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DIFFERENTIATION OF NEUROBLASTOMA CELLS UNDER THE INFLUENCE OF CYTOCHALASIN B

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Several lines of neuroblastoma cells capable of growth in culture have been described [2, 5, 6]. Undifferentiated and actively proliferating cells of these lines can be converted, under the influence of various factors (serum-free medium, dimethyl sulfoxide, analogs of cyclic nucleotides [6, 7, 8]) into differentiated cells, morphologically similar to neurons. Neuroblastoma cultures are thus a convenient object with which to study factors controlling differentiation of tumor cells.

The object of this investigation was to study the possibility of inducing differentiation of neuroblastoma cells by treatment with small doses of cytochalasin B (CB), an agent with selective action on the actin microfilament system. One of the results of destruction of microfilaments by CB is blocking of the last phase of mitosis, leading to the appearance of multinuclear cells. It was found previously that the multinuclear state leads to partial normalization of the morphology of transformed cells of another type, namely fibroblasts of the L line [1].

EXPERIMENTAL METHOD

A culture of mouse neuroblastoma cells of clone N-18-A, line C-1300 [2, 5] was grown at 37°C on Eagle's medium with 10% neonatal calf serum and gentamycin (80 units/ml). For the experiments the cells were planted on coverslips in the proportion of 10,000 cells to 1 ml medium.

CB in a dose of 1.8 μg/ml (from Serva, USA) was added to the culture medium 24 h after seeding. The cells were incubated with CB for three days, then transferred for 1-4 days to medium without CB. Control cultures were incubated throughout the experiment (7-8 days) without CB in normal medium with serum. Cultures grown for 7-8 days in serum-free medium served as the second control. The morphology of the living cells and their contacts with the substrate were investigated by phase-contrast and interference-reflection microscopy [4].

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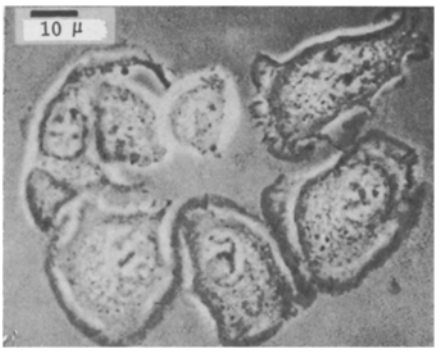

Fig. 1. Undifferentiated neuroblastoma cells cultured under standard conditions (objective 40, ocular 10 × 1.25).

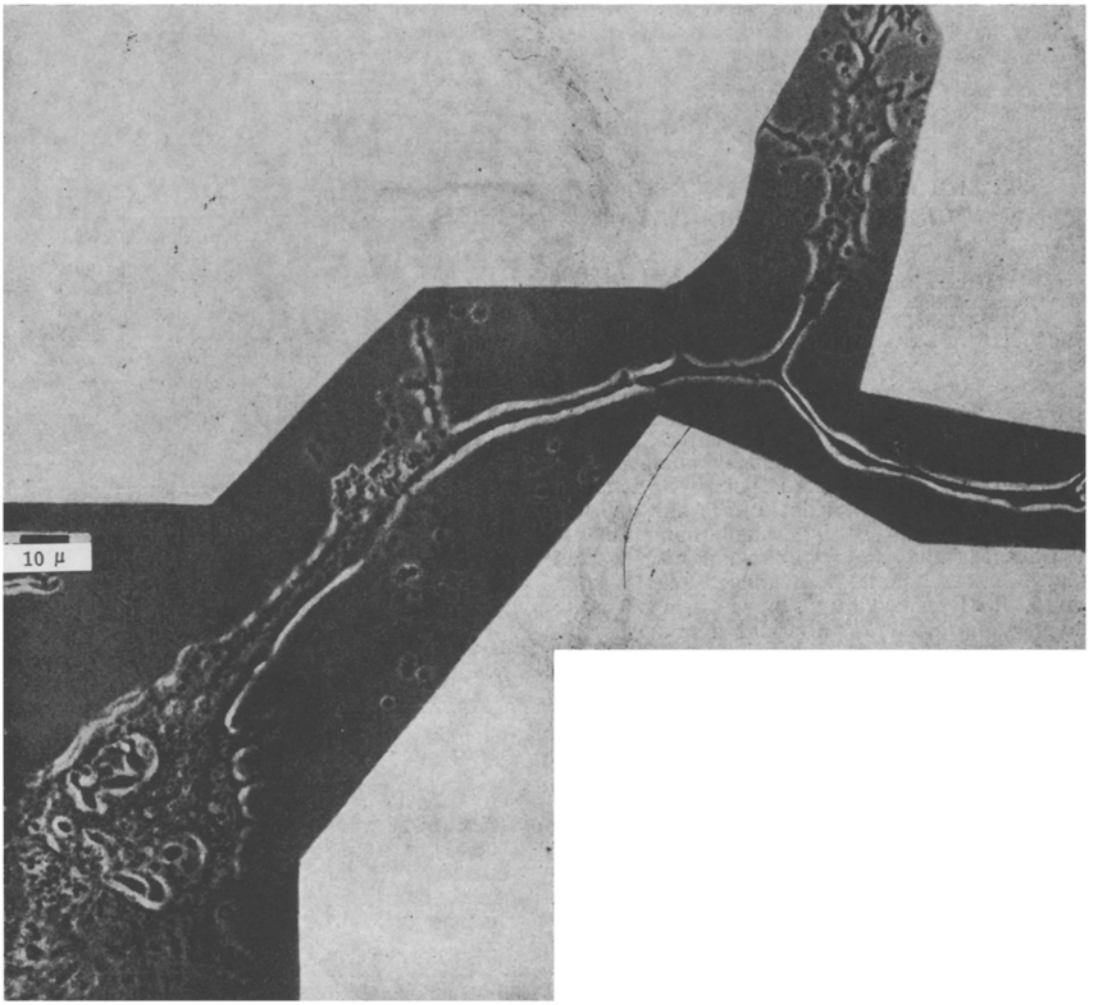

Fig. 2. Morphologically differentiated binuclear neuroblastoma cell with branching axon (3 days of culture under standard conditions after incubation for 3 days in medium with CB).

During the counting, cells in which the length of the primitive axon was not less than twice the diameter of the cell body were regarded as differentiated cells. The distribution of actin was studied by the indirect immunofluorescence method, using antiactin antibodies, as described previously [1]. Fixed preparations were examined in the "Opton" fluorescence microscope.

EXPERIMENTAL RESULTS

Under standard conditions a culture of N-18-A cells consists of collections of round or polygonal cells, with little tendency to spread, and 15-30 μ in diameter (Fig. 1), giving off numerous microprocesses (filopodia) 1-3 μ long. Some cells have a narrow lamellar cytoplasm with an indented border at their periphery. Besides undifferentiated cells, the culture also contains a few morphologically differentiated cells with primitive axons 30-150 μ long.

In cultures treated with CB multinuclear cells appeared after 24 h. After three days of incubation in medium with CB 64.8% of cells had two nuclei, 23.5% had from 3 to 5 nuclei, and 11.7% of cells still remained mononuclear. On replacing the medium with CB by medium without CB (but with serum) the cells spread out readily into a monolayer after 1.5-2 h, and wide lamellae, most frequently circular, appeared. On the 7th-8th day of growth of the culture, after incubation for 3-4 days in medium without CB (after treatment with CB) the number of nuclei per cell increased (up to 15-20), and the cells and nuclei also increased in size. At these times 43.7 $\pm$ 4.1% of the cells in the cultures had long primitive axons (Fig. 2). In contrast cultures not treated with CB and incubated in medium with serum 4.7 $\pm$ 0.6% of differentiated cells were found at the same times. As positive control of the ability of the cells to differentiate, serum-free medium was used; after incubation for 7 days in it, 71.9 $\pm$ 5.1% of cells in the culture were differentiated.

CB thus caused a statistically significant increase in the fraction of differentiated cells. Differentiated cells obtained after treatment with CB, compared with cells differentiated in serum-free medium, spread out into a monolayer better, i.e., they had areas of lamellar cytoplasm and the cones of growth at the ends of the axons were spread out. The formation of processes was most marked in cells with 2-4 nuclei.

Staining cells with primitive axons (differentiated in standard medium with serum after treatment with CB) for actin showed the appearance of long, thin bundles of actin microfilaments in the axons and lamellae. By the technique used, short, needle-like bundles of actin microfilaments could be detected in filopodia in control N-18-A cells, which were poorly spread out. Cells differentiating after treatment with CB formed dark gray hatched contacts of "focal" type with the substrate beneath the cones of growth [4]. Control cells did not form such strongly adhesive contacts with the substrate, but formed only close (gray) contacts with the substrate. Our data thus show that multinuclear cells obtained after treatment with CB acquire morphological features characteristic of differentiated neurons: long axon-like processes containing bundles of actin microfilaments and focal contacts with the substrate beneath the cones of growth.

The new method of inducing differentiation of a neuroblastoma described in this paper differs from other methods in that differentiation takes place in this case in a standard culture medium. Induction of differentiation may perhaps be linked with the multinuclear state of cells incubated with CB, and with their greater ability than that of the control cells to spread out into a layer on the substrate. It was shown previously that multinuclear tumor fibroblasts spread out better on a substrate than mononuclear cells of the same line [1]. The general improvement in monolayer formation may perhaps somehow induce the appearance of a specialized outgrowth. This hypothesis is supported by the fact that the culture medium, by improving spreading capacity, creates better conditions for initiation of axon growth in normal neurons [3]. Another possibility is that induction of neuroblastoma differentiation is connected not with the formation of multinuclear cells, but with certain changes in the system of actin microfilaments, caused by CB.

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