was not 3-dimensional in an invasive situation similar to in vivo models. The cells had a 3-dimensional 'floor' to move across, but lacked the 3-dimensional 'ceiling' necessary to simulate the in vivo situation.

Several authors[16,17] have reported that a cell will respond differently on different substrates and that age and the state of differentiation[18] affect cell movement. Our observations show that cells respond differently in 2-dimensional and 3-dimensional systems. We would confirm that the classical described features of cell movement in vitro are present in vivo but are greatly diminished. The morphological features normally associated with in vitro cell movement, we feel, are exaggerations of normal movements encountered in ovo because of the differences encountered between a 2-dimensional and 3-dimensional environment.

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Promotion of epithelial keratinization by N-methyl-N'-nitro-N-nitrosoguanidine in rat forestomach in organ culture[1]

H. Fukamachi and S. Takayama[2]

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, and Department of Experimental Pathology, Cancer Institute, 1-37-1. Kami-Ikebukuro, Toshima-ku, Tokyo 170 (Japan), 25 September 1978

Summary. The effect of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) on epithelial differentiation of fetal rat forestomach was investigated in organ culture. When forestomach tissues removed from 16.5-day fetuses were treated with 5 µg and 3 µg of MNNG per ml for 1 h, epithelial keratinization was observed after 4 and 5 days, respectively, whereas it occurred after 6 days in control cultures. A clear dose-response relationship was found in the promotion of epithelial keratinization by MNNG.

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) has been known since the report of Mandell and Greenberg[3], to be a powerful mutagen in microorganisms and viruses and it has also been widely used as a potent chemical carcinogen. When given to animals in their drinking water, MNNG induces malignant tumors in the digestive tract, especially in the glandular stomach and forestomach[4]. The reaction of MNNG with biological materials has been extensively investigated at the molecular level[5]. We report here that MNNG promotes epithelial keratinization in fetal rat forestomach in organ culture.

Materials and methods. Forestomachs were removed from the fetuses of inbred Fischer 344/DuCrj rats (Charles River Japan Inc., Japan) at 16.5 days of gestation. MNNG (Aldrich Chemical Co., USA) was dissolved in doubly distilled water (DDW) at appropriate concentrations, and 0.5 ml of the solution was added to 4.5 ml of Hanks' solution in dishes with the tissue fragments. Only DDW was added to control dishes. After incubation with MNNG for 1 h at 37 °C in the dark, tissue fragments were washed 3 times with Hanks' solution, soaked for 2 h in Hanks' solution containing 50% fetal calf serum, and finally

Effect of MNNG on the time-course of epithelial differentiation in organ culture

Concentration of MNNG (µg/ml)	Epithelial differentiation	Incubation time (days) 1	2	3	4	5	6	7
5	Not keratinized	4/4	6/6	12/18	1/17	0	0	0
	Partly keratinized	0	0	5/18	4/17	1/19	0	0
	Keratinized	0	0	1/18	12/17	18/19	6/6	13/13
3	Not keratinized	-	-	7/7	3/8	2/7	0	0
	Partly keratinized	-	-	0	3/8	2/7	0	0
	Keratinized	-	-	0	2/8	3/7	6/6	3/3
1	Not keratinized	-	-	2/2	5/5	3/6	0	0
	Partly keratinized	-	-	0	0	3/6	3/5	0
	Keratinized	-	-	0	0	0	2/5	5/5
Control	Not keratinized	3/3	2/2	14/14	17/17	11/18	1/10	0
	Partly keratinized	0	0	0	0	6/18	1/10	1/10
	Keratinized	0	0	0	0	1/18	8/10	9/10

-, Not examined.

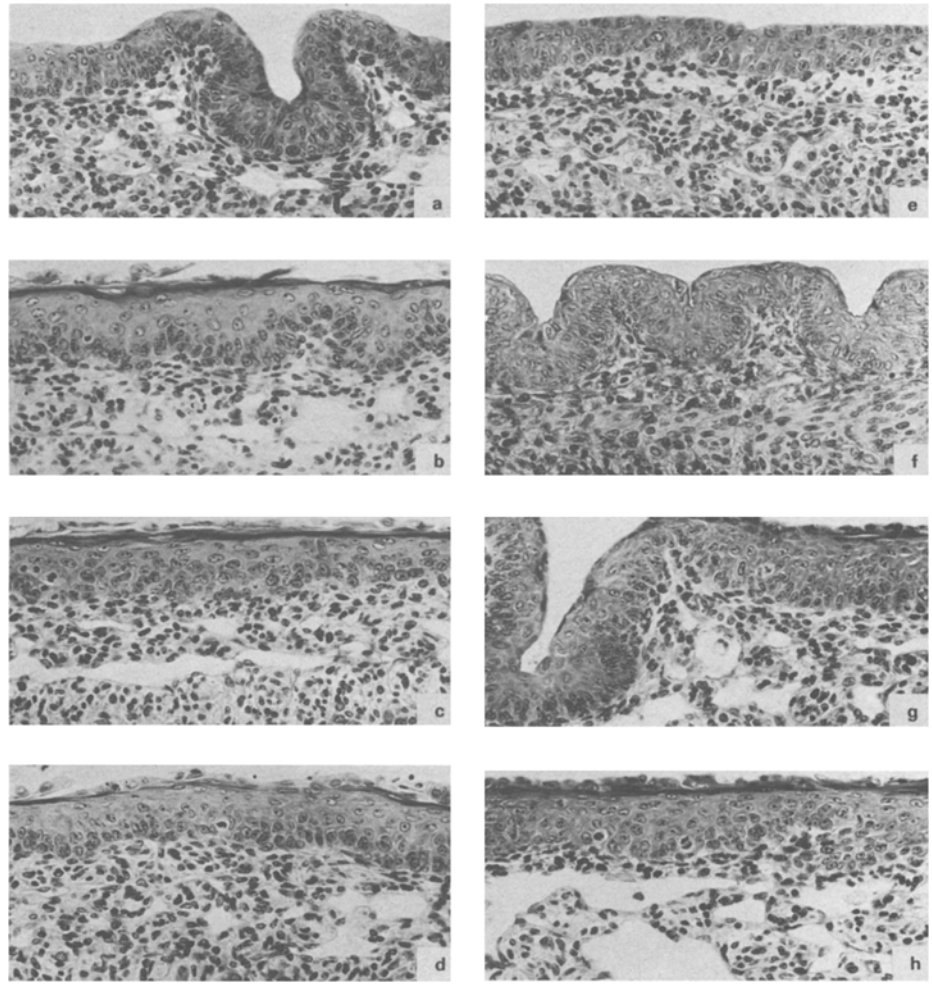
washed with fresh Hanks' solution. The concentration of MNNG in DDW was checked by measuring the optical density at 402 nm. After treatment, the tissue fragments were cultured on millipore filters (Millipore Corp., USA, Type HA, 0.45 µm) laid on stainless steel grids in 35-mm culture dishes containing 3 ml of medium 199 supplemented with 10% fetal calf serum (Microbiological Associates, USA). Explants were cultured under 5% CO_2 in air in a humidified atmosphere at 37 °C for 1–7 days. After cultivation, explants were fixed with Bouin's fluid, embedded in paraffin, sectioned serially at 5 µm, and stained with hematoxylin and eosin.

Results. Tissue fragments treated with 20 µg/ml of MNNG for 1 h did not survive in 7-day organ culture. Those treated with 10 µg/ml of MNNG did not show good differentiation, probably because mesenchymal tissues became necrotic due to the toxicity of MNNG. No toxic effect was noted when the tissues were treated with 5 µg/ml of MNNG. The time-course of epithelial differentiation is summarized in the table.

1. Explants treated with 5 µg/ml of MNNG. At the start of incubation, the epithelium consisted of simple columnar cells. The epithelial cells began to stratify after 24-h incubation (day 1). The superficial cells became flattened on day 2. Keratinization was first noted on day 3, although many explants were still not keratinized (figure a). On day 4, keratinization was seen in almost all explants (figure b), and on day 5, all the epithelium was keratinized (figure c).
2. Explants treated with 3 µg/ml of MNNG. Epithelial keratinization was first noted on day 4. On day 5, some explants were fully keratinized while others were not keratinized at all. The process of keratinization was completed on day 6.
3. Explants treated with 1 µg/ml of MNNG. Epithelial keratinization was first noted on day 5, and completed on day 7. The time-course of epithelial differentiation in vitro was the same as that of control tissue.
4. Controls. On day 1, no difference was noted between treated and control cultures. Superficial cells of the epithelium became flattened on days 3–4 (figures e, f). Keratinization was first noted on day 5 in about $\frac{1}{3}$ of the explants (figure g). Most explants showed keratinization on day 6 (figure h), and all explants were keratinized on day 7.

Discussion. The mutagenicity and carcinogenicity of MNNG have been reported by many investigators, but promotion of epithelial keratinization by MNNG has not been reported. The relationship between carcinogenesis and dedifferentiation has been discussed by many workers[6]. The present work showed that MNNG, a potent carcinogen, promoted epithelial differentiation in fetal rat forestomach in organ culture. This system should be useful in further studies on the relationship between carcinogenesis and differentiation.

A dose-response relationship was noted in the promotion of epithelial keratinization by MNNG: treatments with 5 µg and 3 µg of MNNG/ml resulted in the appearance of keratinization 2 days and 1 day, respectively, earlier than in control cultures. These findings show that MNNG pro-



Time-course of epithelial differentiation of fetal rat forestomach in vitro. *a–d* Tissue fragments treated with 5 µg/ml of MNNG. *e–h* Controls. *a* and *e* on day 3, *b* and *f* on day 4, *c* and *g* on day 5, and *d* and *h* on day 6. × 200.

motes keratinization. It is well known that some chemicals, such as vitamin A, influence epithelial differentiation: addition of vitamin A to organ cultures of hamster trachea after development of keratinized squamous lesions causes reversal of the process of keratinization[7]. We are now investigating the problem of whether the effect of MNNG on keratinization can be modulated by such chemicals.

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The distribution of carcinogens, 4-nitroquinoline-1-oxide and 4-hydroxyaminoquinoline-1-oxide, in the nervous system and its possible neurotoxicological significance[1]

T. Takasu

Department of Neurology, Institute of Brain Research, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo (Japan), 7 August 1978

Summary. 4-NQO-^{14}C can enter the grey matter parenchyma of the central nervous system of mice after i.v. injection. The level of its uptake by the central grey is higher than that taken up by the central white and by the trigeminal and spinal dorsal root ganglia. This pattern of distribution is strikingly different from that obtained after i.v. injection of 4-HAQO-^{14}C, suggesting the possible occurrence of 4-NQO encephalomyelopathy having entirely different sites of lesions from those of 4-HAQO neuropathy.

4-Nitroquinoline-1-oxide (4-NQO) is a well-known carcinogenic agent[2]. In animal bodies it is converted to its reduced form, 4-hydroxyaminoquinoline-1-oxide (4-HAQO), before acting as a carcinogen[3].

In 1971 Hayashi, Hasegawa and Toyoshima[4] reported in this journal on the degeneration of axons observed in the posterior column, as well as in the spinal tract of the trigeminal nerve, in rats after repeated i.v. injection of 4-HAQO. In 1977 Takahashi, Agari and Nakamura[5] have reconfirmed the facts. One of the most interesting points in this dying-back type of neuropathy is the progressive nature of degeneration recognized after completion of the administration of the agent, and this nature has been ascribed to the conceivably irreversible changes occurring in the DNA of the nerve cells[4,5]. On the one hand, no reports have ever appeared on the nervous disorders induced by 4-NQO.

Recently the author of this communication has observed the distribution of both these agents in mice. It has been concluded from these experiments that the pattern of distribution of 4-NQO-^{14}C in the nervous system is strikingly different from that of 4-HAQO-^{14}C after their i.v. injection. This finding suggests the possible occurrence of an encephalomyelopathy by 4-NQO having entirely different sites of lesions from those of 4-HAQO neuropathy.

Materials and methods. Male adult mice of ddy strain, around 10 weeks in age, were given i.v. either 4-NQO-5, 6, 7, 8, 9 and 10-^{14}C (6.2 mCi/mmole) suspended or 4-HAQO-5, 6, 7, 8, 9 and 10-^{14}C hydrochloride (3.2 mCi/mmole) dissolved in hydrochloric acidic saline. The doses were 9.5 and 9.1 μCi/mouse, respectively. After a certain length of time (i.e. 1, 5 or 24 h), the animals were sacrificed by chloroform inhalation, and whole-body macroautoradiograms were made, according to Matsuoka's modification of Ullberg's method, from the 40-μm-thick section of the frozen mice. The density distribution developed in the industrial X-ray films were examined by naked eye, aided by densitometry of the films. The details of the method were described elsewhere[6].

Results and discussion. The results were remarkable. Thus, it was in the grey matter of the central nervous system (CNS) that the level of 4-NQO-^{14}C uptake after 5 h was highest of all the parenchymal organs in the body (figure 1a). Differing from the case of methyl mercury[7], the level was relatively low in the cerebellar cortex and white matter, and its uptake by the spinal dorsal root ganglia was at quite a low level compared with that by the central grey (figure 2a). To the author's knowledge, there has never appeared a report on the distribution of 4-NQO in the nervous system.

On the one hand, the uptake of 4-HAQO-^{14}C by the brain and spinal cord was at a relatively low level in the body (figure 1b), and the level of its uptake by the spinal dorsal root ganglia was definitely high in comparison with that by the CNS (figure 2b). These results seem to be compatible with the occurrence of a sensory neuropathy of dying-back type in rats[4,5], involving primary sensory neuron. The level of its uptake by the trigeminal ganglia was also higher than that by the CNS and this finding agrees with Hayashi et al.'s with ^{3}H-4-HAQO only briefly mentioned in their review[8].

After 24 h, the basic pattern of distribution of 4-NQO-^{14}C and 4-HAQO-^{14}C remained unchanged although the level of their uptake was generally reduced. The most interesting findings seem to be the predilection of 4-NQO-^{14}C for the CNS and the striking difference in distribution found between 4-NQO-^{14}C and 4-HAQO-^{14}C, as if 1 of the 2 were the positive or negative of the other.

What should be stressed first is the fact that the uptake of 4-NQO-^{14}C by the grey matter of the CNS was much higher than that by the blood. This indicates that the concentration of 4-NQO-^{14}C in the central grey parenchyma must have been much higher than in the blood.

Second to be emphasized is the fact that the level of 4-NQO-^{14}C uptake by the cerebral cortex and by the spinal grey matter, for example, was definitely higher than that by the corpus callosum and by the spinal white matter, respec-