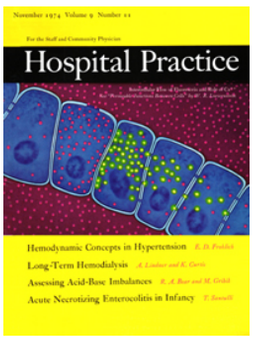

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Cellular Communication by Permeable Membrane Junctions

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Fine channels built into the plasma membranes where cells are joined enable large masses of cells to exchange a variety of substances and thereby to interact and function as a unit rather than as discrete bodies. This finding has major implications for biology and medicine, in particular for our understanding of both normal (e.g., embryonic differentiation) and abnormal growth (e.g., cancer).

For well over a century, biology and medicine have been greatly influenced by the Cell Theory. In one of the theory's earliest formulations, the botanist Schleiden writes in 1838: "Every higher organism is an aggregate of fully individual independent units, the cells." He uses the precise German wording "*in sich selbst abgeschlossene Einheiten*," circumscribed, self-contained units. This has been an extraordinarily productive theory. We are now aware, to be sure, that the self-containment is not absolute. In particular (as earlier articles in this series have discussed at length), cells communicate with and regulate other cells by releasing various signal molecules (e.g., hormones, neurohumors) that trigger receptors on the membrane of the target cell. For all that, however, we continue to think of cells as essentially discrete bodies, whose many interactions with one another do not negate their basically separate character. Indeed, the past 20 years of electron microscopy have given us the picture that all cells are completely surrounded by a membrane, and this membrane was assumed to be a continuous diffusion barrier.

It now appears that this aspect of cell theory must be revised. Cells, it is becoming evident, are typically *not* wholly separate from their neighbors; rather, large masses of cells are connected by fine channels built into the plasma membranes where the cells are joined. These "permeable junctions" enable the cells in a tissue to rapidly exchange a variety of substances, with the result that they can interact and function as a unit rather than as discrete bodies. This finding has obvious and major implications for biology and medicine, many of which are yet to be explored. In particular, it may be relevant to our understanding of growth processes, both normal (i.e., embryonic growth and differentiation, wound healing) and abnormal (e.g., cancer).

My involvement in this field began with a chance observation in 1963, when Yoshinobu Kanno and I were studying the permeability of the nuclear membrane. This entailed inserting micropipettes into the nucleus and cytoplasm of large salivary cells, passing a current through them, and measuring the resulting potential. Much to our surprise, we found that it made little difference whether we measured the potential in the same cell or in an adjacent one, despite the interposition of two plasma membranes in addition to the nuclear membrane itself. Since electrical currents within cells are carried by ions, our finding implied that plasma membranes at the cell junction interfered very little with ionic flow, which contradicted flatly the prevailing ideas of how cell membranes operated. This finding was all the more surprising, since salivary cells, like all epithelial cells, do not normally carry electrical signals.

To be sure, Silvio Weidmann at Cambridge had found in 1952 that certain heart cells are electrically coupled; and Edwin Furshpan and David Potter, then at University College, London, had elegantly demonstrated this in 1957 for certain nerve synapses. These important findings showed a mode of transmission of electrical signals from one heart or nerve cell to another. But, since the heart and nerve cells were long known to specialize in communication by electrical impulses, these investigators properly considered their findings membrane specializations adapted to electrical signal transmission. (Actually, heart muscle, since the turn of the century, had been thought of as a syncytium, and until 1951, synaptic transmission in the central nervous system was widely held to be electrical.) Thus, from the

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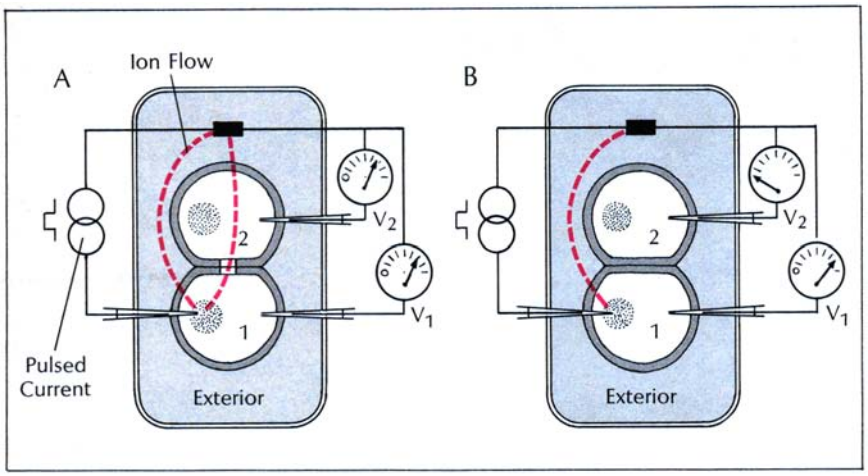
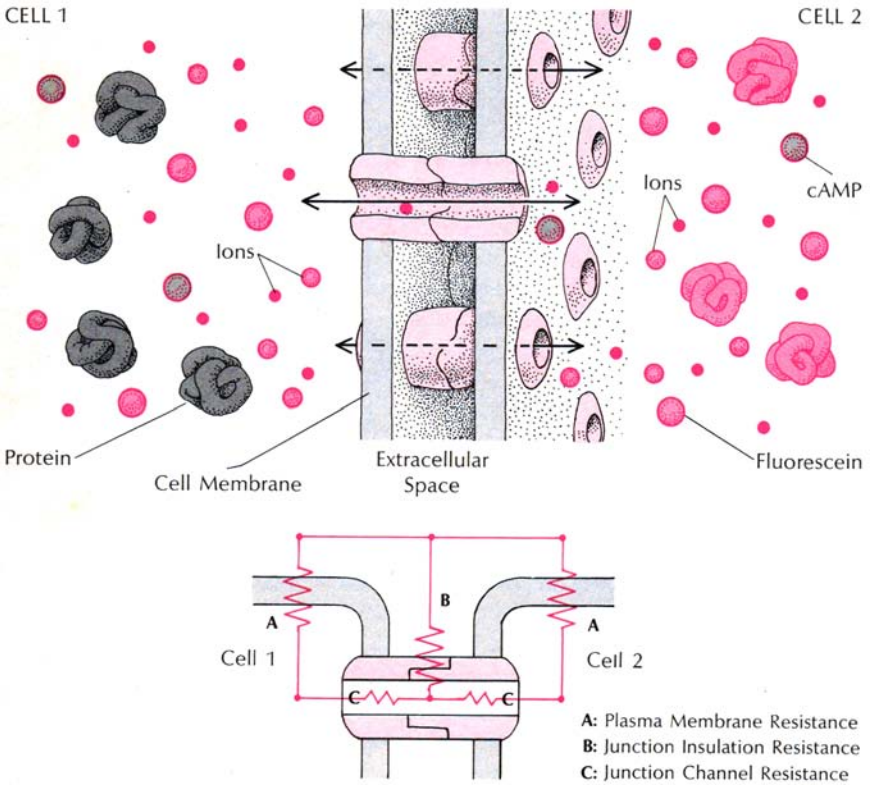


Initial clue to existence of intercellular communication came from experiment in which electrical resistance between two contiguous cells proved to be relatively low. This implied a rather free flow of current-carrying ions across the junctional membranes between the cells. The basic demonstration consists of passing a pulse of current into one cell and measuring resulting voltage in both. When cells are fully coupled by a junctional path of low resistance (A), pulse produces a voltage in cell 2 nearly as high as in cell 1 (see also illustration below). When junction is blocked and cells are uncoupled (B), pulse produces a large voltage in cell 1 but a barely detectable one in cell 2.



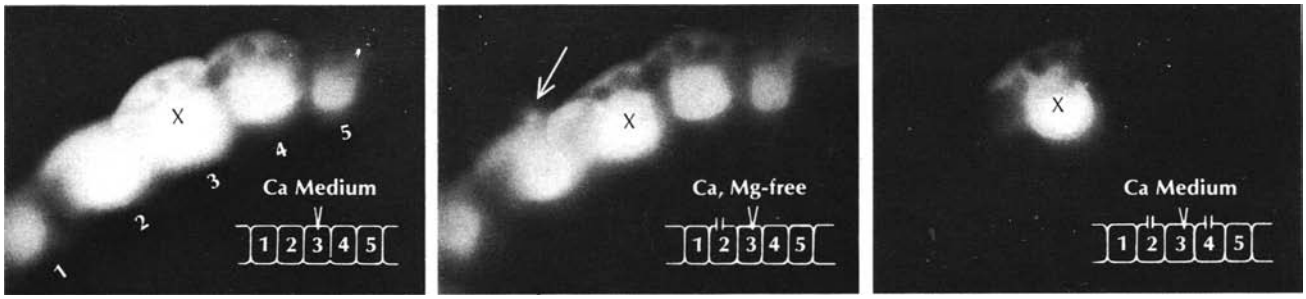
In this schematic view of a permeable junction, pairs of abutting membrane channels form cell-to-cell passageways insulated from the exterior. Channels are pictured as built into protein particles, according to the prevailing concept (cf S.J. Singer, "Architecture and Topography of Biologic Membranes," HP, May 1973). This, as well as the spatial interrelationship between the particles, is merely one of several possible representations of the cell-to-cell passageways, which are defined precisely in their resistive properties by electrical measurement (lower drawing). The size of the channels permits rather free passage of small molecules, such as inorganic ions, fluorescein, or cyclic AMP, but excludes larger ones, such as DNA and most proteins.

point of view of general cell physiology, no one had found these phenomena especially surprising.

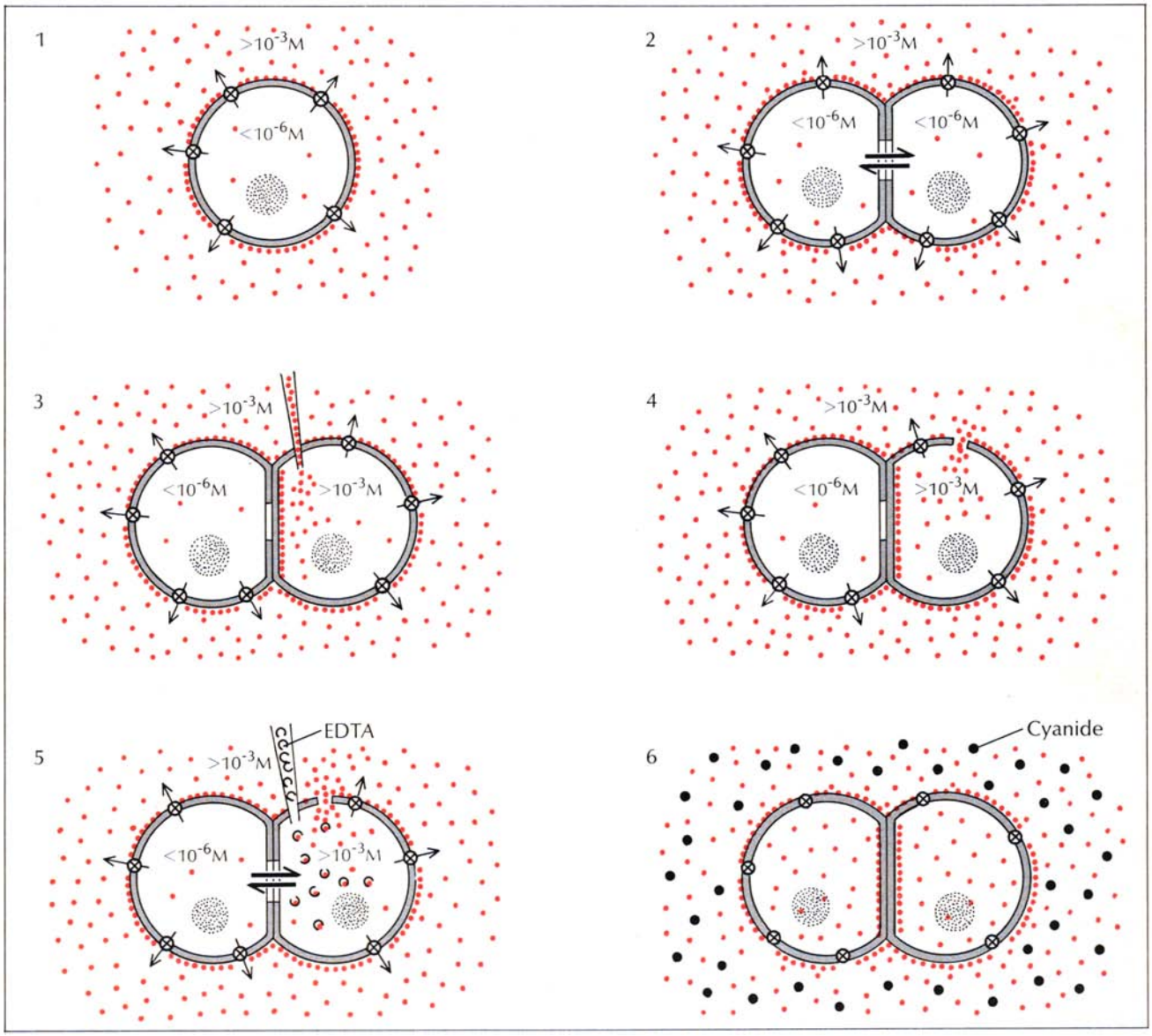
Within a few months, Kanno and I were able to show that the coupling in the epithelial cells was not limited to the small inorganic ions. Studies with the organic molecule fluorescein demonstrated that this molecule, when injected into a salivary cell, could pass from the interior of one cell to that of an adjacent cell, its progress showing clearly in sequential photographs taken under ultraviolet light. The cell-to-cell passage of fluorescein was soon confirmed in other laboratories for several kinds of cells, including the electrically transmitting nerve synapse. As organic molecules go, fluorescein is not especially large, but its molecular weight – about 300 – is still considerably greater than that of the largest inorganic ion. It appeared, therefore, that the cytoplasms of the adjacent cells were in direct communication with one another through channels big enough to permit the passage of the fluorescein molecule.

In subsequent studies, we showed that such channels are present in a wide variety of epithelial tissues including those of liver, kidney, thyroid, skin, urinary bladder, and pancreas. Indeed, the only tissues in which they are not found are those of skeletal muscle and the nervous system, in which the proper transmission of information by electrical impulses demands insulation between parallel information lines. It seems fair to conclude that direct communication via junctional channels represents a very primitive cellular mechanism (it is present even in sponges); humoral transmission in the nerve and muscle systems is probably of more recent vintage.

In a more detailed exploration of the properties of the channels by means of electrical techniques, we have found that these channels are at least 10,000 times more permeable to small inorganic ions than is the bulk of the cell membrane facing the exterior. Moreover, the channels are effectively insulated from the exterior so that the permeating molecules pass from cell to cell with little leakage to the outside. Finally, by the use of

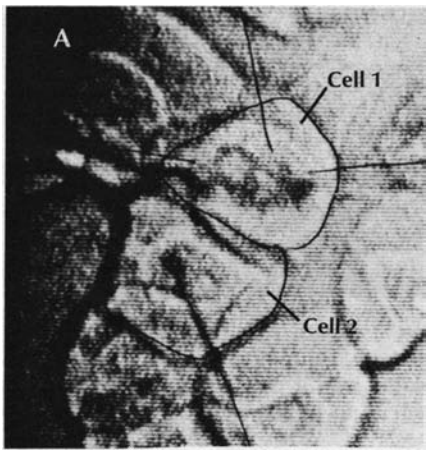


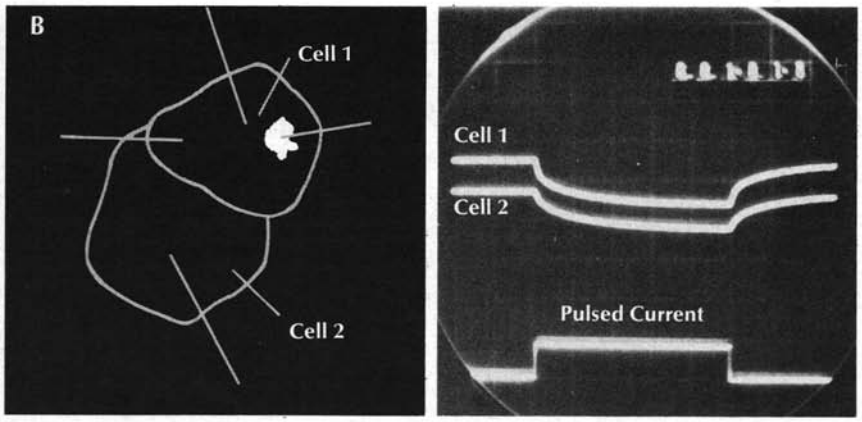
Fluorescent dye injected into one of five adjacent salivary cells (left) diffuses rapidly to the other four. A hole in cell 2 produces no abnormalities of dye flow in a medium free of Ca^{++} (and Mg^{++}), apart from visible leakage (arrow) of dye through the hole (middle). In a Ca-containing medium, with holes punched in both cells 2 and 4, Ca^{++} enters through the holes and the cell-to-cell flow of dye is blocked (from Oliveira-Castro GM and Loewenstein WR, Jn Membr Biol 5:51, 1971).

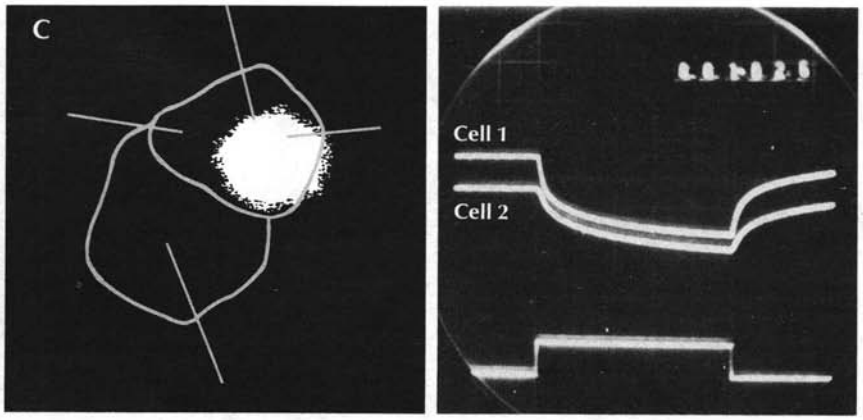


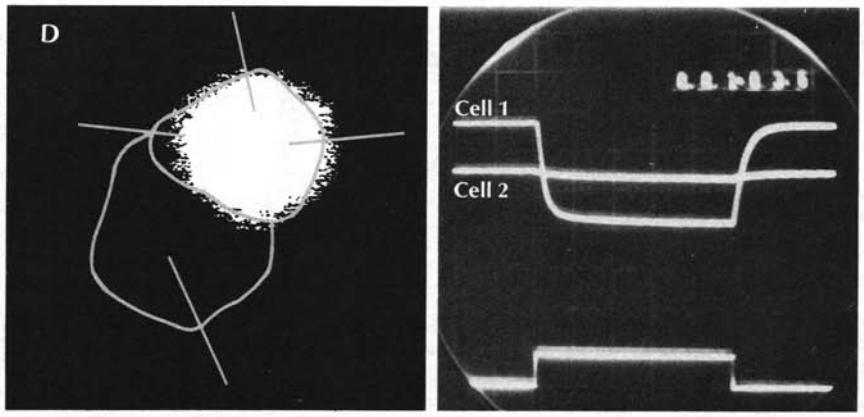
Key role of Ca^{++} ion in intercellular communication is shown in series of experiments. In normal cell (1), Ca^{++} is held at very low levels by calcium "pump"; such cells rapidly set up communication channels when brought into contact (2). Injection of Ca^{++} (3) or leakage of the ion through a hole in the membrane (4) blocks the channels; this can be reversed by injecting EDTA (5), which chelates the Ca. Similar blockage is produced by cyanide treatment (6), which stops the pump.

Calcium ions inside a cell are visualized by the glow of the calcium-sensitive protein aequorin. In photo A the cells are shown in bright field with electrodes in position for electrical measurement. A small injection of Ca^{++} into center of one cell (B, dark field) shows that electrical coupling is not affected, as the oscilloscope record makes clear. A larger injection (C) reduces the channel permeability somewhat, while an even greater amount (D), flooding the junction, blocks the channels altogether (Rose B, Loewenstein WR, XVIth International Congress of Physiological Sciences 1974, Lectures).









several tracer molecules, we learned that the channels will pass molecules with weights of up to about 1,000 (and in some cell junctions up to about 10,000). Into this range fall a wide variety of cellular molecules: the steroid hormones, cyclic AMP, all the common metabolites, such as sugar, amino acids, nucleotides, vitamins, and so forth. On the other hand, the channels will not pass most of the larger molecules such as proteins, RNA, or DNA. With respect to these macromolecules, the carriers of the basic genetic information, the principle of cell individuality is valid.

Ever since this form of intercellular communication was described, electron microscopists have been searching for the related differentiations in membrane structure. Functionally, in terms of their resistive properties to ion diffusion, the cell-to-cell channels are reasonably well defined. They must contain at least two elements: a transmembrane channel in the two joining membranes through which molecules can move and some sort of insulation where the membranes meet, which seals off the passageway from the extracellular medium. Indeed, this is how I defined the channels in 1966 on the basis of electrical measurement made on living cells. Such measurements can be done rather easily in suitably large cell systems. It is, however, a much more difficult task for the electron microscopist, working with dead cells, to demonstrate the channels morphologically. The fixation, staining, and other procedures of present-day electron microscopy may alter the membrane structure and even induce artifactual ones. Thus, unfortunately, there is as yet little structural knowledge at the channel level. Progress has been made, however, at a coarser level, in identifying the membrane regions where such channels may be located. Here the focus has been on the differentiated structures in the membrane junctions between coupled cells that may offer the necessary continuity.

There are various kinds of differentiated structures in cell junctions, but to sort out, on morphologic grounds, which of these structures are mediators of coupling, one needs to have a coupled cell system in which, ideally,

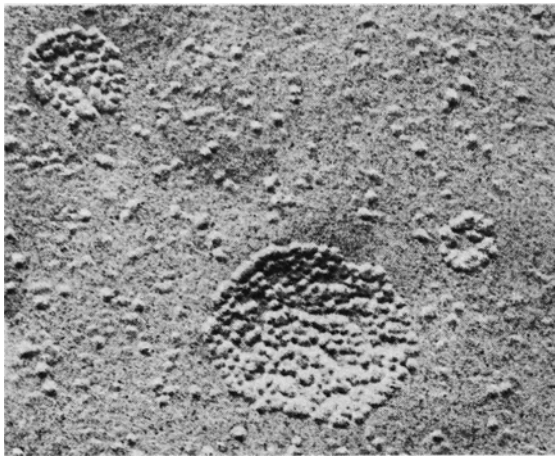
only one type of junctional structure is present at a time (see G. D. Pappas, "Junctions Between Cells," HP, August 1973). One such structure, discovered by David Robertson in an electrically transmitting nerve synapse and by Jean-Paul Revel in epithelial cells is the "gap junction" (which also goes under the name of "nexus"). This structure appears as an aggregation of neatly arrayed membrane particles, which Daniel Goodenough's diffraction studies show aligned on either side of the two joining membranes. It is conceivable that these particles, when properly aligned and joined, form the cell-to-cell passageways; that is, each particle contains a water channel (the membrane channel), and the insulation is given by the hydrophobic particle walls and particle junction.

The evidence implicating the gap junction in coupling is now strong: the structure is widely present among coupling cells (in some it is the only visible differentiated structure) and, as Norton Gilula and his colleagues at Berkeley have shown, it seems to be lacking in certain noncoupling cells. Moreover, when such noncoupling cell strains are rendered coupling by genetic manipulations that I shall describe further on, the gap junction clearly appears again.

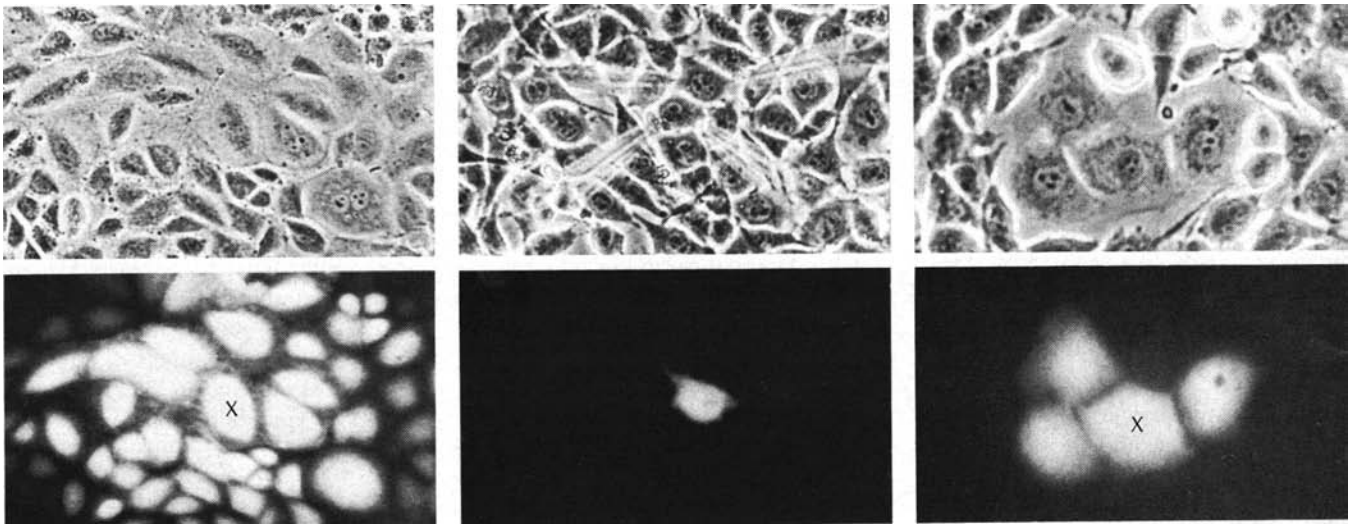
I am citing here only the structures for which the evidence as a mediator of coupling is most compelling. But this by no means concludes the list of structural candidates. There are no reasons to assume that coupling particles could cluster only in one or two kinds of arrays. One possible candidate, for instance, is the septate junction, which contains highly ordered and particularly extensive arrays of membrane particles; and there are other possible coupling structures.

A specially interesting aspect of permeable junction is that the channels are not permanent functional entities in the plasma membrane. Rather, the channels form or become functional when the cells that can make them are brought into contact and disappear or are blocked even more rapidly than they form if the cells are separated, with all of the cell membrane returning to the impermeable state. Nor is their development limited to any special region of the membrane. The first clue in this respect came from experiments with sponge cells, in which I manipulated single pairs of cells into contact at random spots; permeable junctions formed wherever the contact happened to be. Later experiments, conducted with Shizuo Ito on vertebrate embryo cells, were even clearer: coupling channels formed when we brought the cells into contact; they self-sealed and then re-formed when in contact at other points.

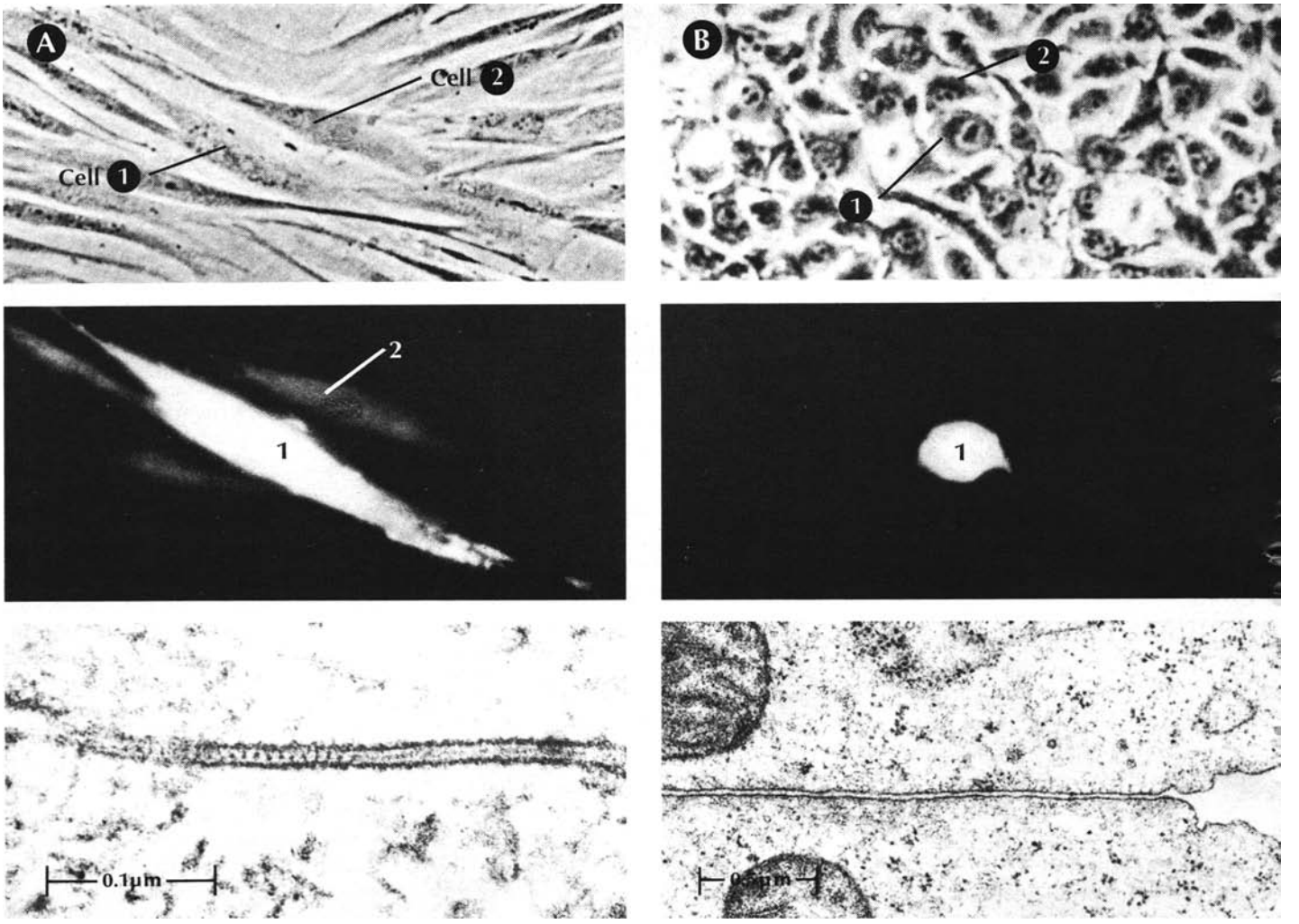
Thus it appears that junction formation is a capacity of most and perhaps all parts of the plasma membrane in these cells; its only immediately necessary condition seems to be



Electron micrograph by William Larsen of three freeze-fractured gap junctions shows the typical clusters of particles in a split membrane junction of coupling human-mouse hybrid cells. The particles here all belong to one membrane; the overlying contiguous membrane has split off. These hybrid cells are also shown on pages 118 and 119.



Contrast in communication between normal cells and a malignant strain of hepatoma cells is demonstrated by fluorescein injection. Same cells are shown in both bright and dark fields. In normal cells (left), dye injected into the one marked "X" rapidly diffuses into the rest, even though they are of different types and from different species (large are rabbit lens; small, rat liver). When injected into malignant cell (middle), dye does not spread beyond it. In mixed culture of normal liver and malignant cells (right) dye diffuses only in normal ones (from Azarnia R and Loewenstein WR, Jn Membr Biol 6:368, 1971).

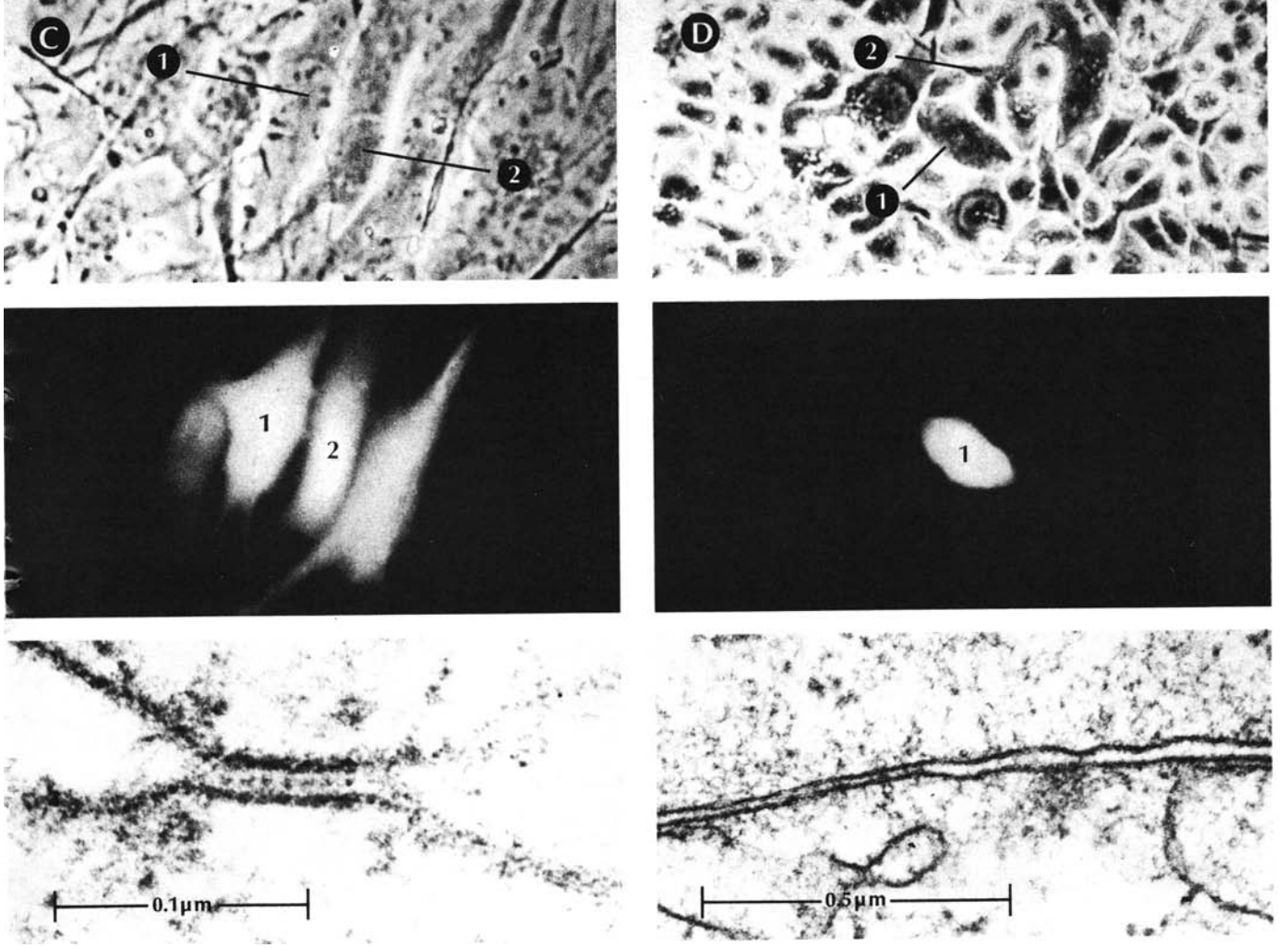


Crossing malignant, junction-defective mouse cell with normal human fibroblast can correct junctional defect. In the normal fibroblasts (A, top), cell-to-cell fluorescein flow proceeds normally (middle) and electron micrograph of contiguous plasma membranes shows typical gap junction (bottom). In the malignant cells (B), dye flow is blocked; membrane has no gap junc-

intimate contact (adhesion) between the two membranes. Surprisingly, the membranes need not be equal for junction formation. In experiments in which Wolfgang Michalke and I paired cells from different organs and from different species in culture, the cells turned out to establish good coupling. For example, a lens cell from a rabbit made a perfectly viable junction with a liver cell from a rat or a human skin fibroblast.

Thus far, these interorganic and interspecific junctions have only been demonstrated on cultured cells that may have undergone some de-differentiation. It would be dangerous therefore to conclude that cells in well-differentiated tissues necessarily behave this way too. But the fact that such heterologous junctions can form under any conditions is surely remarkable. The cultured cell pairs tested were clearly genetically different; they had different morphologic features, made different enzymes, and had different immunologic properties. Yet, they were capable of a coupling process that must require at least some membrane symmetry, such as alignment of channels. This points up once again how basic and general the mechanism of junctional formation is.

The formation of permeable junctions takes on the order of 10 minutes in various cell types. In newt embryo cells, for example, where we have recently been able to monitor the coupling process continuously and precisely by measurement of electrical resistance, the first signs of intercellular communication appear between four and 25 minutes after the cells are placed in contact, with communication attaining its full extent over the next 10 to 30 minutes; the process is equally rapid whether the cells are forced into contact by micromanipulation or are allowed to make contact by their own spontaneous movements. Two important events could be detected during the process of junction formation: the resistance to ion movement across junctional membrane fell gradually until it reached its normal low level, and the resistance to ion movement across junctional insulation increased gradually to a peak, to settle finally at a level somewhat below peak. A plausible and simple explanation for these progressive events is that more and more individual channels develop between cells during junction formation and that junctional insulation improves progressively during the early phase. Unfortunately, we have as yet no electron microscopic information to go with these electrical measurements. However, there are the suggestive observations by Gilula on septate junction in sea urchin embryo cells and by Sheridan and Johnson and their colleagues on gap



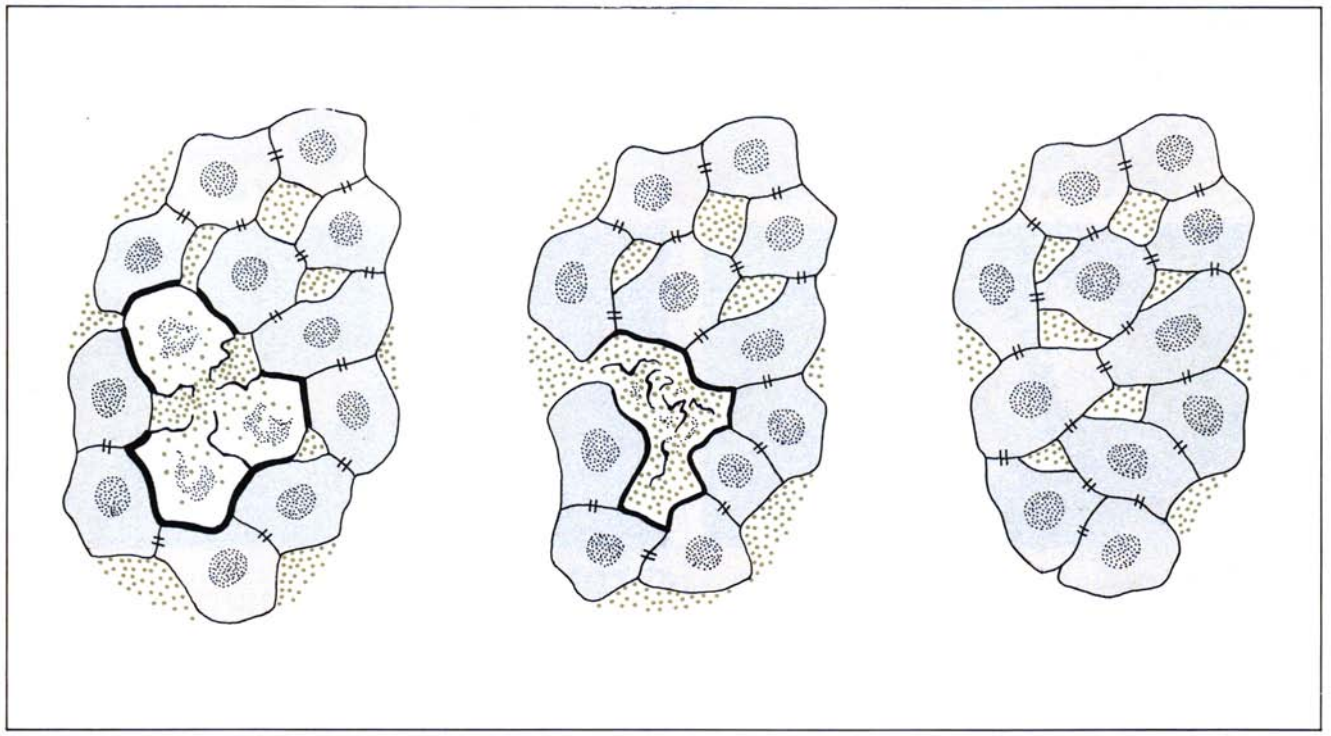
tion. Crosses between the two strains, produced by cell fusion (C), manifest both the normal dye flow and the junction-forming capacity of the human parent. As the culture reverts to malignancy through chromosome loss (D), fluorescein flow is blocked and the gap junctions disappear (Azarnia R, Larsen W, Loewenstein WR, Proc Nat Acad Sci *71:880, 1974).*

junction in cultured rat fibroblasts that the number of membrane particles increases progressively with development. To establish the correlation, one needs a cell system in which both the electron microscopy and the measurement of electrical resistance can be done (measurements of electrical coupling are not sufficient). This is technically difficult – but, no doubt, the right system will be found before long.

In the regulation of the permeability of the channels, the calcium ion seems to play a key role. Here it must be remembered that at their internal face the junctional membranes are exposed only to whatever calcium is in the cytoplasm, where its concentration (in free, ionized form) is normally below the order of 10^{-6} molar. If, however, the cytoplasmic free calcium level is raised, the permeability of the channels falls very steeply. In fact, at concentrations approximating the normal level in the extracellular fluids (10^{-3} molar), the channels virtually disappear as functional entities.

This experimental "dechannelization" can be accomplished in a number of ways – most simply, as in experiments with Gilberto Oliveira-Castro, by punching a hole into the plasma membrane with a microneedle and allowing the cytoplasm to equilibrate with the high exterior levels of calcium (swamping, so to speak, the cytoplasm with calcium ions) or, more elegantly, by injecting calcium into the cells with a micropipette. Similar results are obtained, as shown by my colleagues Alberto Politoff and Sidney Socolar, when the membrane's calcium "pump" or that of the mitochondria – which maintains the intracellular level of the ion at the normal low level – is blocked by poisoning with cyanide or dinitrophenol.

The most revealing results on the action of calcium were recently obtained in collaboration with Birgit Rose (who in between experiments doubles as my wife). These experiments involve the use of aequorin, a protein that luminesces in the presence of calcium, and an electronic image intensifier to make the luminescence visible. The aequorin is injected into a pair of coupled salivary gland cells and serves as an indicator of the cytoplasmic free calcium, and the image intensifier scans the cytoplasm and tells us where inside the cells the cytoplasmic calcium concentration is changing and by how much. With this method, a small injection of calcium is seen as a luminescent puff in the cytoplasm that does not spread much beyond the micropipette. This is because the excess calcium is rapidly swept up and sequestered by mitochondria. If such an injection is made into the cell center, the junc-



First reaction to skin injury is sealing of cell junction at the wound border by the calcium mechanism (left); this protects intact cell population against leakage. As the wound gap closes (middle and right), coupling is reestablished among intact cells.

tional channels are not affected. The channels, however, are promptly blocked if such an injection is made close to a junction, or if a large calcium injection into the cell center swamps the mitochondria, raising visibly the calcium concentration in the cytoplasm around the junction. The channels open up again spontaneously as the excess calcium is pumped out of the cell. Other experiments, in which the calcium influx is stepped up by incorporating a calcium-transporting ionophore into the plasma membrane, show that the junctional channels are blocked whenever the cytoplasmic calcium concentration rises above a certain level. Finally, the drop in permeability can be reversed by injection of EDTA, a compound that avidly binds the calcium.

Having described (to the extent that present data permit) the genesis and the physiologic properties of permeable junction, let us now consider some of their implications for cell biology and medicine. To begin with, there is every reason to think that the reaction of the junctional membrane with calcium plays an important part in the organism's response to injury. The above experiment of blocking a junction by the influx of calcium through a hole punched into the plasma membrane is a perfect micromodel of what happens during ordinary tissue injury: the intact tissue is sealed off from the injured cells. If it were not for this fast mechanism of junctional blockage, a tissue like skin with widely interconnected cells could not survive an injury, or a liver (in which most cells, perhaps all, are interconnected by junctions) could not survive the death of even a single cell; the intact tissue would be leaky at the wound borders. This mechanism has actually been demonstrated in skin wounds, where the intact cells at the wound border remain sealed off from the exterior until they make contact with one another as the wound closes, at which point they form permeable junctions within 30 minutes.

Perhaps the most interesting possibility to consider is that of the junction playing a role in the regulation of cellular growth and differentiation. Before pondering this possibility, let us briefly sum up what junctional communication implies: a common intracellular milieu, including many sizable molecules, for a large community of cells – meaning large portions of an organ and perhaps (in some cases) the entire organ. There is thus ample opportunity for concerted interaction by regulatory molecules within such a community.

The exciting possibility then to be considered is that the size range of molecules passing through the junctions includes substances involved in the regulation of gene activity – i.e., of cell growth and differentiation. Such gene regulators have not yet been identified in higher organisms, but the past 15 years of bacterial genetics have left the lesson that in these organisms, at least, genes can be regulated by a number of quite simple metabolites such as galactose or tryptophane – molecules of only a few hundred molecular weight. Moreover, the results of the past 30 years of experimental embryology clearly imply that embryonic differentiation involves interaction between cells at close range, mediated by diffusible substances. Here we have in junctional communication an obvious can-

didate for such close range interaction, namely a system in which molecules can flow directly from cell to cell with little loss to the exterior.

On purely *a priori* grounds, a cell system connected by junctions is in many ways ideally suited for disseminating information through the community on the number and position of its constituent cells. The key point here is the presence of a sharp boundary, namely the continuous diffusion barrier made of the plasma membrane plus the junctional insulations. Because of this boundary, the connected cell system has a finite volume and hence could respond to simple cues of chemical concentration. For instance, if the processes controlling cell growth were cued to a concentration level of a signal molecule, then the size of a growing cell population could be self-regulated as the (asynchronously) proliferating population enlarges its effective volume, diluting the molecules. Self-regulating growth models of this kind can be readily envisaged. But equally important and, perhaps, even more unusual is the potential of a junction system for conveying information on cell position. Because of the presence of the sharp diffusion boundary, cues about the location of a given cell or a group of cells within the community could be provided by simple time-dependent parameters of signal concentration relative to the boundary. The two kinds of controls, regulation of cell number and cell position, are essential to any normal growth process.

On the experimental side, there is the fact of extensive junctional connection in embryonic development. My colleague Ito and, at Harvard, Furshpan and Potter, were the first to show this. Ito showed that at the morula stage in newt embryo most and probably all the cells are interconnected. Even more interestingly, the Harvard group found at a later developmental stage in squid embryo that there is extensive interconnection between cell groups when these are acquiring visible differentiations. By now the embryos of many different species have been investigated, and it is clear that widespread junctional communication is a general feature of embryonic organisms.

All this, of course, is far from proving that permeable junctions are involved in growth control and differentiation. It is merely the condition *sine qua non* for such an involvement. To demonstrate an involvement would be simple enough if we knew the signal molecules in question. But the lack of knowledge of these signals is precisely what hampers the field of developmental research and the related field of cancer. Thus handicapped, the workers in these fields are forced to take indirect approaches; and how hard it is, under these circumstances, to sort out relevant etiologic phenomena from epiphenomena is all too obvious when one reads the literature of these fields.

My colleagues and I are in no way better off. We too had to take an indirect approach, and only recently have we been able to cut the risk that we may be chasing an epiphenomenon by the use of genetic analysis. Our approach is to search for defects in junctional connection among cancerous tissues – in which growth control is defective by definition – and to determine by genetic analysis whether the growth defect and the junction defect are correlated.

The rationale is as follows: the control of growth, like any controlled system, involves four elements. – 1) the control signals, 2) the signal transmission system, 3) the signal receptor, and 4) the effector process triggered by the signal. In principle, uncontrolled growth – cancer – can arise from a defect in any of these elements. Thus, if the junction is indeed an element of transmission for growth-controlling signal molecules, transmission block (*uncoupling*) by genetic defect should produce uncontrolled growth. This simple idea has guided our work into the cancer field, where our focus is on the etiologic category of signal transmission.

Before describing our work in this area, I should like to say what we do *not* expect to find. The term cancer is an umbrella for many different forms of uncontrolled growth. Each of the four etiologic categories above (and their possible subcategories) constitutes *a* sufficient cause and, for the particular cancer form, *the* necessary cause of uncontrol. We do not expect, therefore, to find junction defects in categories 1, 3, or 4. In fact, we know now of at least seven types of cancer cells that have permeable junctions. The question that concerns us rather is whether there are junctional defects in cancer cells belonging to category 2; or stated in terms of experimental approach, we are searching for *some* types of cancer cells that are junction-defective.

The search was encouraged by our earlier findings that junctional coupling is labile; uncoupling can be readily produced by experimental physicochemical manipulations. In fact, how vulnerable a junction is to uncoupling is evident from considering only the dependence of the junctional permeability on the cytoplasmic calcium level and the many ways this level can be altered. It seemed therefore not unlikely that uncoupling could be induced by genetic defects, and we hoped that such defects might be frequent enough to give us a reasonable chance of finding some kinds of uncoupled cancer cells. In fact, we have thus far found six such cell strains – four derived from rat hepatomas, one produced by x-irradiation of hamster embryo cells, and one a malignant derivative of a mouse L cell.

All six strains are clearly abnormal in their growth patterns. They do not show the density-dependent growth of normal cells in culture, and in animals they produce fatal tumors. Their junctional coupling is no less abnormal; in contrast to normal cells, which (as already noted) will couple not only with their own kind but with cells from different organs and different species, cells from any of these strains will not couple even with one another. This has been demonstrated in three different ways. First, electrical measurements showed that transfer of small ions is blocked; second, studies with fluorescein showed that passage of this molecule is blocked; and, finally, radioautographic studies with labeled nucleotides, such as hypoxanthine, and their derivatives show these molecules too are blocked.

But these results, though they show that the cancer cells in question are undoubtedly junction-defective,

still do not tell us whether this property is any way related to the growth abnormality. To find this out, Roobik Azarnia and I analyzed the defects genetically.

We hybridized through fusion the abnormal cells with normal cells and examined the ability of the hybrids to make permeable junctions and tumors. Mary Weiss, J. Todaro, and Howard Green (then at New York University), Henry Harris and George Klein and their colleagues at Oxford, and the Karolinska Institute had already established that density-dependent growth (contact inhibition) is resumed and tumorigenicity reduced when cancer cells of various types are hybridized with normal cells. The question for us then was whether the normalization of growth properties would go hand in hand with normalization of junctional properties.

In the three types of hybrids we have thus far examined, the results are simple: correction of the growth defect is paralleled by correction of the junction defect. In one system, for example, the partners for fusion (the parent cells) were a normal liver epithelial cell and an epithelial hepatoma cell, both from rat. The initial contrast between them could not have been more striking. The liver cell was "coupling" – i.e., it formed normal junctions, it grew to densities of 10^4 cells/cm^2 in cultures, and it was not tumorigenic. The hepatoma cell was noncoupling, achieved densities in excess of 10^6 cells/cm^2, and was highly tumorigenic: inocula of only 100 cells produced fatal tumors. Hybrids between these cells took after the normal parent in all these respects.

Even more persuasive evidence has come from fusion of mouse and human cells, where we could carry the analysis one step further. Here one parent cell was a skin fibroblast from a patient with Lesch-Nyhan syndrome; apart from its characteristic enzyme defect (which was useful in the selection of the hybrid) it was altogether normal in growth and junction: it was density-dependent, coupling, and, indeed, as our colleague William Larsen found under the electron microscope, it had the characteristic gap junction. The abnormal mouse parent cell – a malignant derivative of an L cell – was not density-dependent, noncoupling, and lacked gap junctions. Again, the hybrids took after the normal human parent.

We then continued to grow the hybrids for a number of generations and found that (as is characteristic for the human-mouse cross) they tended to lose the human chromosomes with continued replication. Eventually clones appeared that had reverted to the growth-defective state. These clones had also reverted to the junction-defective state, showing neither coupling nor gap junctions. In every case, the reversions to the growth defect went hand in hand with reversion to the junction defect, although these properties had segregated from a number of other morphologic and biochemical ones that originally occurred together in the mouse parent. Evidently, the normal human cell contributed a genetic factor (probably linked to one of its chromosomes) that simultaneously corrected the growth defect and the junction defect. The junction defect and the growth defect in vitro are thus clearly correlated in this cell system. It remains to be shown whether the correlation applies also to tumorigenicity, as we know it does at least in the unsegregated hybrids of the liver/hepatoma cell system. Tumorigenicity tests with heterospecific cells with unstable chromosome complements are complex and difficult, but, if all goes well, we should soon have the answer.

These findings on the relationship between cell coupling and cell growth clearly do not begin to exhaust the possible implications of communication by permeable junction, which as an old and general cellular phenomenon is likely to have adapted to a host of other cell functions. I have here selected only those functions on which we are getting the first experimental glimpses. The interested reader will find a discussion of other possibilities in an article in *Perspectives in Biology and Medicine* (see Selected Reading box). But I should not like to end without mentioning at least that junctional communication may well be at the root of the familiar phenomenon in medicine that diseases generally affect whole organs or large parts of them, rather than the single cells. Since the days of the Greek philosophers, it has been customary to liken a living organism to human societies, and the comparison is apt: both consist of interacting units with organized activity. The very word "organism," rather new in our language, implies this; and, indeed, it was precisely the concept of organization that led the French naturalist Buffon to introduce the term in the early 19th century.

Clearly the existence of organization requires the exchange of information between the constituent units, and one can confidently say that the health of an organism, like that of a society, depends on how well the units communicate with each other. It seems no less clear that permeable junctions must take their place as one of the primary communication systems of living organisms. Precisely what they communicate, and how, and when, will no doubt be revealed by further research, which may well develop into a whole new field of physiology. □

Selected Reading

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