LOW-RESISTANCE PATHWAYS BETWEEN MITOTIC AND INTERPHASE EPIDERMAL CELLS IN VITRO

FRANCIS V. CAVOTO and B. ALLEN FLAXMAN. 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 is a well-known phenomenon occurring between both electrically and non-electrically excitable normal cells and between some types of cancer cells (see reviews by Furshpan and Potter, 1968; Gilula et al., 1972; Johnson and Sheridan, 1971; Cavoto and Flaxman, 1972). Coupling has also been demonstrated between dividing and adjacent nondividing fibroblasts in vitro (O'Lague et al., 1970). This latter finding is of interest because dividing cells appear to lose intimate contact with surrounding interphase cells when examined by light microscopy. The purpose of the present study was to determine whether electrotonic coupling exists in vitro between dividing and interphase cells of epithelial origin since the former also appear to lose intimate contact with their neighbors as judged by morphologic criteria.

MATERIALS AND METHODS

Epithelial cultures of human epidermal cells were propagated on the bottom of plastic Petri dishes according to methods described elsewhere (Flaxman

et al., 1967). Electrical measurements were made on cultures immersed in Gey's balanced salt solution at room temperature. Cells were visualized with the aid of phase contrast optics and an inverted microscope. Microelectrodes (micropipettes), filled with 3 M KCl, were inserted into cells by means of a pair of three-axis micromanipulators. The electrical and recording apparatus was standard and is described in detail elsewhere (Cavoto and Flaxman, 1972). A current of 2×10^{-7} A and 10 msec duration was passed into either an interphase or dividing cell and transmembrane potential differences were measured with the recording electrode in a contiguous cell of the opposite type. Microelectrodes were positioned visually but actual impalement of the current-injected cell was determined by a drop in potential to approximately − 25 mV.

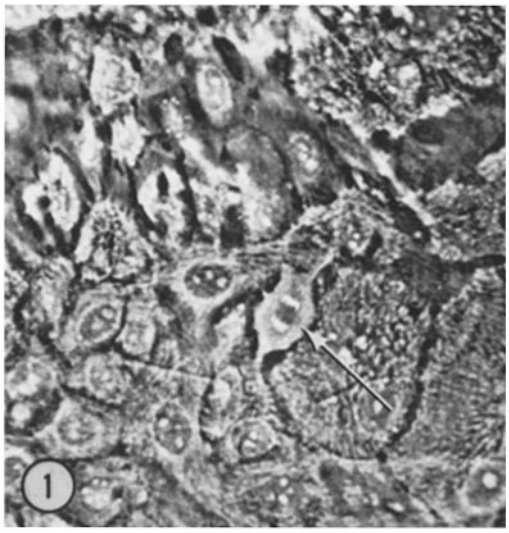

FIGURE 1 Phase contrast light micrograph of dividing epidermal cell (arrow) linked to surrounding interphase cells by fine processes. Diameter of interphase cells ranges from 50 to 150 μm. × 150.

RESULTS

Interphase and dividing cells were readily distinguished (Fig. 1). The former were flat, polygonal, closely apposed and had oval nuclei. The latter were identified by the condensed chromosomes usually in the metaphase or early telophase arrangement. These cells appeared to be separated from surrounding interphase cells by wide spaces. However, fine processes were seen linking dividing cells to their interphase neighbors. Electrical measurements were made only on metaphase and telophase cells. The presence of low-resistance junctions between cells was indicated when, after impalement of a cell by the recording electrode, a concomitant hyperpolarizing transient occurred that was synchronous with the stimulus input (Fig. 2). In experiments on nine consecutive cell pairs, the electrotonic potential differences recorded in a cell contiguous

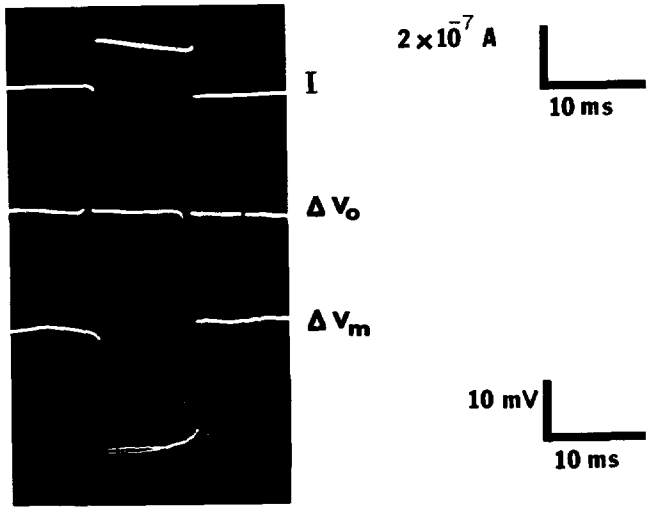

FIGURE 2 Oscilloscope tracings demonstrating electrical coupling between dividing and interphase cells. *I* is a current pulse of 2×10^{-7} A and 10 ms duration injected into one cell of the pair. ΔV_o is the potential difference measured with the recording electrode in the extracellular space. ΔVm is the potential difference measured with the recording electrode within the second cell of the pair.

to the current-injected cell was 25 ± 3 mV (SEM). The difference in potential was observed regardless of whether the stimulating electrode was in a dividing or interphase cell. This value compares favorably with coupling between two interphase cells which also averages about 25 mV.

DISCUSSION

The results extend observations of O'Lague et al. (1970) showing coupling between dividing and interphase fibroblasts in vitro. It would have to be concluded, therefore, that dividing epidermal cells retain important relationships with interphase cells such that low-resistance pathways for the passage of current continue to persist during at least part of the mitotic process (metaphase and telophase). During mitosis in vitro, it is generally accepted that cells become less "tightly" attached to their substratum and to their neighbors, a phenomenon that serves as one basis for obtaining synchronously dividing cell populations (Stubblefield, 1968; Romsdahl, 1968; Scharff and Robbins, 1966). Weakening of cell attachments need not extend to total dissolution of contacts, however. The electron microscope data of O'Lague et al. (1970), showing thin protoplasmic connections between dividing and interphase fibroblasts, are compatible with this hypothesis. Points of contact between the cells could be the site of persistent gap junctions which appear to mediate electrical coupling between interphase cells (Johnson and Sheridan, 1972; Gilula et al., 1972; Payton et al., 1969). Such junctions have previously been demonstrated for fibroblasts (Pinto da Silva and Gilula, 1972) and epidermal cells in vitro (Cavoto and Flaxman, 1972).

This research was supported by grants from The National Cancer Institute, Number 1 PO 1 CA 11536 and The National Institute of Arthritis and Metabolic Diseases, Number 1 PO 1 AM 15515.

Received for publication 20 November 1972, and in revised form 26 March 1973.

REFERENCES

1. Cavoto, F. V., and B. A. Flaxman. 1972. Communication between normal human epidermal cells *in vitro*. *J. Invest. Dermatol.* **59**:370.
2. 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.
3. 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.
4. Gilula, N. B., O. R. Reeves, and A. Steinbach. 1972. Metabolic coupling, ionic coupling and cell contact. *Nature* (*Lond.*). **135**:262.
5. Johnson, R. G., and J. D. Sheridan. 1971. Junctions between cancer cells in culture: ultrastructure and permeability. *Science* (*Wash. D. C.*). **174**:717.
6. O'Lague, P., H. Dalen, H. Rubin, and C. Tobias. 1970. Electrical coupling: low resistance junctions between mitotic and interphase fibroblasts in tissue culture. *Science* (*Wash. D. C.*). **170**:464.
7. 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.
8. Pinto da Silva, P., and N. B. Gilula. 1972. Gap junctions in normal and transformed fibroblasts in culture. *Exp. Cell Res.* **71**:393.
9. Romsdahl, M. D. 1968. Synchronization of human cell lines with colcemid. *Exp. Cell Res.* **50**:463.
10. Scharff, M. D., and E. Robbins. 1966. Polyribosome disaggregation during metaphase. *Science* (*Wash. D. C.*). **151**:992.
11. Stubblefield, E. 1968. Synchronization methods for mammalian cell culture. *In* Methods in Cell Physiology. D. M. Prescott, editor. Academic Press Inc., New York. **2**:25.