CELL POTENTIALS OF A CANCER TISSUE CULTURE

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UDC 612.6.054.014.423+616-006.6-018-073.97

The study of the mechanisms of malignant transformation requires the use of every facility of modern experimental biological science, including methods of physiological investigation of the cell.

The results of the authors' observations using a microelectrode technique have demonstrated certain specific features of the electrophysiological properties and reactivity of the malignant cell by comparison with the cell of normal epithelial tissue [5-9]. Results obtained for the electrophysiological indices of the malignant cell have been confirmed in the laboratory directed by K. P. Balitskii [1].

The further analysis of the problem requires a microelectrophysiological investigation of a cancer tissue culture.

Reports in the literature of microelectrode investigations of cancer tissue cultures, as of cultures of nonmalignant tissue in general, are limited to the well known paper by Schaefer and Schanne [12]. These authors give interesting figures showing the cell potentials of a culture of two different human carcinomas. However, they do not discuss the question of malignant transformation.

The present article describes the result of the authors' first microelectrode observation on a cancer tissue culture.

EXPERIMENTAL METHOD

The test object was a tissue culture of the carcinoma HEp-2 isolated from a carcinoma of the human larynx. The culture was grown in glass flasks; a cell suspension was seeded on the surface of the glass, and a layer of cells was formed, readily accessible to microelectrode investigation. The nutrient medium contained 10% human blood serum, 10% aminopeptide-2, and 80% medium No. 199.

During passage the culture was seeded with cell suspension in several flasks simultaneously and these were used successively in the experiment at intervals of 24 h. Each individual series of experiments thus included several different stages of growth of the tissue culture within the limits of one passage.

Before the experiment, the flask was broken and the pieces of glass with the tissue culture were fixed in a special chamber with a glass bottom filled with Tyrode's solution. The cells of the HEp-2 culture were investigated by a microelectrode method, described in detail in several papers from the authors' department [3,4,11]. The diameter of the point of the microelectrode did not exceed 0.5 μ. The resistance of the microelectrode varied between 5 and 20 mΩ. The microelectrode was introduced into individual cells of the monolayer culture under microscopic control (microscope MBR-3). Cells from the central portion of the monolayer of tissue culture cells were punctured.

EXPERIMENTAL RESULTS

The investigations showed that a characteristic sign of the cells of the human carcinoma, as also of the cells of transplanted malignant tumors in animals, is the low magnitude of the membrane potential (MP).

Details of the MP of normal and malignant epithelial cells of man and animals obtained by the statistical analysis of data collected in the authors' laboratory during recent years are summarized in Table 1. This information fully confirms the results of previous investigation.

The results of the microelectrode investigations of the cancer tissue culture cells confirmed this basic conclusion.

Department of Physiology, A. I. Gertsen Pedagogic Institute, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR D. A. Biryukov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 9, pp. 93-95, September, 1966. Original article submitted January 6, 1965.

TABLE 1. MP of Malignant and Normal Epithelial Tissue (M ± m)

Object	MP (in mV)
Human carcinoma (carcinoma of the stomach)	14.5 ± 0.7
Transplanted malignant tumor of animals (Ehrlich's carcinoma of an albino mouse)	10.8 ± 0.4
Noncancerous epithelium of a cancer patient	18.7 ± 1.3
Normal epithelial tissue of a warm-blooded animal (albino mouse)	26.9 ± 0.6
Normal epithelial tissue of a cold-blooded animal	
Epithelium of a frog's esophagus	16.0 ± 0.9
Epithelium of a frog's swimming membrane	20.0 ± 1.2

TABLE 2. MP of a Cancer Tissue Culture on Various Days after Seeding (M ± m)

Object	MP (in mV)	Number of MP values below 15 mV (in %)
HEp-2		
2 days	13,8±0,7	60
3 "	11,3±0,6	71,5
4 "	11,7±0,8	73
5 "	12,7±1,45	62,2
6 "	12,1±1,8	80
7 "	9,9±1,2	82

The amplitude of the MP of the cancer tissue culture was much lower than that of normal epithelial tissue of man and warm-blooded animals. The mean MP of the HEp-2 culture did not exceed 12-13 mV, i.e., it was close to the mean MP of the human carcinoma. However, although the mean values of the MP were sufficiently striking, the absolute values of the MP of individual cells in the HEp-2 culture showed important individual variations from very low (4-5 mV) to relatively high values (25-28 mV). The results of the microelectrode investigations of the cells of a 3-day HEp-2 culture are shown in the figure.

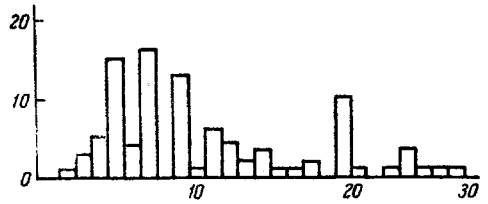
The limits of the individual variations of the MP in the cancer tissue culture cells were much wider than in the cells of human malignant tumors and, in particular, of transplanted malignant tumors of animals. The MP of a cancer tissue culture depends on the time of its growth in an unchanged medium.

The mean values of the MP were highest in a culture on the second day of growth after seeding. On the following days of growth of the culture (3rd and 4th days) the values of the MP fell, and on the 5th day they again rose fairly considerably. Subsequent growth of the culture was associated with a further fall in the MP (Table 2). The differences between the values of the MP of the culture at various stages of growth were confirmed by changes in the absolute number of cells characterized by low MP values (Table 2).

It may be concluded from these investigations that the main physiological characteristic held in common by the cells of a cancer tissue culture and the cells of malignant tumors developing in the human or animal body is their low MP. The low polarization of the cell may therefore be regarded as one of the manifestations of the process of its malignant transformation, one of the indications of the physiological nature of this process.

Modern theoretical electrophysiology regards the low values of the MP of cancer tissue (the depolarization of the malignant epithelial cells by comparison with the normal nonmalignant cell) as an indication that the cancer cell lives in conditions of permanent and distinctive activity. The nature of this activity may to some extent be explained by the study of the dynamics of the MP of cancer tissue when treated by various external agents.

It may be concluded from comparative physiological investigations conducted during recent years, including special studies made by the present authors of the problem of individual development of living structures [2,10], that the relatively high MP values of various tissues of the living organism are the result of the phylogenetic and age evolution of their structural and functional properties. The low values of the MP of cancer tissue may be taken as an indication of the structural and physiological differentiation of cancer tissue, and the loss of its functional specialization.



MP of a 3-day culture of HEp-2. Along the axis of abscissas — MP (in mV); along the axis of ordinates — number of cases (in %).

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