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PRELIMINARY REPORT

IN VITRO TRANSFORMATION OF HAMSTER WHOLE EMBRYONIC CELLS BY 4-NITROQUINOLINE-1-OXIDE

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Although many factors such as chemical carcinogens, viruses and radiation, etc. have been found carcinogenic in animal experiments, no agents have been known to convert normal cells definitely to neoplastic cells *in vitro* except oncogenic viruses. In studying the mechanism of carciongenesis at cellular level it would be of great advantage to establish an in vitro system of neoplastic conversion of normal cells with chemical carcinogens. BERWALD and SACHS (1) reported the *in vitro* transformation of hamster embryonic cells by carcinogenic hydrocarbons, but their results have not been reproduced by others. This paper concerned with the transformation of hamster embryonic cells by a potent carcinogenic compound, 4-nitroquinoline-1-oxide (4 NQO) (2) *in vitro*. The specificity of the *in vitro* transformation by 4 NQO was examined by taking one of the derivative, 6-nitroquinoline-1-oxide (6 NQO) as control.

Tissue cultures were prepared by trypsinization of 10 to 12-day-old whole golden hamster embryos according to the modified Dulbecco's method (3). The suspensions containing 5×10^4 cells per ml were prepared, and seeded in 32 mm plastic petri dishes (Falcon Plastic Co., Los Angels, Calif.) containing 2 ml medium. The plates were incubated in a humidified atmosphere with 7% CO_2 at 37°C. The medium used in this experiment consisted of Eagle's minimum essential medium with 4 times the usual concentration of amino acids and vitamins supplemented by 10% calf serum. The media of all cultures were changed 3 times weekly. When the confluent sheet developed, serial passages of the growing cells were carried out by trypsinization of the cell sheet and transferring the cells into new petri dishes. Cultures were examined at various intervals under the phase contrast inverted microscope.

The application of 4 NQO or 6 NQO dissolved in medium was done 24 hours after the plating of the cells at the final concentrations of 10^{-6}, 5×10^{-7} and 10^{-7} M. The periods of treatment with the chemical agents were 1, 3 or 8 days, at the end of which cultures were washed several times and refed with fresh medium. The normal controls were cultures of untreated cells. Five plates were used with each dilution and period of treatment.

The application of 4 NQO at the final concentration of 10^{-6} or 5×10^{-7} M caused in the course of 1 to 3 days marked reduction of growth rate as well as various cytopathic changes characterized by cytoplasmic vacuolation and granulation, which resulted in necrosis and detachment of the greater part of the cell population. And the degree of the cytopathic

effects depended both on the concentration of 4 NQO used and the duration of treatment. The remaining cells were generally fusiform and distributed at random. On the other hand, all of the cells in the 6 NQO treated cultures revealed neither decrease of growth rate or specific cytological changes, so were the case also with the untreated cultures. Four to six days after removal of 4 NQO from the medium the survived cells apparently initiated to proliferate. On succeeding days the growth rate of these cells progressively recovered to the level of control cells, whereas cells treated with 4 NQO at the concentration of 10^{-6} M could not be carried further than the 2nd passage probably because of its toxicity. About 20th day after application of the carcinogen some polynucleated giant cells made their appearance containing more than ten nuclei in 4 NQO treated cultures (Fig. 1). About this time a few spindle shaped cells were observed among normal shaped cells, especially in 3 of 5 cultures treated with the concentration of 5×10^{-7} M of 4 NQO for about 8 days (Fig. 2). While the giant cells were in the fate of disappearance, the spindle shaped cells gradually increased in number with every passage, until about 35th day they acquired the capacity of forming several crisscross colonies. By 55th day these cells predominated the cultures, showing characteristic piling up and crisscross arrangements in the center of such a transformed colony (Fig. 3a and b). On 55th and 88th day the cells were removed from the plastic by trypsinization, washed several times with phosphate buffered saline, counted and dispersed in serum free medium. Aliquots of 0.5 ml suspensions containing the cells ranging from 10^3 to 10^6 were inoculated subcutaneously into the back of 1-month-old male hamsters respectively. Groups of 3 to 5 animals were inoculated with each dilution. As shown in Table 1, these cells derived from the cultures treated with 4 NQO 5×10^{-7} M for 8 days were already transplantable by 55th day at a dose of 10^6 cells per animals, and the cells kept in culture for 88 days after the application of 4 NQO produced tumors at a cell dose as low as 10^4. All tumors developed at the site of injection, being first evident at 18–30 day, and attaining a diameter as large as 2.5 cm in 1 month. The tumors were removed for histological examination and also for the preparation of tumor cell cultures. Grossly, in all animals there was no evidence of metastases. On histopathological examination they proved to be undifferentiated fibrosarcoma with abundant mitosis and focal necrosis. The fibroblastic cells obtained from the tumors could be easily passaged back in vitro.

On the other hand, the 6 NQO treated and the untreated cultures showed a rather remarkable decrease in growth rate at about 40–60 days, with simultaneous enlargement of cells and formation of vacuoles and granules in the cytoplasm. Around 60 and 80 days, however, the cell population seemed to show very little growth, though they survived these conditions

TABLE 1 *Tumor induction in adult hamsters by hamster cells transformed in vitro with 4-nitroquinoline-1-oxide (4 NQO)*

Days after the application of 4 NQO	Passage level	No. of cells inoculated	No. of hamsters with tumors/ No. of hamsters inoculated	Appearance of first tumor (days)
55	13 th	10^6	2/3	21
88	22 th	10^6	4/4	18
		10^5	2/4	25
		10^4	1/4	30
		10^3	0/5	—

for further several weeks, before they degenerated completely. Any other lines of cells could not be established than that obtained from the cells exposed to 4 NQO 5×10^{-7} M for 8 days.

More detailed studies are now in progress.

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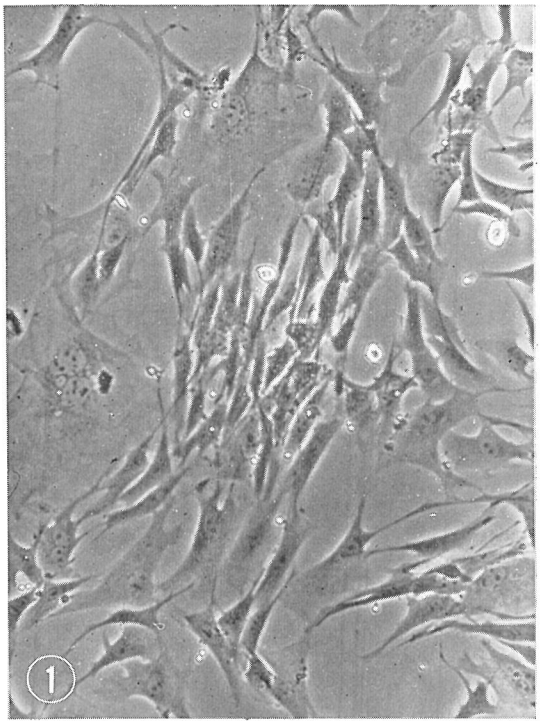
* Figures 1, 2 and 3 Hamster embryonic cells treated with 4-nitroquinoline-1-oxide (4 NQO) 5×10^{-7} M for 8 days. All phase contrast, unfixed, and unstained.

FIGURE 1 Twenty days after application of 4 NQO.

FIGURE 2 Twenty two days after application of 4 NQO.

FIGURE 3 Fifty five days after application of 4 NQO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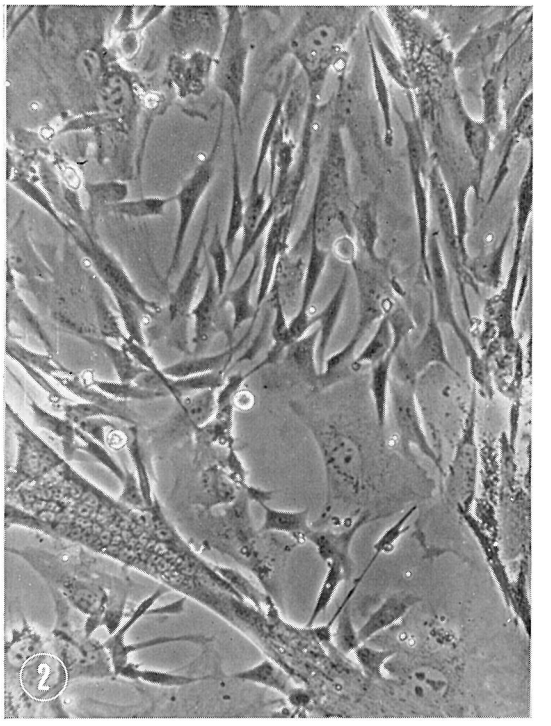
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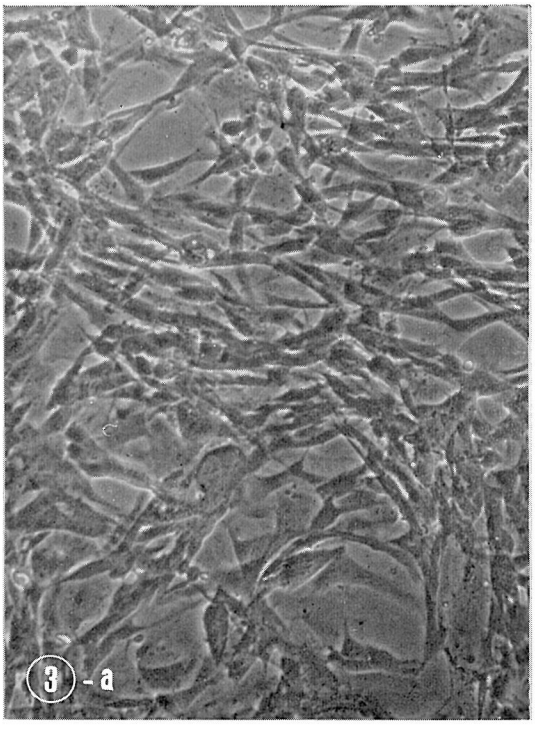
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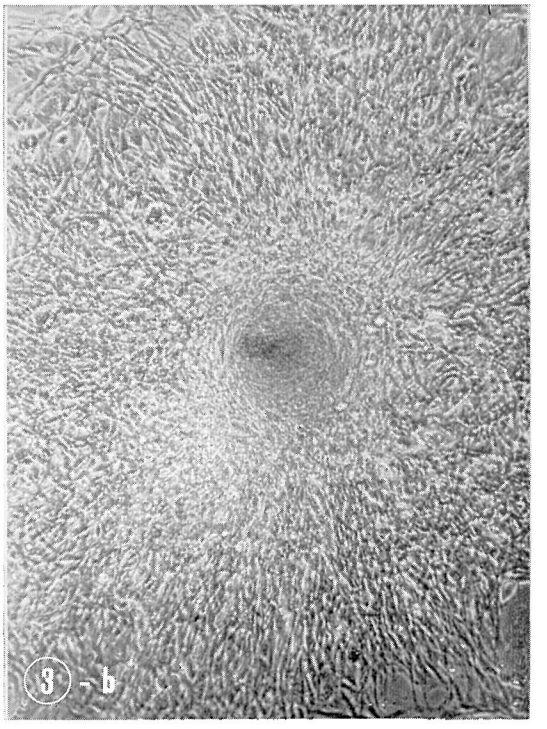
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