filter paper to supply sufficient humidity. After 10 days cultivation at 25 °C, the extent of spore germination and mycelial growth was judged with the naked eye and under a microscope.

Results and discussion. Assessment of biological activity of trichopolyns A and B is summarized in the table. Trichopolyns were found to be ineffective on the following organisms up to a concentration of 100μg/ml: *Escherichia coli, Salmonella typhosa, Shigella flexneri, Shigella sonnei, Klebsiella pneumoniae, Proteus vulgaris, Proteus morganii, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Citrobacter freundii and Enterobacter aerogenes.* Trichopolyns have strong inhibitory activity against fungi, gram-positive bacteria and acid-fast bacteria, which is quite different from cyclosporins[17]. The maximal tolerated dose of a 1:1 mixture of trichopolyns A and B in mice was 5 mg/kg when administered i.p. It is noteworthy that MIC of trichopolyns A and B against *Flammulina velutips* is exceedingly high as compared to those against other hymenomycetes in the agar dilution method, while in the paper disk method it remains on nearly the same level as other hymenomycetes. The observed fact is well explained by assuming that trichopolyns inhibit rather the mycelial growth than spore germination of *Flammulina velutipes*.

Though the mol.wt of trichopolyns A and B have not yet been settled, determination by gel filtration gave a value of about 2000 for both compounds. Moreover, infrared spectra of these antibiotics showed very similar absorption bands characteristic of polypeptides (ν_{max}^{KBr}; 3300, 1670 and 1535 cm^{-1}). Amino acid composition of trichopolyns A and B was determined to be [(α-amino isobutyric acid)$_4$, (Ala)$_2$, (Ile)$_1$, (Pro)$_1$]n. It is interesting that cyclosporins contain a number of methylated amino acids which are missing in trichopolyns. In the hydrolysates of both trichopolyns A and B with 6 N HCl the presence of α-methyl capric acid, and a ninhydrin-positive compound whose structure has not been determined, were recognized. Trichopolyn A was easily converted to trichopolyn B by stirring its acetone solution with saturated aqueous sodium chloride solution, while trichopolyn B, on stirring its acetone solution with 10% aqueous silver nitrate, gave trichopolyn A. These interconversions clearly indicate that these 2 antibiotics have the same basic skeleton but only differ in counter anions, that is NO_3^- for trichopolyn A and Cl^- for trichopolyn B. A more detailed investigation on the structure of trichopolyns is currently underway.

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Quantitative studies on in vitro transformation of hamster chondrocytes[1]

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27 June 1977

Summary. Hamster chondrocytes could be transformed in a quantitative assay system which used X-irradiated feeder layer cells. Morphological transformation occurred on addition of, 4NQO, but not in control cultures. Differentiation was classified into 3 types (good, poor and none); normal and transformed colonies contained similar proportions of the 3 types.

Since the 1st report of chemical carcinogenesis in vitro by Berwald and Sachs[3], various mammalian cells have been transformed by a number of chemical carcinogens. However, almost all studies have been done with fibroblastic cells which have no specific differentiated characters. Recently, several epithelial cell systems, such as rat liver parenchymal cells[4–6], rat submandibular gland cells[7], and rat urinary bladder cells[8], have been established, but they have not yet been used as model systems to investigate the relationship between carcinogenesis and differentiation. We reported previously that hamster chondrocytes, which originate from mesoderm but which have clearly differentiated characters, could be transformed into neoplastic cells with chemicals[9,10]. From our findings we concluded that carcinogenesis and differentiation are more or less incompatible. However, it was not clear whether transformation (or the initial step of carcinogenesis) and differentiation were incompatible, because we only observed a close relationship between malignancy and dedifferentiation. This paper reports the in vitro transformation of hamster chondrocytes with 4-nitroquinoline-1-oxide (4NQO) in a system for quantitative assay of transformation.

Chondrocytes were obtained from suckling hamsters as described previously[10]. Primary cultures of chondrocytes were trypsinized and stored in liquid nitrogen for use as target cells. The method for quantitative assay of transformation was essentially as reported previously[11]. The feeder layer cells used were cryopreserved hamster cells derived from 14 gestation days embryos. When the cells became confluent, they were irradiated with 5,000 R,

1 This work was supported by a grant from the Ministry of Education, Science and Culture, Japan.
2 We thank Miss M. Tanaka for her technical assistance.
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Transformation of hamster chondrocytes by 4NQO

Treatment (μg/ml)	Number of dishes**	Surviving colonies Total	Differentiation good	poor	none	Colony forming efficiency (%)	Transformed colonies Total	Differentiation good	poor	none	Transformation (%)
0.004	9	566	185 (32.7)***	209 (36.9)	172 (30.4)	12.58	4	1	2	1	0.71
0.002	9	620	226 (36.5)	235 (37.9)	159 (25.6)	13.78	3	1	1	1	0.48
0.001	9	604	205 (33.9)	244 (40.4)	155 (25.7)	13.42	3	1	1	1	0.50
0	9	610	186 (30.5)	260 (42.6)	164 (26.9)	13.56	0				0

* Treatment was for 8 days; ** each dish was seeded with approximately 500 chondrocytes; *** numbers in parentheses indicate percentages.

trypsinized, and seeded at 3×10^4 cells/ml in 2 ml of medium in 60 mm plastic dishes. Secondary cultures of chondrocytes were trypsinized when the cultures were 80–90% confluent. About 500 chrondrocytes in 2 ml of medium were seeded into dishes which had been seeded 24 h previously with X-irradiated feeder layer cells. The next day, graded doses of 4NQO were added to the dishes in volumes of 4 ml to obtain final concentrations of 0.001, 0.002, and 0.004 μg/ml of 4NQO, and 4 ml of medium only was added to control dishes. The culture medium used was Dulbecco's Modified Eagle Medium supplemented with 20% pretested fetal bovine serum (Microbiological Associates, USA). The cultures were incubated at 37 °C for 8 days without refeeding. Then they were washed twice with Hank's solution, fixed with methanol, and stained with 0.1% toluidine blue (pH 6.4 for 10 min). After examination for metachromatic staining, the cultures were stained with Giemsa.

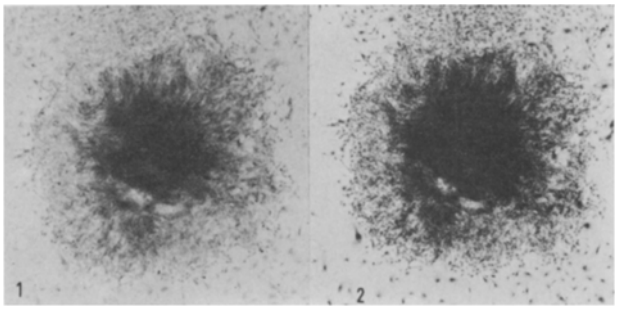

Fig. 1. Transformed colony of hamster chondrocytes (well differentiated type). Note metachromasia in the central region. Toluidine blue ×20.

Fig. 2. Same colony as for figure 1. Giemsa ×20.

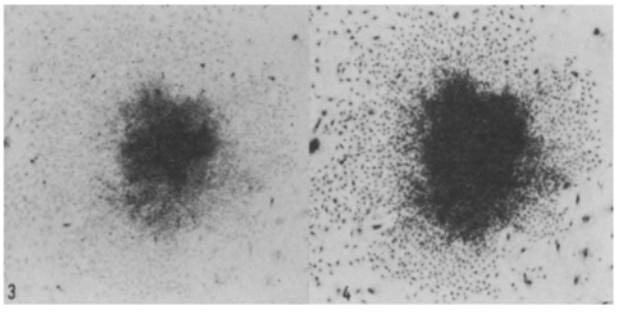

Fig. 3. Normal colony of hamster chondrocytes (well differentiated type). Toluidine blue ×20.

Fig. 4. Same colony as for figure 3. Giemsa ×20.

The colony forming efficiency (CFE) of cells treated with 0.001 and 0.002 μg/ml 4NQO was similar to that of control cultures, but the CFE of cultures treated with 0.004 μg/ml 4NQO was slightly less. As shown in the table, transformed colonies were obtained at all doses of 4NQO used, but no transformation occurred in control cultures. The rate of transformation was highest on treatment with 0.004 μg/ml 4NQO, but no apparent dose-response for transformation was observed. Morphological transformation of chondrocytes was seen as random orientation and decreased cytoplasmic spreading of cells at the periphery of colonies (figures 1 and 2); in contrast to the oriented growth and flattening of the cells at the periphery of normal colonies (figures 3 and 4). About 30% of the colonies in control cultures were well differentiated (metachromasia, ++), 40% were poorly differentiated (metachromasia, +), and 30% were undifferentiated colonies (metachromasia, −). The proportions of these 3 types did not change in transformed colonies, or in normal colonies on 4NQO treatment; namely, 3 of 10 transformed colonies were well differentiated, 4 were poorly differentiated, and 3 were undifferentiated. Therefore, morphological transformation is not incompatible with differentiation. Transformation of differentiating myoblasts [12,13] and differentiated chondrocytes [14] of chick embryos by a temperature-sensitive mutant of Rous sarcoma virus showed that transformation and differentiation were somewhat incompatible. However, viral transformation may involve both steps of carcinogenesis (initiation and promotion). These experiments demonstrate that it is possible to transform hamster chondrocytes in vitro in a quantitative assay system. This system seems to offer a convenient new assay system for rapid detection of the carcinogenicity of chemicals, because the cells are homogeneous.

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