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Intercellular Communication and Tissue Growth

IV. Conductance of Membrane Junctions of Normal and Cancerous Cells in Culture*

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Summary. Epithelial cells of normal rat (adult) liver and hamster embryo in tissue culture communicate through membrane junctions: the membrane regions of cell contact are highly ion-permeable. Cancerous counterparts of these cells, cells from Morris' and Reuber's liver tumors and from x-ray-transformed embryo cultures, do not communicate under the same experimental conditions. These cells also fail to communicate with contiguous normal cells. Cancerous fibroblastic cells from a variety of tissues, including cells transformed by virus, x-radiation and chemicals, communicate as well as their normal counterparts; this is so for long- and short-term cell cultures. Communication in some fibroblastic cells is sensitive to components of blood serum: normal and transformed hamster embryo fibroblasts, which communicate when cultured in medium containing fetal calf serum, appear to lose communication in medium containing calf serum; the converse holds for hamster (adult) fibroblasts and 3T3 cells.

Earlier studies from this laboratory have indicated that cells in certain epithelial tumors lack the kind of communication through specialized membrane junctions which is characteristic of cells in normal epithelia (Loewenstein & Kanno, 1966, 1967; Penn, 1966; Loewenstein & Penn, 1967; Jamakosmanovic & Loewenstein, 1968). These studies were done on cells inside solid tumors and organs. We have now extended these studies to epithelial cells in tissue culture where communication can be measured in cancerous and normal counterparts under more closely matched conditions, particularly with respect to the number and distribution of cells, composition of the extracellular medium, and mechanical properties of the cell substratum.

We also examine here the communication between cultured fibroblast cells isolated from tumors. Potter, Furshpan, and Lennox (1966) have

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already shown that cells of fibroblastic strains transformed by simian virus (SV-40) and polyoma virus are in good electrical communication. These cells were in long-term culture and transformed in vitro. Our aim here is to look into the pattern of communication between cancerous fibroblasts shortly after their isolation from animal tumors so as to minimize the effects of possible differences in selection pressures, between tumors and cultures.

A preliminary report has already appeared (Higashino, Borek & Loewenstein, 1969).

Methods

Cell Cultures

The following cell material was used. (1) *Normal liver (epithelial) cells*: cells cultured from rat liver with Coon's (1969) technique after three clonal isolations, diploid; and a rat liver cell line ("clone 9"), similarly cultured, kindly provided to us by Dr. E. Kaighn. The cells of both lines were differentiated hepatocytes, producing serum proteins (as determined by radio-immunoelectrophoresis) and glycogen; they gave identical results of communication. (2) *Cancerous liver cells*: (a) Cells from Morris' H-5123 rat tumor (Morris, 1965; Novikoff & Biempica, 1966), pseudo-diploid, cultured and cloned as the normal liver cells. Both normal and Morris' cells were from the same rat strain (Buffalo). The Morris' cells produced tumors when reinjected into animals. (b) Cell line H_4IIEC (Pitot, Periano, Morse & Potter, 1964). (3) *Normal epithelial cells*: in a primary culture of a mixed population of epithelial and fibroblast cells from 12-day-old hamster embryos. (4) *Normal fibroblasts:* (a) *Embryonic*, secondary and tertiary cultures of #3, predominantly fibroblastic, (b) *Adult*, primary cultures obtained from newborn hamster or rat, predominately fibroblastic. (5) *X-ray-transformed fibroblasts:* (a) Transformed by irradiation (300 r) in vitro of #4, after 10 subcultures following the irradiation. (b) Cell line 4-years-old transformed as #5a, pseudo-diploid (Borek & Sachs, 1966, 1967). (6) *X-ray-transformed epithelioid cells:* a variant clone of epithelioid morphology, isolated from #5b. (7) *Fibrosarcoma cells:* (a) Cells from a primary hamster tumor (P-68/31B and P-68/45B) induced by simian virus (SV-40) (Defendi, Carp & Gilden, 1966). The cells were derived from five contact-uninhibited clones of the primary culture of the tumor. (b) Cell line H 68/23, 3 months in culture, isolated from a primary hamster fibrosarcoma induced by SV-40 by Dr. V. Defendi. The cells were tumor antigen (nuclear)- and transplantation antigen-positive. (8) Fibroblastic cells from *Novikoff's tumor* (Novikoff, 1957). (9) *Virus-transformed cells:* mouse embryo 3T3 cell line, transformed in vitro by SV-40, tumor-antigen positive (Todaro, Green & Goldberg, 1964; Todaro, Habel & Green, 1965).

The normal and cancerous tissues from which the cell cultures were derived were minced, and the cells (except for those of liver and Morris' hepatoma) were dissociated by treating the tissue fragments with a 0.25 % trypsin solution which was Ca-, Mg-free (Grand Island Biological Co.). The cells were cultured in Eagle's medium (Eagle, Oyama, Leuy & Freeman, 1957), as modified by Vogt and Dulbecco (1960), supplemented with calf or fetal calf serum (10 % v/v) and equilibrated with a 5 % CO_2-air mixture (pH 7). The liver and hepatoma cells were dissociated with 0.1 % trypsin — 0.1 % collagenase — 1 % chicken serum in Hank's solution which was Ca-, Mg-free. They were cultured for three clonal passages in Ham's F-12 medium containing a twofold concentration of amino acid and 5 % fetal calf serum, and then continued in the same media (gener-

ally with fetal calf serum supplement) as the other cells. All cells were grown in plastic petri dishes (60-mm diam., Falcon Plastics) at 36.5 °C. The seeding levels ranged from 10^5 to 10^6 cells per dish, except for the cultures used for electrical measurements on nonosculating cells which were seeded at a level of 5×10^4 per dish. Unless stated otherwise, the culture media were changed for fresh ones every 3 days and at the start of a measuring series of communication.

The cell cultures used for measurements of junctional communication were grown on the surface of a soft dielectric resin (Sylgard 184, Dow Corning) coating the bottom of the petri dishes. The resin-coated plates were sterilized by application of ethyl alcohol (100 %) and ultraviolet irradiation (30 min); the resin surface was conditioned for culturing by bathing it with culture medium at 37 °C for 2 days before cell seeding. The resin offered the advantage of a cell base which microelectrodes could enter without breaking. Moreover, its electrical resistance, very much higher than that of the cell interior, allowed easy and precise recognition of the cell boundaries in the vertical dimension at all times during the measurements of junctional communication.

Electrical Measurements

Junctional communication was measured by pulsing currents (i) with a microelectrode between one cell interior (I) and the exterior, and by recording the resulting voltages (V) with a second microelectrode inside a contiguous cell (II) (Fig. 2, diagram; Loewenstein & Kanno, 1964). The corresponding V_{II}/i curves were nearly linear (Fig. 2) and their slopes (*transfer resistances*) were discernible down to 0.05 MΩ. The criterion for uncoupling was a transfer resistance of less than 0.05 MΩ. Transfer resistances of normal cells in favorable media were at least one order and generally two orders of magnitude greater. The measurement of transfer resistance was followed by two steps of *input resistance* measurement (V_I/i_I, V_{II}/i_{II}) with both electrodes first in cell I and then in cell II[1]. The two latter steps were obligatory in the case of uncoupled cells. They provided the criterion for surface membrane (nonjunctional) integrity, an input resistance of 0.5 MΩ. Cases with lower input resistances in either cell I or II were rejected, thus eliminating surface membrane damage as a possible cause of uncoupling (*see* Loewenstein & Kanno, 1967). The 0.5 MΩ limit was chosen because the input resistance in the various types, measured in widely scattered cells not in contact, ranged from 0.5 to 60 MΩ.

The ratio $V_{II}/i_I : V_I/i_I$ (hereafter *communication ratio*) provided a convenient index of communication. It is in principle the same index as that used in the preceding papers of this series, but the experimental derivation of the ratio differs in that V_{II} and V_I are determined successively, not simultaneously. This method yielded more data in the case of coupled cells where each step in the measuring sequence thus provided useful statistics, even if subsequent steps failed.

For estimations of specific membrane resistance, cell surface areas were determined by planimeter measurements in the focal plane of photomicrographic enlargements of the cells. No allowance was made for the depth dimension or for surface infoldings. The areas and, hence, the specific resistances (Table 2) are therefore all underestimated.

To minimize the danger of unrecognized cell overlap, the measurements of communication were taken on small groups of single-layered cells or an just-confluent single layers of cells, such as shown in Fig. 1 (*1*), (*2*), and (*4*)[2] where cell boundaries were visible

1 Input and transfer resistances were calculated from curves with many points, such as illustrated in Fig. 2.

2 Fig. 1 (*3*) only serves to illustrate the type and the contact-uninhibited character of the x-ray-transformed cells; populations so dense were not used for measurements of communication.

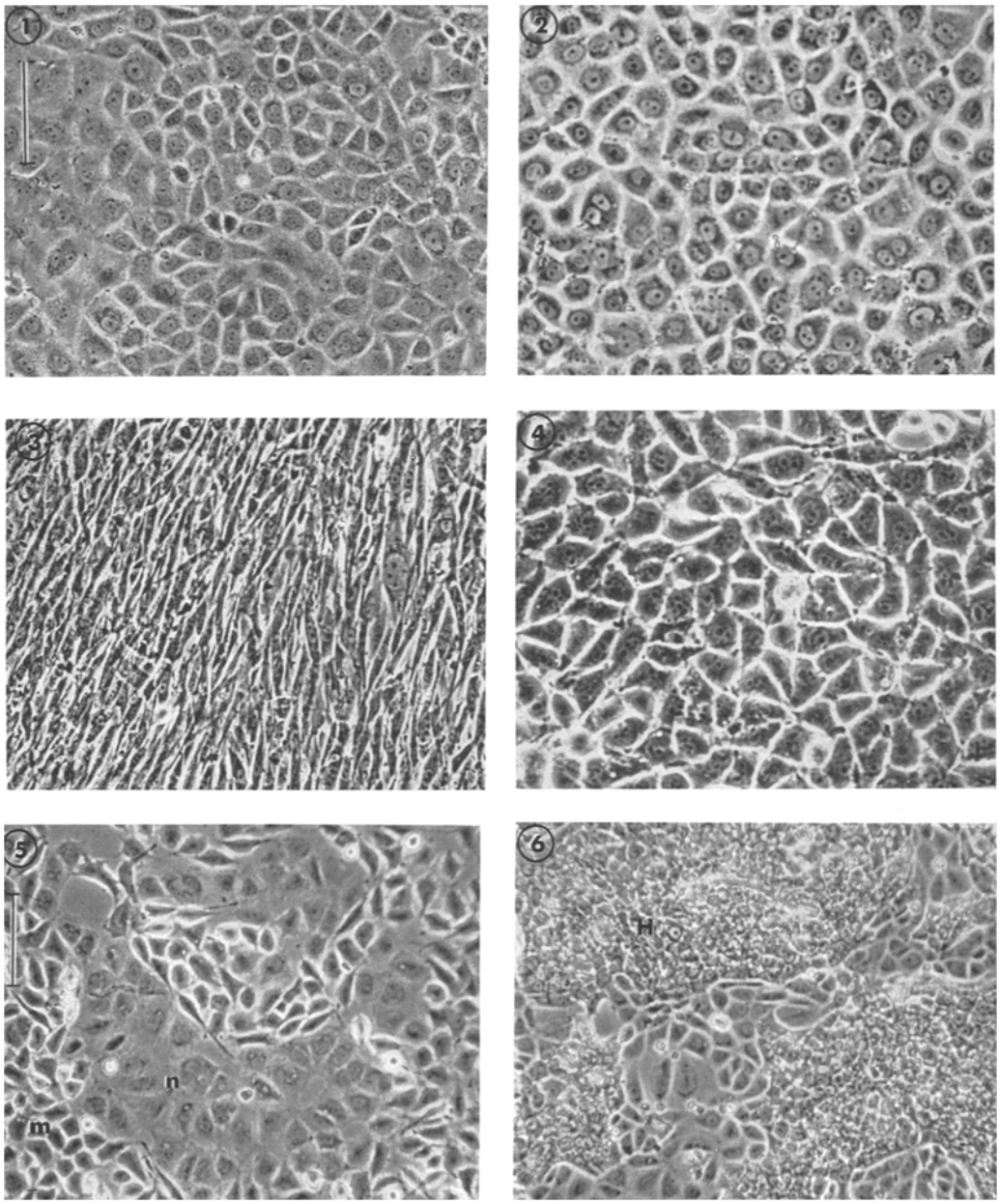

Fig. 1. (*1*) Cultures of normal epithelial cells of adult rat liver, and (*2*) of Morris' H 5123 rat hepatoma cells. First passages after establishment of the clones in three consecutive isolations. (*3*) X-ray-transformed hamster embryo fibroblasts. Tenth passage after establishment of the line; 90 days after x-irradiation. (*4*) Epithelioid variant isolated from a culture of a 4-year-old, x-ray-transformed, hamster embryo fibroblast line. (*5*) Mixed culture of normal rat liver (*n*) and Morris' H 5123 hepatoma cells (*m*). The two cell kinds have been cultured together for 2 days. (*6*) Mixed cultures of normal rat and H_4IIEC hepatoma cells, cultured 4 days together. Phase contrast photomicrographs in vivo. Upper calibration: 100 μ for (*1*), (*2*), (*3*), and (*4*); Lower calibration: 100 μ for (*5*) and (*6*)

in the focal plane. The only exception to this was a measuring series on crowded foci of H_4IIEC cells specially mentioned in the Results. The measuring series lasted up to 2 − 1/2 hr, generally 2 hr, with fibroblasts, and up to 1 hr with epithelial cells. Each culture dish generally yielded data on 3 to 10 junctions. There were no noticeable changes in communication within these spans. All measurements were taken at room temperature ranging from 25 to 27 °C.

Table 1. *Parameters of*

Cell type	Culture stage[a]		Resistance 10^6 Ω[b]	
	Cloned	Uncloned	Input (V_I/i_I)	
			F-C serum	C serum
Normal				
epithelial, liver (rat)	1st, 6th, >20th		3.4±0.6 (23)	3.4±0.4 (10)
fibroblast (hamster embryo)		2nd, 3rd	7.4±1.4 (26)	3.3±0.5 (29)
epithelial (hamster embryo)	1st		1.7±0.4 (15)	
fibroblast (hamster adult)	1st		2.8±0.9 (16)	2.8±0.5 (18)
fibroblast (rat)	1st		4.8±1.4 (14)	3.3±0.59 (13)
3T3 (mouse embryo)[d]			6.9±2.3 (10)	8.2±2.7 (7)
Cancerous, from animal tumor				
Morris' H 5123 liver (rat)[g]	2nd, 3rd, >20th		8.8±1.2 (27)	4.1±0.8 (9)
H_4IIEC liver (rat)[g]	[d]		2.5±0.4 (19)	3.1±0.3 (11)
fibrosarcoma P-68/31B and P-68/45B[g]				
SV-40-induced (hamster)[g]	1st		3.8±0.3 (50)	6.3±0.9 (23)
fibrosarcoma H-68/23[g]				
SV-40-induced (hamster)		[e]	6.3±1.2 (30)	11.3±2.1 (17)
Novikoff fibroblast (rat)	1st		5.0±0.6 (13)	
Transformed in vitro[g]				
epithelioid, x-ray-induced (hamster embryo)	1st, 2nd, >20th		8.0±1.1 (21)	6.2±1.7 (9)
fibroblast, x-ray-induced (hamster embryo)	[d]		9.2±0.9 (23)	9.2±1.2 (43)
fibroblast, x-ray-induced (hamster embryo)	10th,[f]		3.6±0.4 (23)	
3T3, SV-40-induced (mouse embryo)	[d]		5.2±0.6 (56)	5.9±1.1 (20)

[a] Number of passages in culture from one full dish to another after isolation of the clone ("cloned" column) or after isolation from the animal ("uncloned" column).

[b] Mean values with their standard errors where given as single figures; minimal and maximal values where given as range. In parentheses, the number of cells or cell pairs on which measurements were done. F-C, in fetal calf; C, in calf serum.

Results

Epithelial Cells

Homogeneous Cultures. The results obtained in the various cell types are summarized in Table 1. The picture of junctional communication was relatively simple in the epithelial cell cultures: the normal liver and embryo

communication in osculating cells

Transfer (V_{II}/i_I)		Communication[c] (V_{II}/i_I: V_I/i_I)	
F-C serum	C serum	F-C serum	C serum
3.3 ± 0.5 (21)	2.5 ±0.3 (14)	0.96	0.75
7.2 ±1.9 (25)	<0.05[h] – 7.6 (25)	0.97	<0.005[h] – 0.76
0.75 ±0.2 (15)		0.44	
<0.05[h] – 2.0 (15)	1.7 ±0.3 (20)	<0.01[h] – 0.72	0.62
<0.05[h] – 3.0 (12)	1.9 ±0.4 (15)	<0.01[h] – 0.53	0.56
<0.05[h] – 3.3 (9)	1.8 ±0.4 (9)	<0.005[h] – 0.33	0.22
<0.05[h] (24)	<0.05[h] (7)	<0.005[h]	<0.01[h]
<0.05[h] (18)	<0.05[h] (16)	<0.02[h]	<0.005[h]
<0.05[h] – 16.6 (55)	3.0 ±0.4 (25)	<0.01[h] – 0.6	0.5
<0.05[h] – 15.0 (23)	5.6 ±1.5 (16)	<0.01[h] – 0.35	0.47
2.8 ±0.4 (20)		0.61	
<0.05[h] (19)	<0.05[h] (6)	<0.006[h]	<0.008[h]
4.7 ±0.7 (14)	<0.05 – 6.2 (41)	0.51	0.005[h] – 0.74
1.6 ±0.2 (21)		0.45	
<0.05[h] – 14.4 (42)	3.8 ±0.8 (21)	0.005[h] – 0.67	0.64

[c] $\overline{V_{II}/i_I}$: $\overline{V_I/i_I}$ where given as single figure; V_{II}/i_I: V_I/i_I where given as range.
[d] Cell line in long-term culture.
[e] 2 months in culture.
[f] 90 days after x-radiation.
[g] Growth not inhibited by cell contact.
[h] Limit of resolution of the method. Uncoupled.

cells communicated well, at least as far as their small ions are concerned; the two cancerous types of liver cells and the transformed epithelioid embryo cells did not communicate to any detectable degree.

In the normal cells, a considerable fraction of the probing ion current injected into one cell flowed into the contiguous cells (Fig. 2). This was so in sparse cultures as well as in confluent ones. In the latter, ion communication was detectable over distances of several cell diameters from the current sources, and any given cell was in communication with many, if not all, of its neighbors. These epithelial cells thus appear to establish in culture the same kind of permeable membrane junctions as they do in

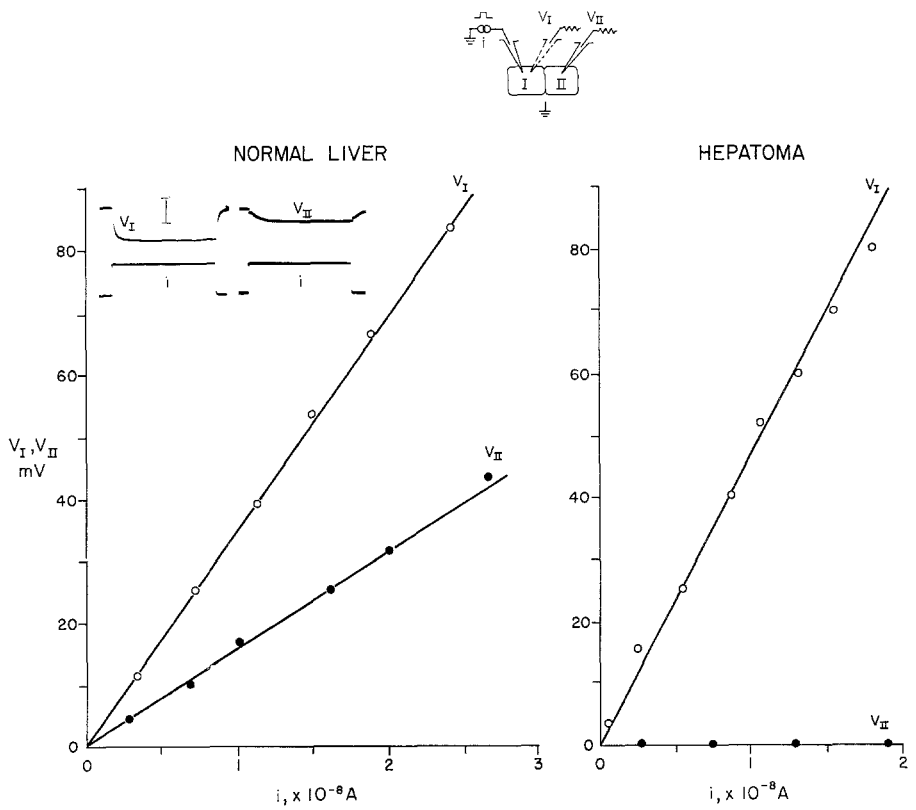

Fig. 2. Membrane current-voltage relations in normal liver and Morris' H 5123 hepatoma cells in culture. Current (i) is pulsed inward between a microelectrode inside one cell (I) and the outside (grounded). The resulting changes in membrane voltage (V) are recorded with a second microelectrode in a contiguous cell (II) and, in a subsequent measuring series, in cell I. Both cell cultures in fetal calf serum. Note the virtually zero V_{II}/i slope (transfer resistance) of the hepatoma cells against that of nearly $1.5 \times 10^6\ \Omega$ of the normal liver cells. *Left inset*; oscilloscope record samples of membrane currents ($i = 7 \times 10^{-9}$ A, 100 msec pulse duration) and voltage in the normal liver cell cultures. Voltage calibration: 20 mV

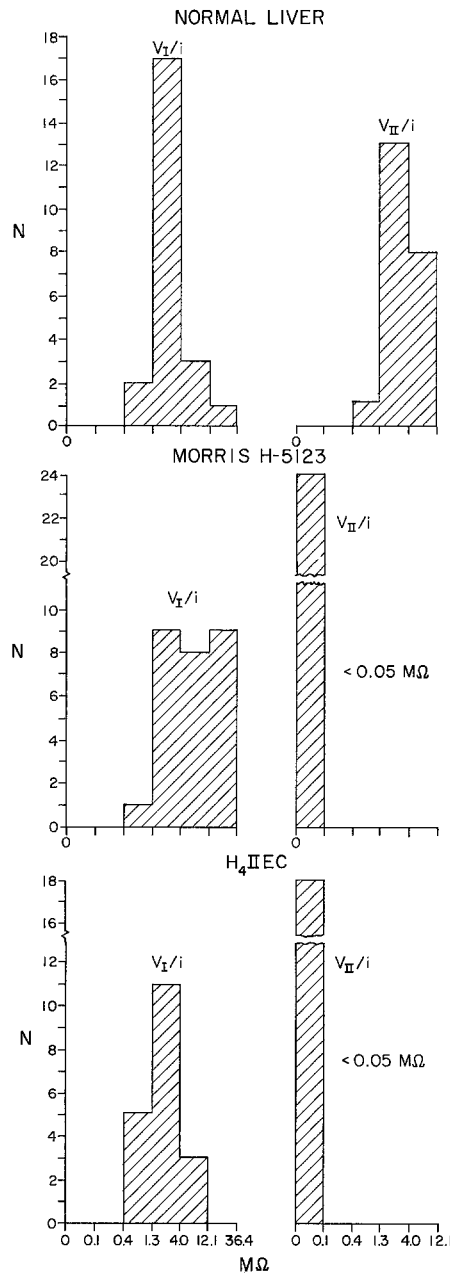

Fig. 3. Junctional uncoupling in cancerous liver cells (rat). Histograms of input (V_I/i_I) and transfer (V_{II}/i_I) resistances in normal liver, H_4IIEC, and Morris' H 5123 cells (in fetal calf serum). *N*, the number of cells or cell pairs on which input or transfer resistances were measured. All transfer resistances in Morris' and H_4IIEC cells are below the limit of resolution of the method (0.05 MΩ); that is, the cells are uncoupled

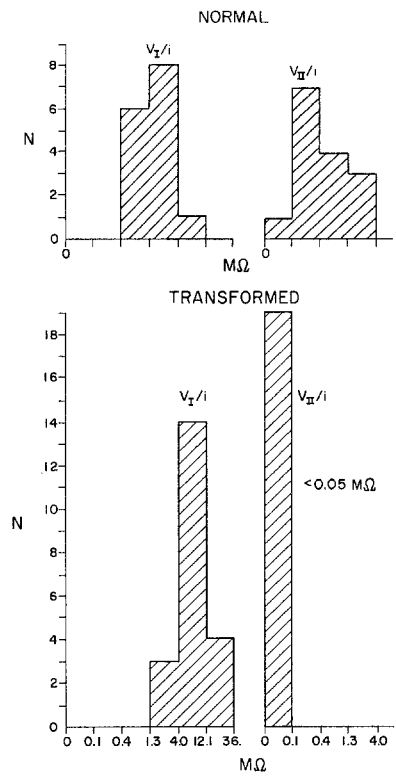

Fig. 4. Uncoupling in x-ray-transformed epithelioid cells (hamster embryo). Histograms of input and transfer resistances. *Top*: in normal epithelial cells. The single transfer resistance case in the interval 0—0.1 MΩ is 0.09 MΩ. *Bottom*: in x-ray-transformed epithelioid cells. All cases in the intervals 0—0.1 MΩ transfer resistance are uncoupled. (Cultures in fetal calf serum)

organized tissues (Loewenstein & Kanno, 1964; Loewenstein, Socolar, Higashino, Kanno & Davidson, 1965; Penn, 1966; Potter et al., 1966; Sheridan, 1968). The corresponding frequency distribution of input and transfer resistances are shown in Figs. 3 (top) and 4 (top). There is considerable scatter in the values, probably reflecting in large part scatter in cell size and in degree of membrane sealing around the electrodes. But the essential point here is that all cases have a substantial transfer resistance. The communication ratios, $\overline{V_{II}/i_I}:\overline{V_I/i_I}$, in media containing calf or fetal calf serum, ranged from about 1/2 to near unity.

In contrast, the transfer resistances of the two kinds of cancerous liver cells and of the x-ray-transformed epithelioid cells were, in all cases, below 0.05 MΩ, the limit of resolution of the method; this was so regardless of the kind of serum used (Figs. 2, 3, and 4).

The aforegoing results were obtained on single-layered cultures in which cell boundaries were well defined [e.g., Fig. 1(*1*) and (*2*)]. In H_4IIEC cultures, there were occasional small foci in which cells were multilayered and cell boundaries were blurred or invisible in the light microscope. In such foci, we got often transfer of current between the measuring electrodes which, using nuclei as markers, appeared to be inside different cells. It is doubtful, however, that this represents current transfer through membrane junctions; the electrodes may have been inside processes of the selfsame cells, overlapping with those of a neighbor, or there may have been actual cell fusion (with membrane dissolution) in such foci.

Rough estimates of specific nonjunctional membrane resistances, made on the basis of input resistances and planimeter measurements on widely separated cells in sparse cultures, appear in Table 2. The values are given here for general information only. They are underestimates (*see* Methods). The extent of the underestimates differs in the various cell types; the values are therefore not comparable. The Morris' hepatoma and normal liver cells are the only class with sufficiently similar shapes in which one may reasonably expect the underestimates to be similar. These cells show no significant difference in specific nonjunctional membrane resistance.

Cancerous – Normal Cell Mixtures. The patterns of communication in homogeneous cell cultures were retained in mixtures of normal liver and Morris' hepatoma cells, and of normal liver and H_4IIEC cells. The two cell kinds could be easily distinguished by their morphology (Fig. 1), and measurements of communication could be made under conditions in which the cells had been growing together for up to 4 days. The location of the cell boundaries in older mixtures was too uncertain to allow reliable measure-

Table 2. *Specific membrane resistances* (*nonosculating cells*)

Cell type[a]	Membrane resistance[b] (Ω cm^2)	
Normal		
epithelial, liver (rat)	240 ± 70	(10)
fibroblast (hamster embryo)	530 ± 150	(11)
Cancerous, from animal tumor		
Morris' H 5123, liver (rat)	160 ± 50	(9)
H_4IIEC, liver (rat)	30 ± 7	(9)
fibrosarcoma P 68/31B, SV-40-induced (hamster)	120 ± 40	(9)
fibrosarcoma H 68/23, SV-40-induced (hamster)[c]	100 ± 30	(10)
Transformed in vitro		
epithelioid, x-ray-induced (hamster embryo)	90 ± 10	(11)
fibroblasts, x-ray-induced (hamster embryo)[c]	910 ± 230	(9)
fibroblasts, x-ray-induced (hamster embryo)[d]	400 ± 150	(13)

[a] Culture stages as in Table 1.

[b] Mean values with their standard errors. In parentheses, the number of cells on which measurements were done. Membrane area uncorrected for infoldings (*see* Methods).

[c] Cell line in long-term culture.

[d] 90 days after x-irradiation.

Table 3. *Communication in cell mixtures*

Junction	Communication $\overline{V_{II}:i}/\overline{V_I:i}$[a]	Number of cell pairs	Number of dishes
Normal-to-Normal	0.83	26	7
Morris-to-Morris	<0.005	21	4
H_4IIEC-to-H_4IIEC	<0.005	14	3
Normal-to-Morris	<0.005	20	4
Normal-to-H_4IIEC	<0.005	25	3

[a] Limit of resolution of method: 0.005.

ments[3]. Junctions of normal-to-normal cells were communicating; junctions of normal-to-cancerous cells and of cancerous-to-cancerous cells were not communicating (Table 3).

The question of ionic communication aside, it is interesting to note that movement and growth of Morris' or H_4IIEC cells, which were not inhibited by contact with cells of their own kind, were also not inhibited by contact with normal cells. The Morris' and H_4IIEC cells grew on top of the normal cells; eventually all normal cells in a dish were covered by the cancerous ones. (Dissociation and reseeding of the cells showed that at least part of the normal ones had survived.) In this behavior, the two types of cancerous epithelial cells here resemble certain types of cancerous fibroblastic cells which are not contact-inhibited by normal fibroblasts (Abercrombie, Heaysman & Karthauser, 1957; Abercrombie & Ambrose, 1958; Temin & Rubin, 1958; – *but see* Medina & Sachs, 1963; Stoker, 1964, 1967; Berwald & Sachs, 1965; Stoker, Sheaver & O'Neill, 1966; Borek & Sachs, 1966*b* for differently behaving cells).

Fibroblasts

Both the normal and the transformed fibroblasts presented good communication in favorable culture media. This was so for cells which had been in culture for years, such as the 3T3 line, as shown before (Potter et al., 1966), as well as in cells which had been in culture for only days, such as some of the fibrosarcoma cells[4] (Table 1). Moreover, it did not seem to matter in this respect whether the transformation was by virus or by x-rays, nor whether the transformation was in vitro or in vivo (Table 1).

3 No measurements were made on mixtures of normal fibroblasts and cancerous epithelial cells for the same reasons.

4 The shortest-term culture on which we were able to make measurements of communication was one of P 68/31B fibrosarcoma cells, 6 days old.

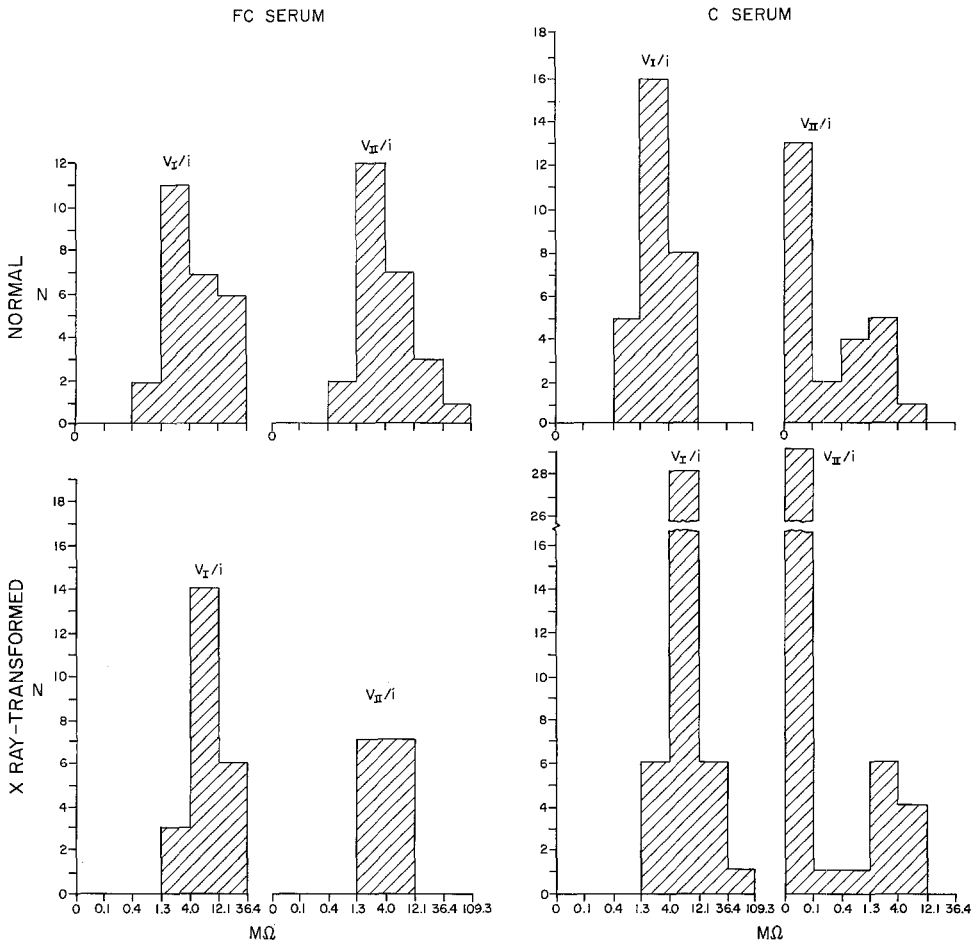

Fig. 5. Coupling patterns in normal and x-ray-transformed fibroblasts of hamster embryos. Histograms of input and transfer resistances of cells. *Left*, cultured in fetal calf serum (F – C): *right*, in calf serum (C). All cases in the interval 0 – 0.1 MΩ transfer resistance are uncoupled. The histograms pool data of cultures which had been in F – C serum 1 to 5 days (both normal and transformed cells), and in C serum 1 to 7 days (normal) and 15 to 24 days (transformed)

Serum Effects. However, the picture was complicated by the fact that communication was sensitive to the serum content of the culture media in at least some kinds of fibroblasts. Fibroblasts of hamster embryos, well-communicating when growing in fetal calf serum, appeared to lose communication in calf serum, in both their normal and transformed states (Fig. 5). On the other hand, fibroblasts of adult hamsters and 3T3 cells and their transformed counterparts, well-communicating in calf serum, tended to lose communication in fetal calf serum (Fig. 6).

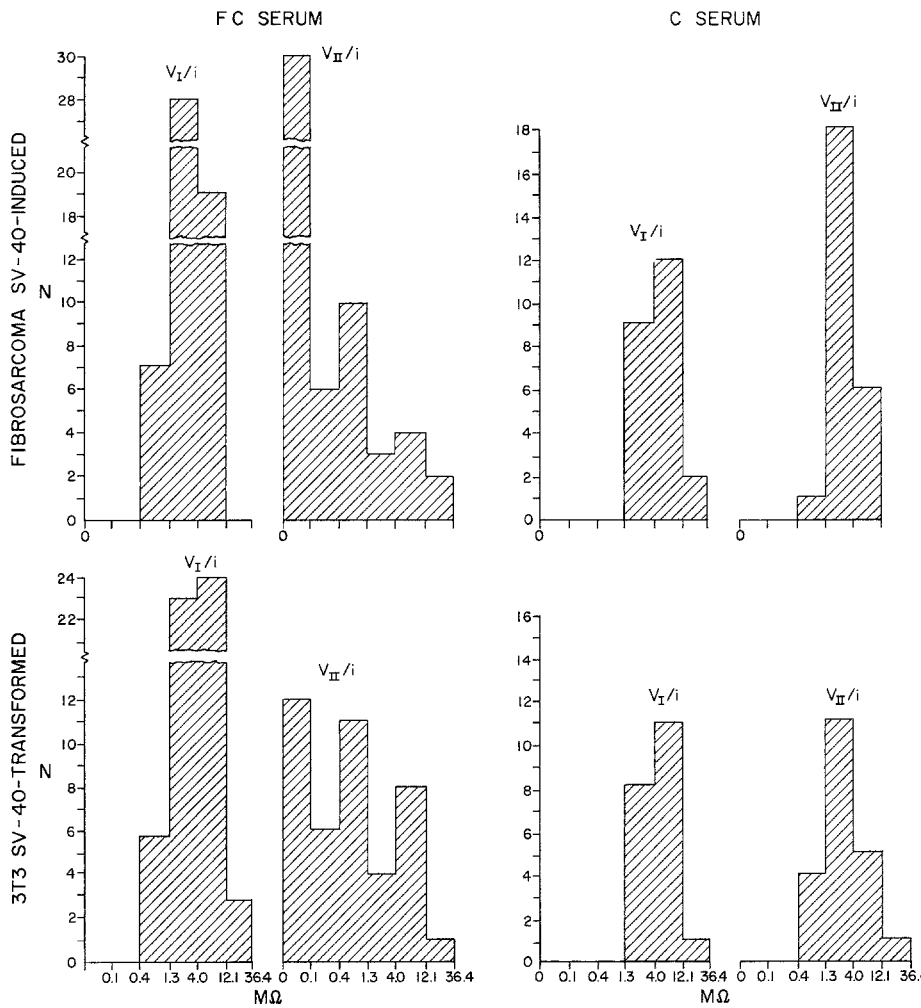

Fig. 6. Coupling patterns in fibrosarcoma (rat) and 3T3 (mouse embryo) cells, SV-40-transformed. *Left:* histograms of input and transfer resistances of cells cultured in fetal calf serum (F−C). All cases in the intervals 0−0.1 MΩ transfer resistance are uncoupled. *Right:* of cells cultured in calf serum (C). Data on fibrosarcoma cells are from primary cultures (6 days old) of tumor P 68/31B. The histograms pool data of cells that had been cultured in F−C serum for 1 to 20 days (both fibrosarcoma and 3T3 cells) and in C serum for 6 to 20 days (fibrosarcoma) and 3 to 20 days (3T3)

These effects were generally noticeable within 3 to 5 days of exposure to the "uncoupling" sera. The probability of finding a noncommunicating contiguous cell pair in a given dish, which was zero in the favorable sera, increased with time (*see* Methods for rejection criteria); and the communication ratios of the still-communicating cells declined. The effects were

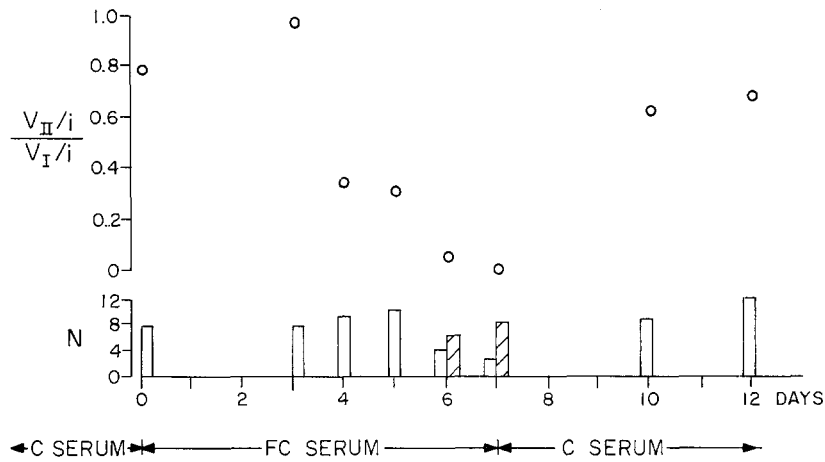

Fig. 7. Effect of serum on coupling among 3T3 cells, SV-40-transformed. The dots are mean communication ratios, $(\overline{V_{II}/i_I})/(\overline{V_I/i_I})$. *Below*, the corresponding numbers (N) of cell pairs with discernible communication (ratios >0.005) and of uncoupled pairs are represented by white and hatched columns, respectively. The arrows indicate the time the cells were cultured in calf serum (C) and fetal calf serum (F−C). Culture media renewed every 2 days

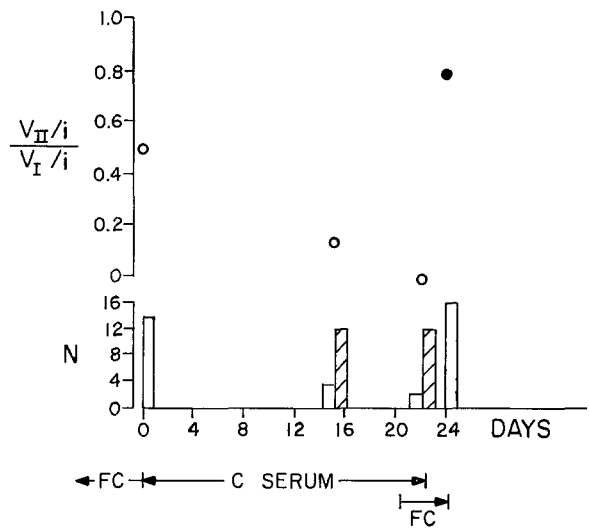

Fig. 8. Effect of serum on coupling among x-ray-transformed fibroblasts (hamster embryo). Notation as in Fig. 7. Upper arrow (C) corresponds with first three points; lower arrow (F−C) with last point (filled)

reversible within 1 to 2 days of return to the favorable sera. Figs. 7 and 8 illustrate time courses of the effects.

The serum effects seemed to be independent of the renewal schedule of the culture media; they ensued when the media were renewed every 1 or 2

days, as well as when renewal was omitted for 5 days (including at the start of the measuring series). The effects are thus different from those found by Furshpan and Potter (1968) on Crocker mouse sarcoma cells where loss of communication appeared to be consequent to medium renewal. The present effects on communication appear to be caused by a serum component. Various serum components are known to affect cell growth (e.g., Eagle, 1960; Todaro, Lazar & Green, 1965; Holmes, 1967; Temin, 1967*a*, *b*; Holley & Kiernan, 1968) and cell adhesion (Lieberman & Ove, 1957; Fisher, Puck & Sato, 1957; Moscona, 1961; Curtis & Greaves, 1965 – *but see* Moscona & Moscona, 1966; Lilien, 1968). Some of the components even act differentially. Orr and Roseman (1969) have just shown that a protein component of horse serum promotes adhesion of chick neural retina cells, whereas a component of calf or chicken serum inhibits adhesion of these cells. This appears especially interesting in the light of the present differential serum effects on communication, because cell adhesion and junctional communication (in particular, perijunctional insulation) are intimately related (*see* Loewenstein, 1967*b*). We have as yet no clues to the nature of the serum conponent or to the mechanism of interruption of communication involved in the present effects, but it may be worthwhile looking into the possible action on junctional communication of serum factors of the Orr and Roseman kind.

Discussion

The results on epithelial cells reproduce in tissue culture, under matched physical and chemical conditions, the contrast in junctional communication offered by certain normal and cancerous epithelial organs and tumors (Loewenstein & Kanno, 1967; Kanno & Matsui, 1968; Jamakosmanoviç & Loewenstein, 1968). This is encouraging as we face the problem of if the absence of junctional communication (*uncoupling*) reflects the actual state in the cancerous cells. The problem arises because the high junctional membrane permeability prevailing in normal epithelia is thermodynamically an unfavorable state; communication is vulnerable to interruption by a variety of factors acting directly on the junctional membranes or perijunctional insulation, or on cytoplasmic processes that provide the energy for maintaining the high state of junctional membrane permeability (Loewenstein, 1966, 1967*a*, *b*; Politoff, Socolar & Loewenstein, 1969). The problem is thus whether the safety margins for communication in respect to these factors rather than the communication itself is lowered in the cancerous cells. For instance, a possibility to be considered is that the safety margins

for perijunctional insulation are lower in the cancerous cells. Perijunctional insulation is closely related to cell membrane adhesion (Loewenstein, 1967*b*; Ito & Loewenstein, 1969), and adhesion is known to be low in certain cancerous epithelia (Coman, 1944; McCutcheon, Coman & Moore, 1948). Thus, uncoupling could conceivably result from mechanical shear at the membrane junction, developed during cell impalement with the microelectrodes. Although this possibility is not excluded by the present results [5], it is reassuring that the Morris' hepatoma H 5123 cells were among the uncoupled cultures. At the junctions of these cells, the shear forces may be expected to be weakest. Of all epithelial cells examined, the Morris' hepatoma cells were most easily impaled (much more easily and with much less deformation than the normal liver cells) [6]. This encourages us in the belief that the uncoupling here reflects the actual state and strengthens our suspicio that it plays an etiological role in the cancerous condition of these cells (*see* Loewenstein, 1968*a*, *b*).

The cells of the transformed fibroblast cultures show essentially the same degree of electrical communication as do their normal counterparts under similar conditions, and this includes results on cells which had been in culture for only 6 days after their isolation from the tumors. P. O'Lague and H. Rubin (*personal communication*) have obtained similar results on short-term primary cultures of fibroblasts transformed by Rous sarcoma virus. This reduces the likelihood that the prevalence of communication is the result of selection pressures in tissue culture. Several possibilities are open to account for the difference in communication patterns between the fibroblasts and epithelial cells. These possibilities are analyzed in detail elsewhere (Loewenstein, 1968*a*, *b*, 1969). Here we shall touch upon some of them only insofar as they bear directly on the present results.

An obvious possibility is that, in the fibroblasts, the cancerous transformation involves growth-regulating processes different from and independent of junctional communication. A further possibility is that uncoupling has indeed taken place, but that it is transient and too brief to be detected, or that it is limited to molecules above a certain size. The finding of electrical communication here does not eliminate, of course, the latter possibility, which is particularly worth considering because communication

5 The possibility also applies, of course, to the uncoupling effects by sera. The measurements of input resistance exclude, however, in all cases, the possibility of damage to non-junctional surface membranes as the cause of uncoupling. *See* Methods.

6 The Morris' H 5123 was also more favorable in this respect than most fibroblast cultures; only the cells of the x-ray-transformed fibroblast line were penetrable with comparable ease. The Morris' cells and x-ray-transformed fibroblasts gave also the stablest electrical membrane resistances and potentials.

in various cell systems seems to involve molecules of a wide range of sizes (Loewenstein & Kanno, 1964; Loewenstein, 1966, 1968*b*; Potter et al., 1966; Bennet, 1966; *see* Furshpan & Potter, 1968). A positive finding of communication by the electrical technique – unlike a negative finding which is very informative – means only that there is communication for the small cellular ions that carry the probing currents, but it gives no clue about the quality of communication for larger and perhaps more relevant particles. This point will have to be investigated with methods that probe junctional permeability over a wide range of molecular sizes.

The results obtained on cell cultures of Novikoff's tumor are different from those obtained earlier on cells of this tumor in situ (Loewenstein & Kanno, 1967). However, it is quite possible that the results are not comparable. The present measurements were all taken on fibroblastic cells [7], whereas the earlier measurements may have been predominantly on epithelial cells. Moreover, it is possible that the tumors themselves, which came from different immediate sources, were different. Novikoff's tumor is not as well characterized and as constant as Morris' 5123, or Reuber's H-35 tumors from which the H_4IIEC cells were derived (*see* Morris, 1965; Pitot et al., 1964; Novikoff & Biempica, 1966).

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7 The cultures of this tumor were rapidly dominated by the fast-growing fibroblastic cells. Work is now in progress on culturing the epithelial cells of this tumor in isolation.

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