the leakage from between the adjacent membranes to the bathing fluid. It will be supposed that no such leakage takes place. Additional presumptions in the theoretical model are: (*a*) the potential in the bathing fluid is supposed to be zero and (*b*) the resistivity of the cytoplasm is neglected. The consequences of all the assumptions made are discussed in the section called Results and Discussion.

In the following, $R_1$ and $R_2$ (MΩ) will denote the membrane resistances of cell 1 and cell 2; $A_1$ and $A_2$ ($cm^2$), the membrane areas. If the surface-contact-area is $\Delta A_1$, then $x_1 = \Delta A_1/A_1$ and $x_2 = \Delta A_1/A_2$ (surface-contact-fractions). Fig. 6 shows also the equivalent electrical circuit in which $R_a = R_1/(1-x_1)$, $R_b = R_1/x_1$, $R_c = R_2/x_2$, and $R_d = R_2/(1-x_2)$. Assuming equal specific membrane resistances for both amoebae, it follows that $A_1/A_2 = R_2/R_1$. Hence, $R_a = R_2/(R_2/R_1 - x_2)$ and $R_b = R_2/x_2$. The input resistances $Z_1$ and $Z_2$ of the system, which are measured with the help of the stimulating electrode and the recording electrode, respectively, are equal to

$$Z_1 = \frac{2R_1 - R_1 x_2}{2-(R_1/R_2+1)x_2} \tag{1}$$

$$Z_2 = \frac{2R_2 - R_1 x_2}{2-(R_1/R_2+1)x_2}. \tag{2}$$

Hence

$$R_2/R_1 = \left(1-\frac{x_2}{2}\right)\frac{Z_2}{Z_1} + \frac{x_2}{2}. \tag{3}$$

Taking into account the equal specific membrane resistances for both amoebae, it follows that

$$x_1 = (R_1/R_2)\cdot x_2. \tag{4}$$

If the stimulation current is $I$ then $V_1$, the induced voltage change in the stimulated cell, is $I \cdot Z_1$. If the voltage change in the adjacent cell is $V_2$,

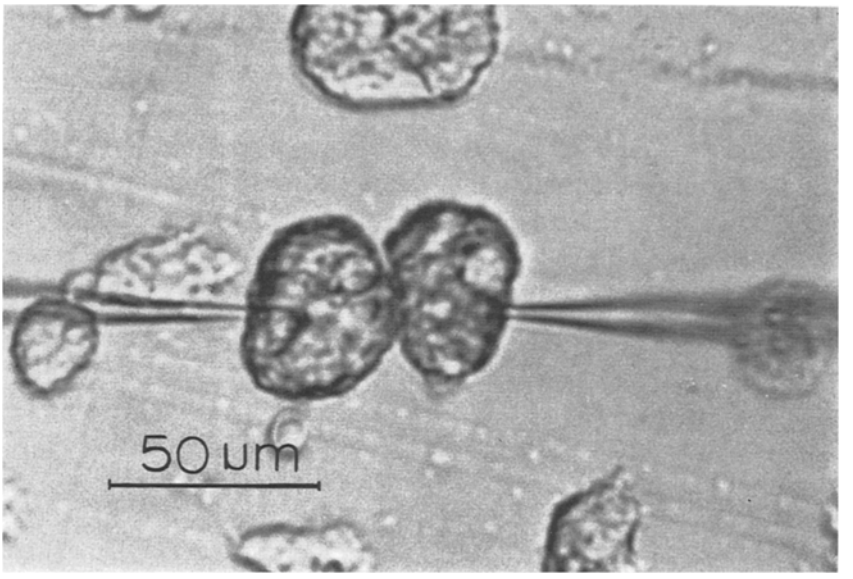


Fig. 6. Photograph of an aggregate of *A. castellanii* cells, consisting of two cells, with two microelectrodes

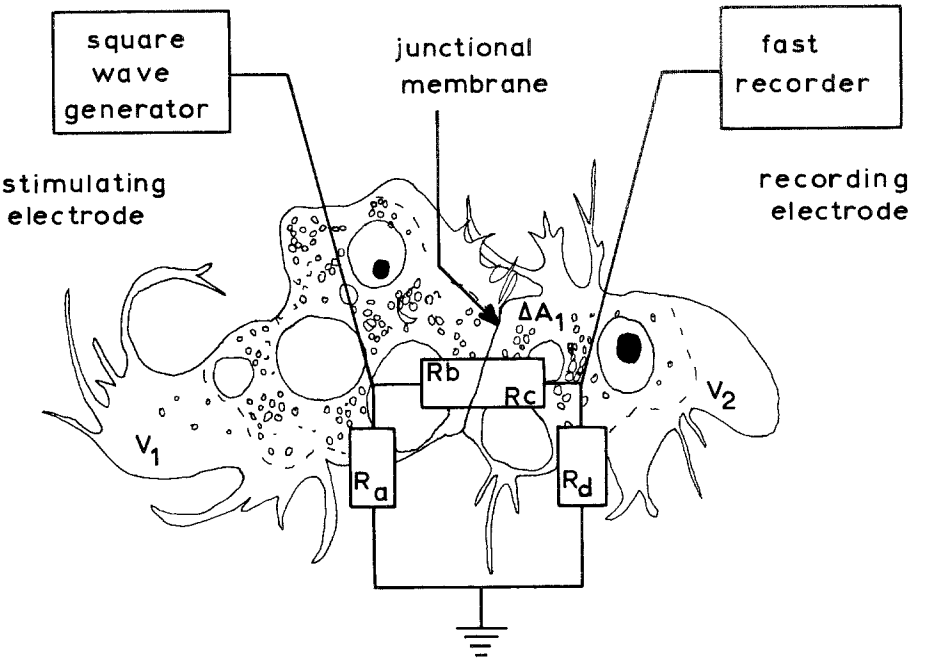


Fig. 7. Drawing from a phasecontrast preparation of an aggregate consisting of two cells. The equivalent electrical circuit is indicated in the figure. Ra and Rd, resistances across the noncontacting cell surface membranes. Rb and Rc, resistance across the surface-contact-area

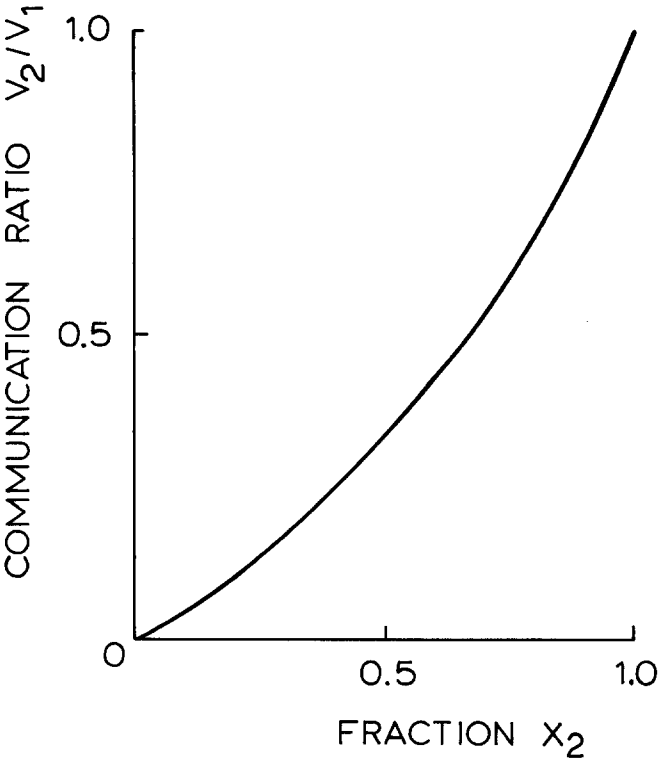


Fig. 8. Theoretical relation between the communication ratio $V_2/V_1$ and the surface-contact-fraction $x_2$ according to Eq. (8)

then the communication ratio

$$V_2/V_1 = V_2/I \cdot Z_1. \tag{5}$$

From the model it follows that $V_2/V_1$ can also be written as $V_2/V_1 = R_d/(R_b + R_c + R_d)$. Since $R_b = R_c$

$$R_c/R_d = \tfrac{1}{2}(V_1/V_2 - 1) \tag{6}$$

$$R_c/R_d = (A_2 - \Delta A_1)/\Delta A_1 = 1/x_2 - 1. \tag{7}$$

Combination of Eqs. (6) and (7) will result in

$$1/x_2 = \tfrac{1}{2} V_1/V_2 + \tfrac{1}{2}. \tag{8}$$

Eq. (5) represents the "communication ratio" as introduced by Loewenstein. If $V_2/V_1 = 1$, then there exists an ideal communication between the coupled cells; if the cells are uncoupled $V_2/V_1 = 0$.

Eq. (8) shows the relation between the surface-contact-fraction $x_2$ on the one hand and $V_2/V_1$ on the other hand. Fig. 8 shows graphically the theoretical relationship between the communication ratio $V_2/V_1$ and the surface-contact-fraction $x_2$ according to Eq. 8.

## Results and Discussion

The values obtained for intracellular potentials and input resistances of differently treated *A. castellanii* cells are shown in Fig. 9 together with mean values and standard deviations.

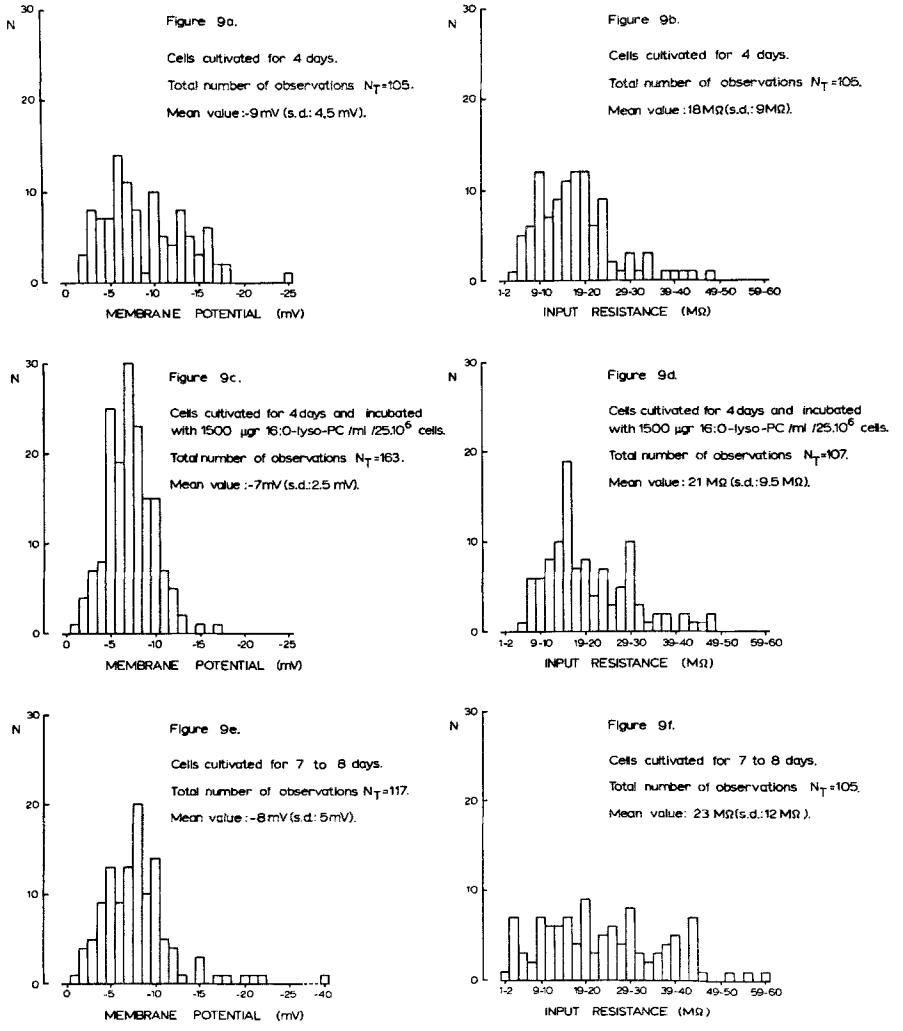


Fig. 9a–f. Histograms showing the distribution of the membrane potentials and input resistances obtained after impalement of treated and untreated *A. castellanii* cells. The abscissae show the stable potential value and input-resistance, respectively. The ordinate gives the total number in each group. The mean values of the negative potentials as well as of the input-resistances (± standard deviation) are indicated in the figures. In the case of *A. castellanii* cells of a 4-day-old aerated culture, incubated with 16:0-lyso-PC, aggregates consisting of two cells were impaled; in control suspensions only single cells were impaled. Single cells as well as aggregates from 7- to 8-day-old cultures were impaled. The aggregates consisted of two cells

Values found for the seeming increase of the electrode resistance when impaling a cell system were used for estimating $Z_1$ and $Z_2$. In a number of cases the stimulating electrode as well as the recording electrode could be inserted into the same cell. The values obtained in this way for membrane resistances were in agreement with the values found for the input resistances.

The spread in the resistances is partly due to the spread in diameter of the individual cells and possibly to cell damage caused by microelectrode impalement (Lassen, Nielsen, Pape & Simonsen, 1971). Also, differences in specific membrane resistance of individual cells would cause differences in input-resistances. Besides, in aggregated cells the input resistances $Z_1$ and $Z_2$ of the system will depend on the amount of communication between the cells.

By measuring $I$, $Z_1$ and $V_2$ theoretical values of the surface-contact-fraction $x_2$ can be computed with the help of Eq. (5) and Eq. (8). Use of Eq. (3) together with values of $Z_2$ yield a value for $R_2/R_1$. Then $x_1$, the surface-contact-fraction of the stimulated cell, can be computed with the help of Eq. (4).

The theoretical values for the surface-contact-fractions $x_1$ and $x_2$ are shown in Table 1 and Table 2, together with the communication ratios $V_2/V_1$.

From the morphological studies by Spies *et al.* (1972) it can be deduced that the surface-contact-fractions $x_1$ and $x_2$ are far below 0.01, probably of the order of magnitude of 0.001. Any voltage change $V_2$ in the recording cell which, according to the model, is related to values of $x_1 \geqslant 0.01$ and/or $x_2 \geqslant 0.01$ proves that the chosen model is to be rejected. So our model is clearly wrong since the computed values of $x_1$ and $x_2$ (Table 1) are indeed incompatible with the surface-contact-fractions observed.

Experiments by Lucy (1970) and Poole, Howell and Lucy (1970) showed that fusion of avian erythrocytes is brought about by the presence of lysolecithin. Croce, Sawicki, Kritchevsky and Koprowski (1971) observed induction of homokaryocyte, heterokaryocyte and hybrid formation by lysolecithin in other cell types. If in *A. castellanii* cells in addition to the observed tight, focal-tight and gap junctions cell fusion would occur too, then the findings of coupling would be trivial. However, we want to emphasize that Spies *et al.* (1972) throughout their experiments established by light microscopical observation of the cell clumps, by serial sectioning and by goniometer tilting of the sections, that fusion of amoeba cells never occurred. The contrast of the membrane contact site is so high that the absence of cytoplasmic continuity above the resolution of the electron-microscopic technique was always evident. Gross cytoplasmic channels would certainly have been found (*see* Fig. 2).

Table 1. Cell aggregation after incubation of *Acanthamoeba castellanii* cells with 16:0-lyso-PC

| $Z_1$[a] (MΩ) | $Z_2$[b] (MΩ) | $V_2$[c] (mV) | $x_1$[d] | $x_2$[e] | $V_2/V_1$[f] |
|---|---|---|---|---|---|
| 5 | 20 | 0.14 | 0.01 | 0.05 | 0.03 |
| 42 | 18 | 3.05 | 0.30 | 0.14 | 0.07 |
| 10 | 30 | 3.18 | 0.19 | 0.48 | 0.32 |
| 9 | 10 | 3.67 | 0.54 | 0.58 | 0.41 |
| 25 | 47 | 14.62 | 0.48 | 0.74 | 0.58 |
| 32 | 24 | 4.78 | 0.33 | 0.26 | 0.15 |
| 30 | 21[g] | 6.26 | 0.47 | 0.35 | 0.21 |
| 21[g] | 21[g] | 1.22 | 0.11 | 0.11 | 0.06 |
| 14 | 46 | 16.01 | 0.52 | 1.07 | 1.14 |
| 25 | 44 | 16.01 | 0.53 | 0.78 | 0.64 |
| 24 | 21[g] | 0.18 | 0.02 | 0.02 | 0.01 |
| 21[g] | 21[g] | 10.72 | 0.68 | 0.68 | 0.51 |
| 38 | 16 | 17.97 | 1.06 | 0.64 | 0.47 |

[a] $Z_1$: input resistance measured by way of the stimulating electrode.
[b] $Z_2$: input resistance measured by way of the recording electrode.
[c] $V_2$: voltage change in a cell due to passage of current from a stimulating electrode in an adjacent cell.
[d] $x_1$: surface-contact-fraction of the stimulated cell.
[e] $x_2$: surface-contact-fraction of the cell impaled by the recording electrode.
[f] $V_2/V_1$: communication ratio.
[g] When for technical reasons the input resistance could not be determined, then the average input resistance of 21 MΩ was taken.

To facilitate a possible fusion between the cells, the incubation time as well as the lysolecithin concentration was increased. Increasing the incubation time resulted in disaggregation of the cell clumps into single cells within 24 hr; these cells can be reaggregated again by the same inductor. The process is therefore reversible, whereas cell fusion generally is an irreversible process eventually leading to the formation of multinucleated giant cells. Increasing the concentration of the lysolecithin resulted in lysis of the cells without any evidence of fusion. On the other hand, in our experiments in all cases cell coupling was demonstrated. It may be concluded from this that the observed coupling is not resulting from cell fusion.

The high theoretical surface-contact-fractions are not a result of the assumption in the model that there is no leakage of ions from between the adjacent membranes to the bathing fluid. In case of leakage one would predict even higher values.

It is obvious that if the resistivity of the cytoplasm had not been neglected, then also higher values for the theoretical surface-contact-fractions would have been found. A justification of the neglection of the cytoplasmic resistivity was given by a unique observation. Two amoebae (pos-

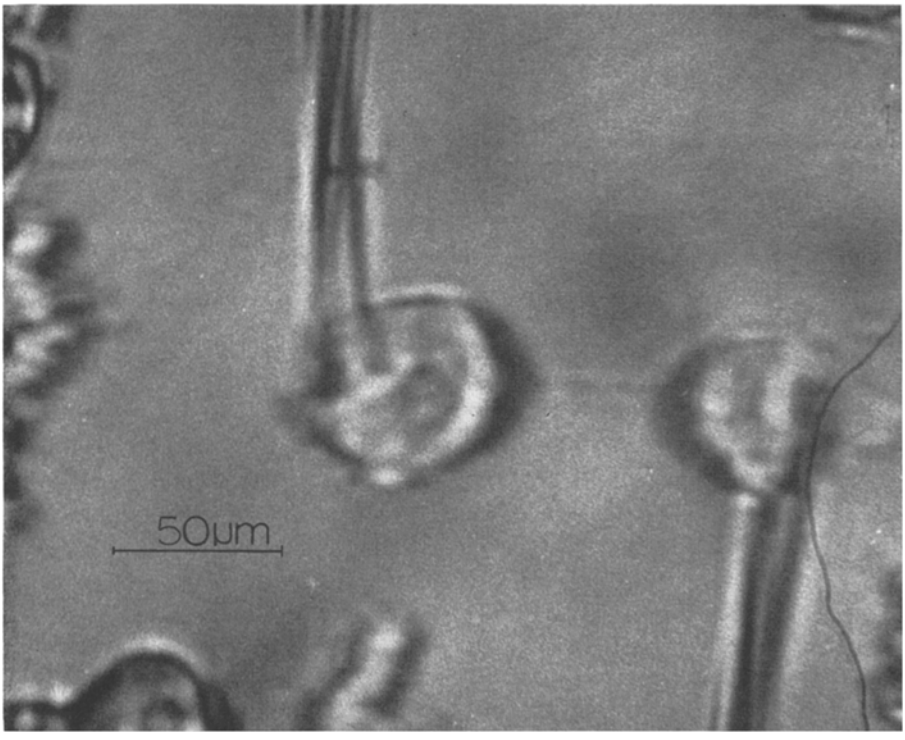


Fig. 10. Aggregate consisting of two cells. After successful impalement the cells were separated very gently so that they stayed in contact by means of a thin thread

sibly a dividing cell system) were separated very gently and stayed in contact by means of a thin thread (Fig. 10). In this particular case the induced voltage change $V_2$ is related, according to the model, to theoretical values of $x_1 = 0.47$ and $x_2 = 0.35$; the communication ratio $= 0.21$ (*see* Table 2, footnote b).

If we assume that the connecting thread is a duct filled with cytoplasm, its resistance equals

$$R_x = \frac{\rho l}{\pi r^2} = \frac{0.11}{r^2}\ \Omega\,\mathrm{cm}^2$$

in which $\rho$ = specific resistance of the cytoplasm $\approx 100$ Ωcm, $l$ = length of the duct $\approx 35$ µm, and $r$ = radius of the duct. Using the measuring results it can be computed that $R_x = 130$ MΩ, suggesting a radius $r = 0.3$ µm. This result does not disagree with the microscopic observation, demonstrating that $\rho = 100$ Ωcm is a fairly correct estimate for the specific cytoplasmic resistivity. The contribution of such a resistivity may indeed be neglected here. Also, the assumption of equal specific membrane resistances can not explain the results. Finally, the assumption that the potential in the bathing fluid was negligible was always checked after successful measurement of electro-

Table 2. Spontaneous cell aggregation

| $Z_1$[a] (MΩ) | $Z_2$ (MΩ) | $V_2$ (mV) | $x_1$ | $x_2$ | $V_2/V_1$ |
|---|---|---|---|---|---|
| 44 | 9 | 5.40 | 0.75 | 0.22 | 0.12 |
| 37 | 32 | 9.55 | 0.46 | 0.41 | 0.26 |
| 26 | 30 | 11.33 | 0.55 | 0.61 | 0.44 |
| 14 | 19 | 1.40 | 0.14 | 0.18 | 0.10 |
| 17 | 15 | 7.69 | 0.67 | 0.62 | 0.45 |
| 40 | 28 | 15.18 | 0.70 | 0.55 | 0.38 |
| 39 | 24 | 14.55 | 0.75 | 0.54 | 0.37 |
| 40 | 28 | 8.50 | 0.47 | 0.35 | 0.21[b] |

[a] *See* footnotes a through f of Table 1.
[b] Observation in which two amoebae were separated very gently and stayed in contact by means of a "thin thread" (*see* text).

tonic spread. So the conclusion seems justified that 16:0-lyso-PC is able to aggregate *A. castellanii* cells in a way that communicative contacts are formed, which allow ions to pass from one amoeba to the other, resulting from an increase in membrane permeability at these specific contacts.

No electrotonic spread at all could be detected in control experiments performed with suspensions that did not contain 16:0-lyso-PC and in which the cells were manipulated together.

The aggregation of *A. castellanii* can be generated by the addition of exogenous 16:0-lyso-PC, but it would be interesting if these amoebae could also aggregate spontaneously. In older cultures – 7 to 8 days old – we observed spontaneous aggregation, as has also been described by Neff, Ray, Benton and Wilborn (1964). The results of measurements of electrotonic spread in these cultures are shown in Table 2. Here again the measured voltage changes $V_2$ suggest $x$-values in the model that do not correspond to the observed surface-contact-fractions.

Lysosomal activity resulting from lysis of some cells in older cultures might increase the lysophospholipid content of the medium and therefore of the cell membrane of remaining intact cells. So here again lysophospholipids could be responsible for the cell aggregation, the increase in membrane permeability at the contact area, and thus for the formation of communicative contacts between these amoebae.

Ulsamer, Wright, Wetzel and Korn (1971) made extensive biochemical analyses of the plasma membrane of *A. castellanii* but could not detect any lysophospholipids in the plasma membrane of cells of 5- to 7-day-old cultures. Since spontaneous aggregation occurred in our experiments only after 7 to 8 days of culturing, it seems worthwhile to verify by biochemical

analysis whether at this time the lysophospholipid content of the cell membrane is increased.

Our experiments suggest that lysophospholipids might play an important role in the phenomenon of cell communication in general. The cell membrane has a dynamic character. Many membrane bound enzymes are known that take care of the selection of permeating substances. Phospholipase A (Bjørnstad, 1966; Scherphof, Waite & van Deenen, 1966; Nachbauer & Vignais, 1968; Waite, 1969; Ono & Nojima, 1969; Nurminen & Soumalainen, 1970; Rahman, Verhagen & van de Wiel, 1970; Torquebiau-Colard, Paysant, Wald & Polonovski, 1970; Victoria, van Golde, Hosteler, Scherphof & van Deenen, 1971) as well as acyltransferase (Lands, 1960; Mulder & van Deenen, 1965; Proulx & van Deenen, 1966; Eibl, Hill & Lands, 1969; Sarzala, van Golde, de Kruyff & van Deenen, 1970) activities have been shown to be associated with a variety of membrane structures. The concerted action of these two types of enzymes, situated on the contact area of two cells, could regulate the lysophospholipid content of the junctional part of the membrane, and thus its permeability properties. As phospholipase A as well as the acyltransferase (s) need divalent ions (calcium and magnesium ions, respectively) for their activity, our hypothesis might easily be fitted in the calcium-hypothesis of Loewenstein and co-workers (Loewenstein, 1967*a*, *b*; Loewenstein, Nakas & Socolar, 1967). Low intracellular concentrations of divalent ions will establish the concerted action of the above-mentioned enzymes, resulting in regulation of cell communication. High intracellular concentrations of divalent ions might stabilize the lipid bilayer as a result of cross-linking by these ions of adjacent phospholipid molecules. As a consequence, micelle formation would be inhibited (Danielli, 1967). This micelle formation may be important in the increase of membrane permeability (Lucy, 1964) and also in the phenomenon of cell aggregation.

Our hypothesis also does not contradict the observations that energy is required for the maintenance of normal cell communication (Politoff, Socolar & Loewenstein, 1969). The enzyme phospholipase A and the acyltransferase (s) require energy for their activity as do many enzymes.

In summary, our experiments do not yet justify the conclusion that lysolecithins are crucial to the phenomenon of cell communication; but in any case lysolecithins do induce communicative contacts between *Acanthamoeba castellanii* cells. The morphological basis of these contacts is rather similar to tight, focal-tight and gap junctions. Some recent studies show that ionic coupling is associated with the appearance of gap junctions (Revel & Karnovsky, 1967; Payton, Bennett & Pappas, 1969; Johnson & Sheridan, 1971; Gilula *et al.*, 1972). Spies *et al.* (1972) only claim that some

of the contact regions resemble gap junctions by one morphological criterion, the separation distance of the adjacent membranes. The two other criteria, the lanthanum hydrosol tracing and the freeze-etch aspect were tested as well, but no positive results have been attained as yet. As the lanthanum hydrosol is not really an inert tracer but probably more of a positive stain for surface coat material, the negative result with *A. castellanii* is less surprising, because this cell does not possess a surface coat. Furthermore, it should not be assumed that in all cell types and especially in those that normally do not form organized tissues, the sites for the physiological function of cell communication should of necessity show the complete structure of the gap junction in epithelial tissues. Friend and Gilula (1972) consider the mediation of ionic coupling as a physiological criterion for a gap junction of more importance than differences in fine structure between junctions.

In the light of the above our data may support the hypothesis that specialized membrane regions like gap and/or tight junctions are a site of electrotonic coupling between cells.

Our thanks are due to Mrs. Karin Hogema for her skillful technical assistance. We wish to thank Dr. R. A. Demel (Laboratory of Biochemistry, Utrecht), Dr. F. Spies and Dr. W. A. M. Linnemans (Biological Ultrastructure Research Unit, Utrecht) for useful discussion. Furthermore we wish to thank Dr. F. Spies and Dr. W. A. M. Linnemans for the electron-micrographs and for the supply of *Acanthamoeba castellanii* cells, and Dr. R. A. Demel for the gift of the lysolecithin.

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