

LOW-RESISTANCE JUNCTIONS IN EPITHELIAL OUTGROWTHS FROM NORMAL AND CANCEROUS EPIDERMIS IN VITRO

B. ALLEN FLAXMAN and FRANCIS V. CAVOTO. From Temple University Health Sciences Center, Department of Dermatology, The Skin and Cancer Hospital of Philadelphia, Philadelphia, Pennsylvania 19140 and the Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

INTRODUCTION

Electrotonic coupling has been demonstrated between normal cells from a variety of tissues (see reviews by Loewenstein, 1966; Furshpan and Potter, 1968; Sheridan, 1970; Cavoto and Flaxman, 1972, Van Heukelom et al., 1972 b), but the significance of this type of coupling is not yet clear. The passage of charged molecules from cell to cell implies a role in regulating some aspects of behavior, e.g. during metabolic cooperation

(Gilula et al., 1972). Studies of cancer cells, where coupling might be expected to be absent or diminished when compared with normal cells, have given conflicting results. Both normal and transformed fibroblasts have been found to be coupled in vitro (Potter et al., 1966; Furshpan and Potter, 1968; Borek et al., 1969) and it might be concluded that this aspect of cell function per se is not related to those aspects of a fibroblast's behavior that give it a "cancerous"

profile. For epithelial cancers, however, electrotonic coupling has been reported in some studies (Sheridan, 1970; Johnson and Sheridan, 1971) but not in others (Loewenstein and Kanno, 1967; Jamakosmanovic and Loewenstein, 1968; Borek et al., 1969). Some possible reasons for the differences have been discussed (Sheridan, 1970). In those instances where cancer cells have been found to be coupled, there has been no quantitative comparison with cells of the normal counterpart. Significant quantitative differences could support the notion that defects in coupling are indeed associated with abnormal cell behavior. The purpose of the present study was to compare the extent of electrical coupling between normal and cancerous cells of epidermal origin in terms of the number of cell distances over which coupling could be detected as current flows from cell to cell. No significant differences were found between the normal and cancerous cells.

MATERIALS AND METHODS

Epithelial cell cultures, made from explants of normal human skin and nodules of human epidermal basal cell carcinoma, were grown on the bottom of plastic Petri dishes according to methods described previously (Flaxman et al., 1967; Flaxman, 1972). The normal skin explants, comprising epidermis and a small amount of dermis, give rise to sheets of pure epidermal cells (Flaxman et al., 1967). Tumor nodules can be dissected from basal cell carcinomas in a form that is essentially free from stromal connective tissue (Flaxman and Van Scott, 1968; Flaxman, 1972). Epithelial cells from both tissues grew outward as a circular sheet reaching a maximum diameter of 1–1.5 cm. Within the sheet, cells were arranged as a stratified squamous epithelium centrally and as a monolayer more peripherally. In the latter location the large polygonal cells were readily identified by phase contrast microscopy (Figs. 1, 2) and microelectrodes could be placed in any desired cell. Cells from basal cell carcinoma were, on the average, somewhat larger than those from normal epidermis. Areas were selected where cell outlines were especially clear so that there was no risk of confusion due to overlapping. The few fibroblasts that were occasionally present grew outside of the epithelial sheet and were identified by both shape and lack of coherent growth pattern.

Glass micropipettes were drawn to tip diameters of less than $1 \mu\text{m}$ and filled by vacuum boiling with 3 M KCl. They were coupled to a Grass P16 high impedance electrometer amplifier (Grass Instrument Co., Quincy, Mass.) via a silver/silver chloride wire. Only electrodes with resistances in the range of

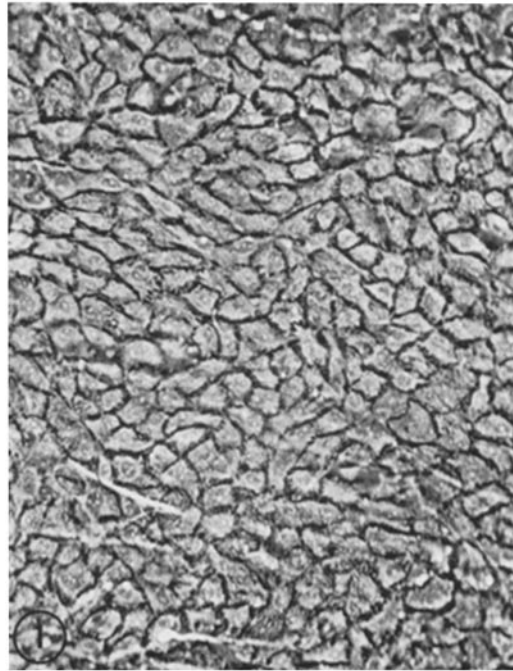


FIGURE 1 Phase contrast light micrograph of monolayer from portion of epithelial outgrowth of normal human epidermis showing distinct cell boundaries. $\times 150$.

16–30 $M\Omega$ were used. Hyperpolarizing current pulses were generated via a Grass SD5 stimulator isolated from ground. The pulses were 2×10^{-7} A in magnitude with a duration of 10 ms.

During studies of communication, cultures were immersed in isotonic Gey's balanced salt solution (pH 7.4) at room temperature. Each microelectrode was positioned by means of a three-axis micromanipulator. Positioning of the microelectrodes was monitored through an inverted phase contrast microscope at $100\times$. The current-carrying electrode was placed within a cell in the monolayer part of the culture. Successful impalement of the cell was indicated by a concomitant rapid drop in potential difference as monitored on the oscilloscope. Both the current pulse and transmembrane potential were displayed on the face of Tektronix 502A dual beam oscilloscope (Tektronix, Inc., Beaverton, Ore.) and photographed with a Polaroid camera using 3000 ASA film. The methods of measuring electrotonic coupling were standard and are described in detail elsewhere (Cavoto and Flaxman, 1972).

RESULTS

There was no observable difference in the apparent resting membrane potential between epi-

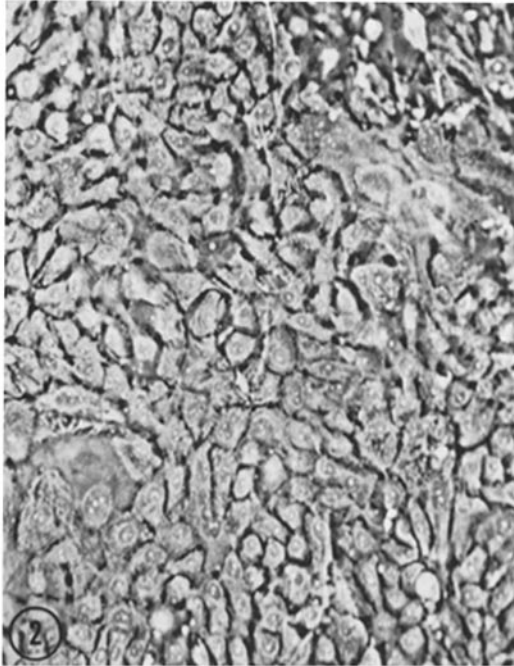


FIGURE 2 Phase contrast light micrograph of epithelial outgrowth from nodule of basal cell carcinoma. Cells are somewhat larger than those from normal epidermis. $\times 150$.

thelial cells from normal epidermis and those from basal cell carcinoma. In both, the apparent resting potential ranged from -25 to -30 mV. When the recording electrode was placed within a contiguous cell with respect to the current-injected cell, the potential difference obtained indicated a similar amount of electrotonic coupling in both normal and cancerous cells (Table I and Fig. 3). When the recording electrode impaled cells 1, 2, 3, and 5 cell-distances from the current-injected cell, the decrease in electrotonus at each point was also similar for normal and cancerous cells (Table I and Fig. 3). When the two impaled cells were separated by six intervening cells, no electrotonic coupling was detectable since the transmembrane potential difference recorded at that distance was indistinguishable from that obtained in the extracellular space.

DISCUSSION

The present study shows that electrotonic coupling is comparable for both normal and cancerous epidermal cells in vitro and that in both cases

TABLE I
Transmembrane Potential Differences (mV \pm SEM)
According to the Number of Cells Between Stimulating and Recording Electrodes

Number of cells between electrodes	Normal (n = 6)	Basal cell carcinoma (n = 4)
*0	25 \pm 2	24.5 \pm 3
1	15 \pm 3	17 \pm 3
2	8 \pm 2	9 \pm 2
3	5 \pm 2	6 \pm 1
5	2 \pm 0.5	3 \pm 1

*0 denotes contiguous cells.

n denotes the number of cell pairs studied to obtain each value.

this is suggestive of low-resistance pathways. The distance over which electrotonic spread occurred is less than for some other epithelia, but may reflect specific tissue or environmental differences (Shiba, 1970, 1971). Coupling has previously been demonstrated between amphibian (Loewenstein and Penn, 1967) and human (Van Heukelom et al., 1972 a) epidermal cells in vivo and between human cells in vitro (Cavoto and Flaxman, 1972), but not between any cancers of epidermal origin.

The human epidermal basal cell carcinoma is somewhat different biologically from other carcinomas in that surface properties that permit metastasis are not evident in most instances (Van Scott, 1962; Flaxman, 1972). The cancer cells, which grow in a variety of histologic patterns, are able to invade and destroy all local tissues. The extent of invasion is related to the length of time that treatment of the cancer is neglected. Metastasis, although reported, is rare (Van Scott, 1962). In addition, certain other behavioral features of these cells should be pointed out. In vivo, cells of basal cell carcinomas show little or no tendency to keratinize whereas in vitro, massive keratinization occurs, especially in older cultures (Flaxman and Van Scott, 1968; Flaxman, 1972). Keratinized cells were carefully avoided in the present study. They were readily identified by their opaque, anucleate appearance when viewed by phase contrast optics and were mainly located centrally, near the explant, rather than at the outgrowth periphery where the electrical measurements were performed. "Reversal" of the characteristic failure to keratinize indicates that in vitro, cells from basal cell carcinoma have

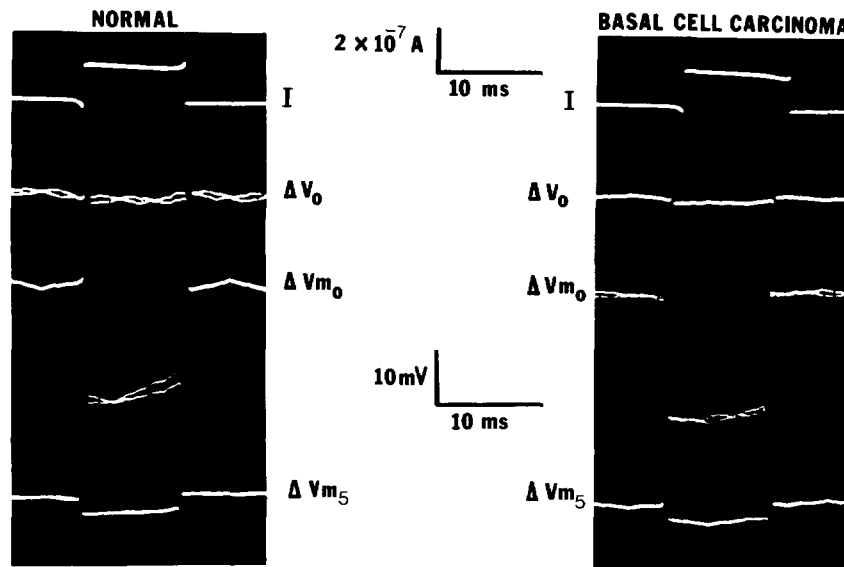


FIGURE 3 Oscilloscope recordings of potential differences measured in epithelial cell outgrowths from normal human epidermis and basal cell carcinoma *in vitro*. *I* refers to current pulse injected into a reference cell (2×10^{-7} A with a duration of 10 ms). ΔV_0 is the potential difference with recording electrode in the extracellular space. ΔV_m is the transmembrane potential difference according to the number of cells between the current-carrying and recording electrodes. ΔV_{m_0} , contiguous cells; ΔV_{m_5} , five cells between.

more "normal" behavior than *in vivo* (although other aspects of cell behavior appear to remain abnormal *in vitro*). Since it is not known whether there is electrotonic coupling in basal cell carcinoma *in vivo*, it is not possible to say whether its occurrence *in vitro* represents yet another aspect of return to normal cell behavior. Gap junctions, which are believed to mediate coupling due to their low electrical resistance and high junctional permeability (Payton et al., 1969; Johnson and Sheridan, 1971; Gilula et al., 1972), have been found between the cells both *in vivo* and *in vitro* (Flaxman, 1972) and thus, it might be suspected that communication does indeed take place *in vivo*.

The present experiments have not explained the divergent results of other investigators (Loewenstein and Kanno, 1967; Jamakosmanovic and Loewenstein, 1968; Borek et al., 1969; Sheridan, 1970; Johnson and Sheridan, 1971) as to the extent of electrotonic coupling between other types of epithelial cancer cells. Some speculative reasons for these differences have been discussed previously and need not be elaborated on further (Sheridan, 1970). Our results clearly support the findings of those who report electrotonic coupling between cancer cells.

SUMMARY

The presence of low-resistance pathways has been studied for normal and cancerous epidermal cells propagated as epithelial sheets *in vitro*. The spread of electrotonic coupling through an epithelium was comparable for both types of cells.

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BIBLIOGRAPHY

1. BOREK, C., S. HIGASHINO, and W. R. LOEWENSTEIN. 1969. Intercellular communication and tissue growth. IV. Conductance of membrane junctions of normal and cancerous cells in culture. *J. Membrane Biol.* 1:274.
2. CAVOTO, F. V., and B. A. FLAXMAN. 1972. Communication between normal human epidermal cells *in vitro*. *J. Invest. Dermatol.* 59:370.
3. FLAXMAN, B. A. 1972. Growth *in vitro* and induction of differentiation in cells of basal cell cancer. *Cancer Res.* 32:462.
4. FLAXMAN, B. A., M. A. LUTZNER, and E. J.

- VAN SCOTT. 1967. Cell maturation and tissue organization in epithelial outgrowths from skin and buccal mucosa *in vitro*. *J. Invest. Dermatol.* 49:322.
5. FLAXMAN, B. A., and E. J. VAN SCOTT. 1968. Keratinization *in vitro* of cells from a basal cell carcinoma. *J. Natl. Cancer Inst.* 40:411.
 6. FURSHPAN, E. J., and D. D. POTTER. 1968. Low-resistance junctions between cells in embryo and tissue culture. In *Current Topics in Developmental Biology*. A. A. Moscona and A. Monroy, editors. Academic Press Inc., New York. 3:95.
 7. GILULA, N. B., O. R. REEVES, and A. STEINBACH. 1972. Metabolic coupling, ionic coupling and cell contact. *Nature (Lond.)*. 235:262.
 8. JAMAKOSMANOVIC, A., and W. R. LOEWENSTEIN. 1968. Intercellular communication and tissue growth. III. Thyroid cancer. *J. Cell Biol.* 38:556.
 9. JOHNSON, R. G., and J. D. SHERIDAN. 1971. Junctions between cancer cells in culture: ultrastructure and permeability. *Science (Wash. D. C.)*. 174:717.
 10. LOEWENSTEIN, W. R. 1966. Permeability of membrane junctions. *Ann. N. Y. Acad. Sci.* 137:440.
 11. LOEWENSTEIN, W. R., and Y. KANNO. 1967. Intercellular communication and tissue growth. I. Cancerous growth. *J. Cell Biol.* 33:225.
 12. LOEWENSTEIN, W. R., and R. D. PENN. 1967. Intercellular communication and tissue growth. II. Tissue regeneration. *J. Cell Biol.* 33:255.
 13. PAYTON, B. W., M. V. L. BENNETT, and G. D. PAPPAS. 1969. Permeability and structure of junctional membranes at an electrotonic synapse. *Science (Wash. D. C.)*. 166:1641.
 14. POTTER, D. D., E. J. FURSHPAN, and E. S. LENNOX. 1966. Connections between cells of the developing squid as revealed by electrophysiological methods. *Proc. Natl. Acad. Sci. U. S. A.* 55:328.
 15. SHERIDAN, J. D. 1970. Low-resistance junctions between cancer cells in various solid tumors. *J. Cell Biol.* 45:91.
 16. SHIBA, H. 1970. An electric model for flat simple epithelial cells with low resistive junctional membranes. *Jap. J. Appl. Phys.* 9:1405.
 17. SHIBA, H. 1971. Heaviside's "Bessel Cable" as an electric model for flat simple epithelial cells with low resistive junctional membranes. *J. Theor. Biol.* 30:59.
 18. VAN HEUKELOM, J. S., D. W. SLAAF, and J. C. VAN DER LEUN. 1972 a. Cell communication in the basal cells of the human epidermis. *Biophys. J.* 12:1266.
 19. VAN HEUKELOM, J. S., J. J. D. VAN DER GON, and F. J. A. PROP. 1972 b. Model approaches for evaluation of cell coupling in monolayers. *J. Membrane Biol.* 7:88.
 20. VAN SCOTT, E. J. 1962. Definition of Epidermal Cancer. In *The Epidermis*. W. Montagna and W. C. Lobitz, Jr., editors. Academic Press Inc., New York. 573.