ABILITY OF NORMAL MUSCLE FIBROBLASTS AND L-CELLS, GROWN IN MIXED CULTURE WITH NORMAL FIBROBLASTS, TO FORM INTERCELLULAR CONTACTS

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Electrical coupling, recorded by intracellular glass microelectrodes, was demonstrated between normal muscle fibroblasts (NMF) in pure culture (79% of connected pairs of cells) and between NMF and transformed fibroblasts (L-cells) in mixed culture (30% of connected pairs). L-cells in pure culture were not electrically connected.
KEY WORDS: normal fibroblasts; L-cells; intercellular contacts.

Normal mammalian cells grown in tissue culture can form highly permeable intercellular contacts in the same way as has been shown for intact tissues [3, 4, 6, 8, 9, 12-14]. Cases of the absence [3, 4] and of the presence [7] of such contacts have been described for epithelial tumor cells from Morris and Novikov hepatomas. Fibroblast-like cells of varied origin usually are well connected together, the only exceptions are transformed mouse fibroblasts, L-cells, which do not form highly permeable contacts with each other. The role of intercellular contacts between cells in culture is not clear. The possibility cannot be ruled out that enzymes or other substances important for cell metabolism may pass through these contacts. It has been shown in the writers' laboratory [10] that during combined cultivation of normal mouse fibroblasts with L-cells the sensitivity of the latter to the carcinogen DMBA is increased; evidently so-called metabolic cooperation exists between normal and L-cells, and this may be based on the highly permeable intercellular contacts [15].

In the investigation described below the possibility of formation of such contacts between normal mouse fibroblasts and neoplastic cells of strain L was studied.

EXPERIMENTAL METHOD

Normal fibroblast-like cells (NMF) obtained by trypsinization of 16-18-day mouse embryos (1st-3rd passage) and neoplastic mouse fibroblasts of strain L were used. The cells were seeded in penicillin flasks (100,000-150,000 normal and 50,000-100,000 L-cells per flask), on the bottom of which was placed a cover slip measuring 5×20 mm. To obtain a mixed culture, from 50,000-100,000 L-cells were transplanted after 2-3 days onto the surface of the layer of normal cells. The nutrient medium (a mixture of 45% medium No. 199, 45% lactalbumin hydrolyzate, and 10% bovine serum) was changed every 2-3 days.

To measure the electrical coupling the cover slip with the culture was transferred to a transparent plastic chamber about 1 ml in volume in a circular flow of nutrient medium kept at a constant temperature of 35-37°C [2]. The measurements were made by two glass microelectrodes (2.8 M KCl) with a tip less than 0.5 μ in diameter by Loewenstein's method [8]. On the passage of a square pulse of current (1×10^{-8} A) through one intracellular microelectrode, a voltage drop ΔV in the neighboring cell was recorded by the other microelectrode. In the present experiments the magnitude of random fluctuations of ΔV (for example,

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TABLE 1. Electrical Coupling between Fibroblastlike Cells (M ± m)

Type of culture	Fraction of cell pairs coupled*	ΔV (mV)	MP (mV)
NMF	26/33 (79%)	13±5	13±0,2
on glass	16/20 (80%)	17±7	12±1
in grooves	10/13 (77%)	8±3	14±0,3
L-cells	1/35 (3%)	3	11±0,4
Mixed culture of NMF + L-cells	9/30† (30%)	32±17	14±1 (Both for NMF and for L-cells)

*Ratio between number of compled pairs and total number investigated.

†Measurements always between cells of different types.

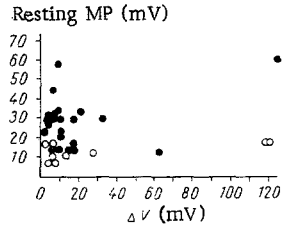

Fig. 1. Absence of correlation between values of ΔV and MP. Filled circles denote pairs of NMF+NMF cells, empty circles pairs of NMF+L cells. Strength of current 10^{-8} A. Remainder of explanation in text.

as a result of extracellular passage of the current of extracellular recording) did not exceed 0.5 mV; in all cases in which ΔV > 0.5 mV was recorded, the cells were therefore regarded as electrically coupled.

EXPERIMENTAL RESULTS

Experiments were carried out on discontinuous cultures (1-3 days of incubation) with clearly distinguishable cell boundaries; for the measurements of electrical connections, pairs of cells with contacting surfaces were chosen. The results are given in Table 1.

In 14 experiments on NMF the value of ΔV varied from 1.5 to 120 mV. A series of experiments was carried out on NMF cultures settling in narrow (about 10 μ in width) grooves scratched in the layer of phospholipids covering the glass.* Under these conditions the cells were in contact only by their appendages. As will be clear from Table 1, the mean value of ΔV and the percentage of connected cells were identical in both modifications of the NMF cultures. The NMF are thus evidently able to establish contact both with their bodies and with their appendages (or only with the appendages), but the effectiveness of the coupling varies from one measurement to another. The causes of this scatter are unfortunately not yet clear. The low values of ΔV are evidently not the result of damage to the cells by the microelectrode, for injury is usually characterized by a fall of membrane potential (MP), and in these experiments there was no correlation between the values of MP and ΔV (Fig. 1). For technical reasons (the absence of simultaneous measurements with 3 intracellular microelectrodes of the changes in MP during passage of an intracellular current for each cell of the pair) we could not determine the coefficient of coupling ($K_{coup.} = \Delta V/\Delta V_0$, where ΔV_0 is the voltage drop in the polarized cell), widely used by some workers [7, 8]. The values of the "transfer resistances" (the ratio ΔV/I) in the present experiments varied from 0.1 to 8 MΩ, in agreement with data in the literature [4] for various cultures of fibroblasts.

Only one case of elctrical interaction was recorded in 12 experiments in a culture of L-cells. This result evidently shows that the L-cells cannot establish highly permeable contacts with one another. The case of contact observed possibly belongs to a pair of cells recently completing mitosis, and although split into two (so that they were taken as a pair of chance neighbors), they had not yet lost their connection with each other. In two cases a high degree of connection was recorded between clearly daughter cells (just after completion of mitosis and before separation): ΔV was 15 and 100 mV.

In eight experiments of mixed cultures effective electrical connection was observed between the NMF and L-cells. The L-cells were easily distinguished morphologically from NMF: they were longer and fuller, with darkly stained cytoplasm and with fewer appendages; the measurements were made only between reliably identified NMF and L-cells with surfaces in contact. The mean value of ΔV and the scatter of this value (from 1.5 to 120 mV) in the mixed culture were similar to the corresponding characteristics for a pure NMF culture.

*These cultures were provided by O. Yu. Ivanova and L. B. Margolis, working in this laboratory, to whom the authors wish to record their gratitude.

Coupling between cells of the two types was thus demonstrated in the mixed culture, but under these circumstances one of the partners in pure culture was unable to form highly permeable contacts with other similar cells of the same type. Previously [1] the writers published data to show that a dye (trypaflavin, mol. wt. 214), injected intracellularly in a pure culture of L-cells, did not leak into neighboring cells. The same dye spread readily between NMF in pure culture, but in mixed cultures, if injected into a L-cell, it leaked into neighboring NMF. For the establishment of highly permeable contacts between two cells it is apparently sufficient if at least one of them is capable of forming such a contact. Further investigations are necessary before a more detailed description can be given of the mechanism by which highly permeable intercellular contacts are formed in tissue culture.

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