J. Membrane Biol. 30, 175–186 (1976)

Intercellular Communication and Tissue Growth: VII. A Cancer Cell Strain with Retarded Formation of Permeable Membrane Junction and Reduced Exchange of a 330-Dalton Molecule

R. Azarnia and W.R. Loewenstein

Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33152

Received 7 July 1976

Summary. A cancer (hepatoma) cell strain is described in which the formation of junctional membrane channels is abnormally slow. The development of electrical junctional coupling following the establishment of contact between these (reaggregated) cells is at least 15 times slower than that between their normal counterparts; and junctional transfer of fluorescein eventually develops, but only in about 5% of the contacts (as against 100% normally). This deviant membrane behavior is interpreted as a retardation in the process of accretion of junctional membrane channels. Its possible etiological role in defective growth regulation is discussed.

Cells in normal tissues and cultures are connected by channels built into their junctional membranes through which molecules can pass from one cell interior to another (Loewenstein, 1966; 1975*b*; Furshpan & Potter, 1968). The upper molecular size limit for the cell-to-cell passage, as determined with synthetic and natural peptides in *Chironomus* salivary gland cell junction, is 1200–1900 dalton (Simpson, Rose & Loewenstein, 1976). Thus, a wide range of molecules, including all cellular inorganic ions, metabolites and nucleotides, can be freely exchanged between the cells. The junctional membrane channels are rapidly made by the cells when these come into contact; functioning channels develop within 1–40 min (*cf.* Loewenstein, 1975*b*). Thus, even when the junctional connections are temporarily broken in a tissue by cell movement or other activities, the intercellular communication can be rapidly restored.

With the thought in mind that growth controlling molecules may be exchanged by the cells via the junctional membrane channels and, hence, that defects in channel connection may lead to uncontrolled growth (Loewenstein, 1968), we have searched for such defects among

cells of cancerous tissues. The search so far has yielded three types of cells which seem incapable of making junctional channels: two strains of cells derived from Morris rat hepatoma H-5123, an X-irradiated cell strain from hamster embryo, a mouse L-cell derivative (clone 1D), and a series of clones of revertant hybrid cells between the latter cell type and a human skin fibroblast. These cancerous cells seem incapable of exchanging small inorganic molecules and fluorescein (330 dalton), as determined by electrical measurements and fluorescent tracer diffusion (Borek, Higashino & Loewenstein, 1969; Azarnia & Loewenstein, 1971); of exchanging endogenous nucleotides or their derivatives, as shown by radioautographic studies (Azarnia, Michalke & Loewenstein, 1972); and, at least in the case of the L-1D cell and its revertant hybrid cells, where an exhaustive electron microscopic junctional search was made, the cells seem also incapable of making gap junctions (Azarnia, Larsen & Loewenstein, 1974), the structure now generally thought to contain the junctional channels.

Such radical junctional defects as channel absence or extreme paucity are technically the easiest to detect, and our efforts have heretofore been only in this direction. But, in terms of the above working hypothesis, any defect in channel connection capable of causing a sufficient reduction in the intercellular flow of growth controlling molecules may, in principle, be expected to give rise to uncontrolled growth (Loewenstein, 1968; 1969). We report here the finding of a junctional defect in a cancerous cell type where junctional channels are present, but where the formation of the channels is abnormally slow and the intercellular flow of a 330-dalton molecule is abnormally limited.

Materials and Methods

Cell Types and Media

The following cell types were used. *Normal liver cells*: RLB rat epithelioid liver cell line (Borek *et al.*, 1969); and RLM rat epithelioid liver cell line, kindly provided to us by Dr. I. McPherson. The results obtained with these two cell lines were similar and are lumped together. *SA-21 hepatoma cells*: A malignant epithelioid derivative of an A-cell culture. The A cells originated from explants of Morris' H-5123 rat liver tumor (Azarnia & Loewenstein, 1971). The A cells tend to grow in compact islands. After several months in culture, a variant appeared which spread better on the dishes and grew more as isolated cells. The SA-21 cells are a cloned derivative of these cells.

The normal liver cells were cultured in Eagle-Dulbecco's medium (Vogt & Dulbecco, 1960) and the SA-21 hepatoma cells in Ham's (1965) F-12 medium; both media were supplemented with 10% fetal calf serum. Except for certain controls (p. 182), the cells were in their respective media in all experiments on junction formation.

Cell Dissociation and Reaggregation

Confluent cultures were dissociated by a 10-min exposure to Puck's modified saline A containing 0.5 g/liter trypsin and 0.2 g/liter EDTA, at 37 °C. The cells were rinsed in fresh medium (with serum) and placed 5–10 min in test tubes containing this medium for; suspensions were then obtained by gentle back-and-forth pipetting of the cells. Aliquots of the cell suspensions were examined in a microscope. The dissociation yield, determined for each cell type on 20 culture dishes, for the normal liver cells was $94 \pm 2.6\%$ (SD) single cells, $5 \pm 2.4\%$ cell doublets, $0.9 \pm 1\%$ triplets, and $<0.1\%$ quadruplets; and for the SA-21 hepatoma cells, $100 \pm 0\%$ single cells (EDTA alone was insufficient for full dissociation).

For reaggregation, the cell suspensions ($5 \times 10^5 - 1 \times 10^6$ cells) were transferred to plastic Petri dishes (Falcon, 60 mm diameter) and incubated for varying times. The dishes were coated with protamine sulfate (0.1%). The cells adhered well to this coat and spread rapidly, developing stable contacts with each other.

Determination of Coupling and Fluorescein Transfer

With the aid of two microelectrodes, rectangular pulses of current (1×10^{-8} A) were passed between the interior of one cell and the medium, and the resulting displacements in membrane potential (V) were measured in this cell and in another one. The two cells containing the electrodes will be referred to as *terminal cells*. The microelectrodes were connected to balanced bridge circuits and served each for passing current and measuring potential (Fig. 2, top). This permitted us to measure input resistance and membrane potential in each terminal cell and to exclude membrane damage by electrode insertion as the cause of nonfunctional junction in the coupling-negative cases. One of the microelectrodes was generally filled with KCl (3 M) and the other, with a mixture of fluorescein-Na (160 mM) and KCl (100 mM). The latter electrode served to iontophorese the fluorescent anion into the cell during the electrical measurement of coupling. In a few cases, the fluorescein was microinjected instead by means of a pneumatic pressure system (Rose & Loewenstein, 1976).

In the measurements of electrical coupling on normal liver cell populations, the electrodes were placed so that there was at least one cell intervening between the terminal cells (in many cases two, three or four). This reduced the chance that a coupling measurement was taken between a set of previously undissociated cells to less than 1:100 (*see Cell Dissociation*). One or more continuous chains of cells in contact, as seen at a 180-times magnification, linked the two terminal cells. In the case of the SA-21 cells, which were all single before their reaggregation, the terminal cells were contiguous.

Time zero. It took on the average 2 min from their deposition into the dishes for the suspended cells to reach bottom. For determination of the time of formation of electrically coupling or fluorescein-transferring junction, we used therefore as time zero, the time of cell deposition plus 2 min. All determinations were done on cells that had spread well on the dishes and were in visible contact, which for some of the cells was the case already at about time zero.

Experimental sequences

The general experimental sequence was: (*1*) cell dissociation; (*2*) exposure to fresh medium and suspension; (*3*) plating for reaggregation; (*4*) determination of electrical coupling and/or fluorescein transfer.

During steps *1* and *3* and most of *2*, the cells were in an incubator at 37 °C (5% CO_2, 95% air). During step *4*, the cell dishes were at 32–35 °C, open to room air on a heated microscope stage. This step was kept to 15 min. During this time the cells looked healthy and maintained stable contacts, resting potentials and input resistances. (Indeed, they could maintain this state for another 15 min; thus we had ample safety margin.)

It took 3 min in the fastest experiments to ready the cell dishes and the various components of the electrical measuring system for step *4*. It was then feasible to take the first measurement about 3 min after time zero.

Scoring Method

Fluorescein transfer. For determination of the proportion of fluorescein transferring junctions in a given cell population, we counted the number of fluorescent cells contiguous to the injected cell (*functional junctions*), and the number of nonfluorescent cells contiguous to that cell, and expressed the score as the proportion of functional junctions. Junctions to higher order cells were not counted. Thus, for example, the score in the representation of Fig. 1*A* equals 40% functional junctions.

Electrical coupling. In SA-21 hepatoma cell populations, where the terminal cells were contiguous, the score of functional junctions was the proportion of coupled cell pairs among all pairs tested.

A special problem in scoring arose with the normal cell population. Here the terminal cells were bridged by one or more cells, and a positive finding of coupling conveys a different degree of information from a negative one. Whereas a positive finding gives certainty that the number of functional junctions equals at least the number of junctions in the shortest chain between the terminal cells, a negative finding provides certainty for only one nonfunctional junction in a chain. To reduce the bias resulting from this difference, we used two bracketing scoring methods in the cases of coupling-positive results. In *Method I,* the number of junctions in the shortest connecting chain was scored as functional. In *Method II,* only the junctions between the terminal cells and the nearest neighbors of the shortest connecting chain were scored. In the cases of coupling-negative results, the number of nonfunctional junctions equalled the number of independent pathways between the terminal cells. Thus, in the example of Fig. 1*B*, the scores by the two methods are 50% and 40% functional junctions, respectively.

The two methods are unavoidably biased. *Method II* is more symmetric in its score of coupling and noncoupling situations, but it underestimates the proportion of functional junctions in the population, except in the case of 100% functional junctions. *Method I* overestimates this proportion for most cell topologies. The true values lie somewhere in between the two scores, and all data of the normal cell population are therefore presented in terms of the two methods. As it turned out, the two methods gave only slightly different results (*see* Fig. 3).

In the cell types studied here, long, fine cell processes, as revealed in many kinds of fibroblastic cells upon fluorescein injection, are generally absent. This simplified the task of identifying contiguous cells and shortest cell chains bridging "terminal" cells.

Results and Discussion

The normal liver cells make functional junctions in relatively short time (Fig. 2). Already at time 5 min, the earliest it was feasible to test

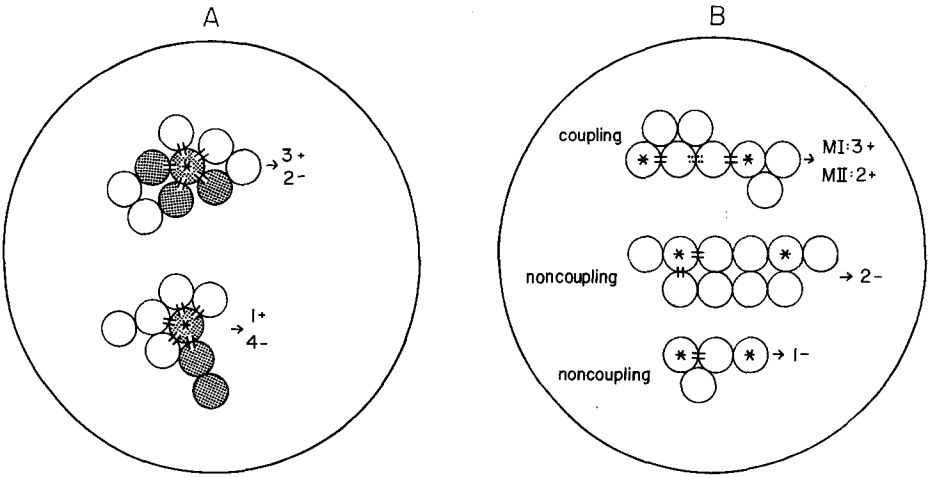

Fig. 1. Examples of scoring method. Cells in contact are diagrammed as osculating circles. Scored junctions are marked (=). Scores of functional (+) and nonfunctional (−) junctions for each test follow the arrows. *(A) Fluorescein transfer:* Cells injected with fluorescein are marked (*); fluorescent cells, grey. Total score equals 40% functional junctions in this population sample. Only first-order junctions are scored (*see* text). *(B) Electrical coupling (normal cell population):* The cells containing the measuring electrodes (terminal cells) are marked (*). On top, a coupling-positive measurement; below, coupling-negative measurements with 2 and 1 independent chains between terminal cells, respectively. Total score by *Method I* (MI) equals 50% by *Method II* (MII), 40% functional cells

coupling, a substantial fraction of the cell contacts in the populations sampled showed electrical coupling. In the interval 5–15 min, on the average 10–13% of the contacts were electrically coupled and by 30–40 min all contacts were coupled (Fig. 3). Cell-to-cell transfer of fluorescein became detectable at time 15 min: 10% of the cell contacts transferred fluorescein at 15–25 min, and all of them at 60–120 min (Fig. 4). These times of formation of functional junction are quite comparable with those estimated for a variety of other normal cell types (Loewenstein, 1967; Ito & Loewenstein, 1969; Bennett & Trinkaus, 1970; Hülser & Peters, 1972; deHaan & Hirakow, 1972; Hammer *et al.*, 1973; Ito *et al.* 1974*a*; Johnson *et al.*, 1974).

The SA-21 hepatoma cells make junctions more slowly. The first electrical coupling between contacting cells occurred somewhere in the interval of 75–125 min, and only after 180–240 min were all sampled cell contacts coupling (Fig. 3). The contrast was even more striking in

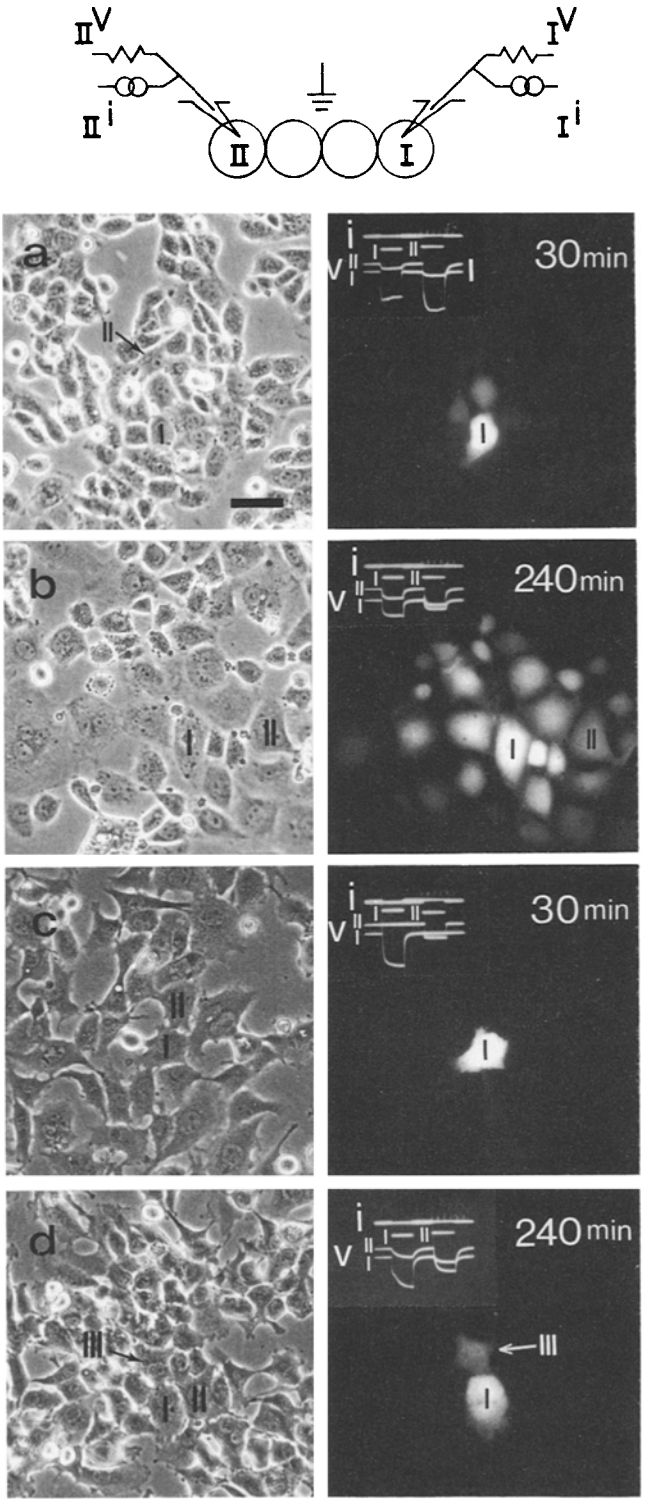

Fig. 2

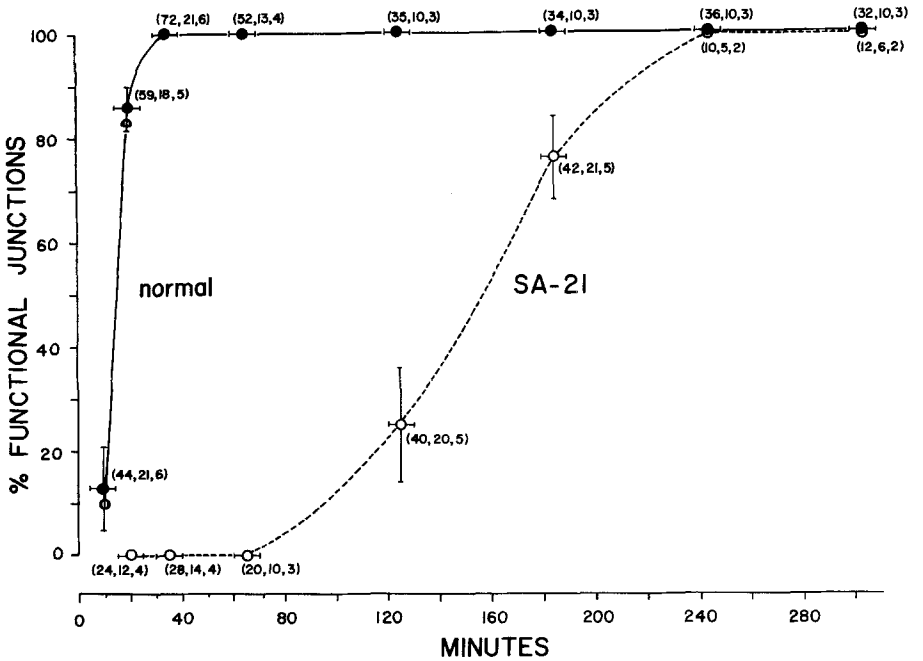

Fig. 3. Development of electrically coupled junctions between normal liver cells (●), and SA-21 hepatoma cells (○). *Ordinates:* percentage of electrically coupled junctions of the sampled populations. Plotted are the means of the various populations (culture dishes), as obtained by scoring *Methods I* (●) and *II* (◍). The vertical bars subtend the standard error; in parentheses are first, the number of functional junctions scored; second, the number of measurements; and third, the number of culture dishes. *Abscissae:* time of junctional testing; time 0 = time of plating +2 min. The horizontal bars give the time span (10 min) of the various tests

Fig. 2. Formation of functional junction in normal liver cells and SA-21 hepatoma cells. *Top:* diagram of electrical arrangement. Current (*i*) is pulsed into cell *I* and, with a 100 msec delay, into cell *II*. The resulting voltages (V) are measured in the two cells (terminal cells). Simultaneously fluorescein (F^-) is injected into cell *I*. The terminal cells in the SA-21 cells are contiguous; in the normal cells they are two or more junctions apart. *Main Figure:* electrical coupling and fluorescein transfer between normal (RLM) liver cells (*a, b*) and SA-21 hepatoma cells (*c, d*) determined 30 min and 240 min after cell aggregation. Micrographs of the cells in phase contrast (*left*), and in darkfield (*right*). Calibration 50 μ. *Insets:* oscilloscope records of *i* and *V*; calibration 100 mV, current pulse duration 100 msec. Terminal cells are marked *I* and *II*. Note that in *d* the electrically coupled terminal cells do not transfer fluorescein; fluorescein is transferred to cell *III* adjacent to one of the terminal cells

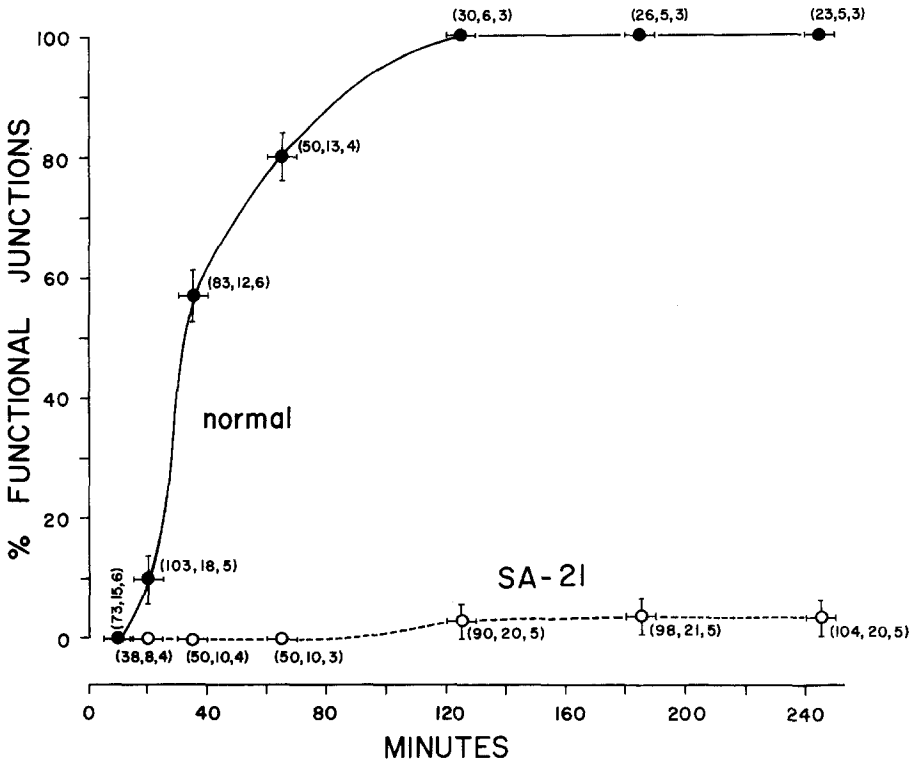

Fig. 4. Development of fluorescein-transferring junctions between normal liver cells (●) and SA-21 hepatoma cells (○). *Ordinates:* mean percentage of fluorescein transferring junctions. *Abscissae:* the test time. Symbols and notations as in Fig. 3

regard to fluorescein transfer. The first transferring junctions were found at 75–125 min when 3–4% of the contacts were functional. This proportion did not substantially change for 4 hr (Fig. 4), although by then all contacts were electrically coupled (Fig. 3). The tests were continued on 130 cells for 24 and 48 hr, and still only 4 and 7%, respectively, of the contacts transferred fluorescein.

There were no obvious differences in movement and contact relations between the normal liver cells and SA-21 cells. The two cell types spread equally well on the coated dish surface and established contact. In both cases, tests of functional junction were carried out only on cells in contact as seen at 180-times magnification in the phase-contrast microscope.

The rate of functional junction formation and the fraction of fluorescein transferring SA-21 cells did not improve in Eagle-Dulbecco's medium (in which the normal cells were grown). Although the SA-21 cells do not divide in this medium, they can be maintained in it for

24 hr without sign of deterioration. Electrically coupled junctions were not found before 120 min, and fluorescein transferring junctions were not seen during the entire 24-hr period in this medium. The converse experiment, exposure of the normal liver cells to Ham's medium (the growth medium of the SA-21 cells) was feasible for periods of 3 hr (the cells visibly deteriorated with longer exposures). Junctions were tested in this medium at 10–15 min and at 50–60 min. During the first test period, a substantial proportion of the cell contacts were electrically coupled; and during the second, many in addition transferred fluorescein. Thus, the deviant junctional behavior of the SA-21 cell is not simply due to the medium.

The present results indicate a marked retardation in the process of permeable junction formation in the SA-21 hepatoma cells. We will discuss these results in terms of present notions of permeable junction formation.

A simple formation picture is that permeable junction develops by an accretion of junctional channel units at the region where the cells come into contact (Ito, Sato & Loewenstein, 1974*b*). This idea is based on the following experimental facts: (*i*) Isolated junction-competent cells have a high cell membrane electrical resistance ($10^4\ \Omega\ cm^2$). Following contact of two such cells at random membrane spots (sponge, newt embryo, fundulus) the resistance falls at the membrane junction (Loewenstein, 1967; Ito & Loewenstein, 1969; Bennett & Trinkaus, 1970). (*ii*) The fall in junctional membrane resistance (newt embryo cells) is progressive with time, as expected in the case of a parallel addition of membrane channel units (Ito *et al.*, 1974*b*). (*iii*) The number of junctional membrane particles in freeze-fractured "gap" junction (generally assumed to contain the channels; *cf.* McNutt & Weinstein, 1973; Goodenough, 1974; Peracchia & Fernandez-Jaimovich, 1975; Gilula & Epstein, 1976) increase with time after aggregation of isolated cells (junction-competent Novikoff hepatoma cells; Hammer *et al.*, 1973; Johnson *et al.*, 1974).

An additional notion is that the junctional channel unit is made of two matching permeable membrane elements on the two adjoining membranes, the membrane channels (Loewenstein, 1966, 1974; Goodenough, 1975; Gilula & Epstein, 1976). Hence, the above picture of formation of permeable junction involves also alignment of the membrane channels, besides their accretion.

Thus, considered in terms of this model of junction formation, the present results may reflect a retarded process of accretion or of alignment of channels in the SA-21 hepatoma cells.

The lag of development of fluorescein transfer behind that of electrical coupling in both types of cells may also be easily explained in this light, if we may assume that, with the present method, detection of fluorescein transfer requires the formation of more junctional channels than the detection of electrical coupling. This is plausible, because, as analyzed elsewhere (Délèze & Loewenstein, 1976), the sensitivity of the electrical measurements for discriminating changes in junctional conductance, that is, in the number of functional channels, is highest in the low range of the coupling ratio. Thus, the general lag in the development of fluorescein transfer would simply reflect the time used for accretion of additional channels.

Along the same lines of reasoning, the result that less than 5% of the SA-21 cell contacts ever show fluorescein transfer may be accounted for if the number of channels present in the steady state exceeds the limit of detection of the fluorescein method only in 5% of these cells. This number of channels could be limited by a paucity of available channel components or by defect in the assembly mechanism that causes the rate of formation of junctional channel units to be slow relative to the (hypothetical) channel breakdown. A single membrane abnormality could account, in principle, for all the observed deviant behaviors.

An alternative is that in addition to retardation of the process of junction formation, the SA-21 cells suffer from an alteration in junctional channel structure (an alteration in the membrane channels themselves or in their alignment) that reduces the effective channel bore, limiting the passage of the 330-dalton fluorescein, but not, or to a much lesser degree, that of the small inorganic ions carrying the electrical currents. To distinguish between the two alternatives, one needs to probe the permeability of the junction with molecules of different size. A reduction of effective junctional channel bore has just been shown to occur in normal cells (*Chironomus* salivary gland) as the result of small elevations of the cytoplasmic free Ca^{2+} concentration (Rose, Simpson & Loewenstein, 1977).

The question of the underlying mechanism aside, the SA-21 hepatoma cells show a pronounced defect in intercellular communication. The defect is not as radical as that presented by several other malignant cells – A-hepatoma cells, certain X-radiated hamster embryo cells, C1/1DL-cells and their revertant hybrids – which appear incapable of making detectable permeable junctions (Borek *et al.*, 1969; Azarnia & Loewenstein, 1971; Azarnia *et al.*, 1972; Azarnia *et al.*, 1974). Nonetheless, the defect severely limits the exchange of a 330-dalton molecule between these cells and,

for cells making new junctions, it delays the onset of such exchange of all permeant molecular species. Thus, viewed in terms of our working hypothesis (Introduction), the junctional defect here may be as important in the etiology of uncontrolled growth as the more radical defect; and the general considerations on cancer etiology and defective junctional communication published elsewhere (Loewenstein, 1968; 1969; 1975*a*) apply here also.

References

Azarnia, R., Larsen, W.J., Loewenstein, W.R. 1974. The membrane junctions in communicating and non-communicating cells, their hybrids and segregants. *Proc. Nat. Acad. Sci. USA* **71**:880

Azarnia, R., Loewenstein, W.R. 1971. Intercellular communication and tissue growth, V. A cancer cell strain that fails to make permeable membrane junctions with normal cells. *J. Membrane Biol.* **6**:368

Azarnia, R., Loewenstein, W.R. 1977. Intercellular communication and tissue growth. VIII. A genetic analysis of junctional communication and cancerous growth. *J. Membrane Biol.* (*in press*)

Azarnia, R., Michalke, W., Loewenstein, W.R. 1972. Intercellular communication and tissue growth. VI. Failure of exchange of endogenous molecules between cancer cells with defective junctions and noncancerous cells. *J. Membrane Biol.* **10**:247

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

Borek, C., Higashino, S., Loewenstein, W.R. 1969. Intercellular communication and tissue growth. IV. Conductance of membrane junctions of normal and cancerous cells in culture. *J. Membrane Biol.* **1**:274

Dehaan, R.L., Hirakow, R. 1972. Synchronization of pulsation rates in isolated cardiac myocytes. *Exp. Cell Res.* **70**:214

Délèze, J., Loewenstein, W.R. 1976. Permeability of a cell junction during intracellular injection of divalent cations. *J. Membrane Biol.* **28**:71

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

Gilula, N.B., Epstein, M.L. 1976. Cell-to-cell communication, gap junctions and calcium. *1975 Symp. Soc. Exp. Biol.* (*in press*)

Gilula, N.B., Reeves, O.R., Steinbach, A. 1972. Metabolic coupling, ionic coupling and cell contacts. *Nature* (*London*) **235**:262

Goodenough, D.A. 1974. Bulk isolation of mouse hepatocyte gap junction. Characterization of the principal protein, connexin. *J. Cell Biol.* **61**:557

Goodenough, D.A. 1975. The structure and permeability of isolated hepatocyte gap junctions. *Cold Spring Harbor Symp. Quant. Biol.* **40**:37

Goodenough, D.A., Revel, J.-P. 1970. A fine structural analysis of intercellular junctions in the mouse liver. *J. Cell Biol.* (*Abstr.*) **59**:130*a*

Ham, R.G. 1965. Clonal growth of mammalian cells in a chemically defined synthetic medium. *Proc. Nat. Acad. Sci. USA* **53**:288

Hammer, M., Epstein, M., Sheridan, J. 1973. Gap junction formation in reaggregating system. *J. Cell Biol.* (*Abstr.*) **59**:130*a*

Hülser, F.A., Peters, J.H. 1972. Contact cooperation in stimulated lymphocytes. II. Electrophysiological investigations on intercellular communication. *Exp. Cell Res.* **74**:319

Ito, S., Loewenstein, W.R. 1969. Ionic communication between early embryonic cells. *Dev. Biol.* **19**:228

Ito, S., Sato, E., Loewenstein, W.R. 1974*a*. Studies on the formation of a permeable cell membrane junction. I. Coupling under various conditions of membrane contact. Effects of colchicine, cytochalasin B, dinitrophenol. *J. Membrane Biol.* **19**:305

Ito, S., Sato, E., Loewenstein, W.R. 1974*b*. Studies on the formation of a permeable cell membrane junction. II. Evolving junctional conductance and junctional insulation. *J. Membrane Biol.* **19**:339

Johnson, R., Hammer, M., Sheridan, J., Revel, J.-P. 1974. Gap junction formation between reaggregated Novikoff hepatoma cells. *Proc. Nat. Acad. Sci. USA* **71**:4536

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.* **19** (*Suppl. 2*):151

Loewenstein, W.R. 1969. Transfer of information through cell junctions and growth control. *In:* 8th Canadian Cancer Conference, Honev Harbor. J.F. Morgan, editor. p. 162. Pergamon Press, Toronto

Loewenstein, W.R. 1974. Cellular communication by permeable junctions. *In:* Cell Membranes: Biochemistry, Cell Biology and Pathology. G. Weissmann and R. Claiborne, editors. p. 105. HP Publishing Co., New York

Loewenstein, W.R. 1975*a*. Intercellular communication in normal and neoplastic tissues. *In:* Cellular Membranes and Tumor Cell Behavior. p. 239. *28 Annu. Symp. Fundam. Cancer Res.* Williams & Wilkins Co., Baltimore

Loewenstein, W.R. 1975*b*. Permeable junctions. *Cold Spring Harbor Symp. Quant. Biol.* **40**:49

McNutt, N., Weinstein, R.S. 1973. Membrane ultrastructure at mammalian intercellular junction. *Prog. Biophys. Mol. Biol.* **26**:45

Peracchia, C., Fernandez-Jaimovich, M. 1975. Isolation of intramembrane particles from gap junctions. *J. Cell Biol.* **67**:330*a*

Revel, J.-P., Karnovsky, M.J. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver *J. Cell Biol.* **33**:*C7*

Rose, B., Loewenstein, W.R. 1976. Permeability of a cell junction and the local cytoplasmic free ionized calcium concentration. A study with aequorin. *J. Membrane Biol.* **28**:87

Rose, B., Simpson, I., Loewenstein, W.R. 1977. Calcium ion produces graded changes in permeability of membrane channels in cell junction. *Nature (London) (in press)*

Simpson, I., Rose, B., Loewenstein, W.R. 1976. Size limit of molecules permeating the junctional membrane channels. *Science (in press).*

Vogt, M., Dulbecco, R. 1960. Virus-cell interaction with a tumor-producing virus. *Proc. Nat. Acad. Sci. USA* **46**:365