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Inductions of Ornithine Decarboxylase and Replicative DNA Synthesis but not DNA Single Strand Scission or Unscheduled DNA Synthesis in the Pyloric Mucosa of Rat Stomach by Catechol

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The possible tumor-promoting and genotoxic activities of catechol were examined. Administration of catechol by gastric intubation at doses of 10 to 90 mg/kg body weight to male F344 rats induced up to 19-fold increase in ornithine decarboxylase activity with a maximum after 8 h and up to 8-fold increase in replicative DNA synthesis with a maximum after 24 h in the pyloric mucosa of the stomach. These results suggest that catechol has tumor-promoting activity in the pyloric mucosa of rat stomach. However, its administration at doses of 37.5 to 90 mg/kg body weight did not induce DNA single strand scission in the pyloric mucosa as determined by the alkaline elution method after 2 and 6 h or unscheduled DNA synthesis examined after 2 and 12 h.

Key words: Catechol — Ornithine decarboxylase — Replicative DNA synthesis — DNA single strand scission — Rat stomach mucosa

Catechol is present in the human environment: it is one of the main phenols in cigarette smoke (21–502 μg/cigarette)[1] and is present in some foods, such as onions, crude beet sugar[2] and coffee.[3] More than one million kg of catechol is used annually in industry.[2] Recently Hirose *et al.*[4,5] reported that catechol strongly promoted 2-step carcinogenesis in the glandular stomach of male F344 rats initiated by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and that catechol itself strongly induced adenomatous hyperplasia in the pyloric mucosa of rat stomach and also tended to induce adenocarcinomas although the latter effect was not statistically significant. Catechol was reported not to be mutagenic to *Salmonella typhimurium*,[6] but to induce sister chromatid exchanges in human lymphocytes in culture[7] and DNA double strand acissions in cultured rat fetal lung cells.[8] Therefore, for understanding the mechanism of glandular stomach carcinogenesis, it was of interest to examine possible tumor-promoting and genotoxic activities of catechol in the pyloric mucosa of rat stomach *in vivo*.

Previously we developed *in vivo* short-term methods for evaluating possible tumor-initiating activity using unscheduled DNA synthesis (UDS)[9,10] and DNA-strand scission determined by an alkaline elution method,[11] as markers and also possible tumor-promoting activity with ornithine decarboxylase (ODC) and replicative DNA synthesis (RDS) as markers.[10,12] In this work, we examined the possible tumor-initiating and -promoting activities of catechol in the pyloric mucosa of rat stomach after its administration to rats by gastric intubation. The results indicated that catechol has possible tumor-promoting activity but does not induce DNA single strand scissions or unscheduled DNA synthesis in the pyloric mucosa of rat stomach.

MATERIALS AND METHODS

Animals and treatment Male Fischer rats (F344/Du Crj: Charles River Japan, Inc., Kanagawa), 7 to 8 weeks old, were given a limited amount of diet (4 g of commercial pellet diet, Nihon Clea, Tokyo, per rat of 200 g body weight) overnight to reduce their dietary stomach contents. The following day they were given 1.0 ml of an aqueous solution of catechol (99% pure, Sigma Chemical Co., St. Louis, MO) by gastric intubation. Under the present conditions, the LD_{50} of catechol was about 150 mg/kg body weight, but a dose of 100 mg/kg body weight sometimes caused convulsions, so a dose of less than 90 mg/kg body weight was used in this work. Control animals were given distilled, deionized water only.

ODC ODC activity in extracts of the pyloric mucosa of the stomach was determined with L-[1-^{14}C]ornithine (2.01 GBq/mmol, New England Nuclear, Boston, MA) as a substrate, as described previously.[10] The protein content of the extracts was determined by micro-assay as described[13] with bovine serum albumin as a standard. Results are shown as means for duplicate assays on pooled materials from four rats.

RDS and UDS After administration of catechol *in vivo*, RDS and UDS in the pyloric mucosa of the stomach were determined in *in vitro* organ culture in the presence of tritiated thymidine ([^{3}H]dThd, 2.96 TBq/mmol, New England Nuclear) without or with 10 m*M* hydroxyurea

(HU), an inhibitor of RDS, as described previously.[9, 10] The DNA fraction was extracted from the tissue, an aliquot was dissolved in ACS II (Amersham Corp., Arlington Heights, IL), and the incorporation of [^{3}H]-dThd into DNA was determined in a Beckman LS-355 liquid scintillation counter. The DNA content of the DNA fraction was determined with 3,5-diaminobenzoic acid (Tokyo Kasei Kogyo Co., Tokyo) as described[14] with calf thymus DNA (Worthington Biochemicals Co., Freehold, NJ) as a standard. Values for five individual rats were determined in each experiment.

Alkaline elution A modification of the method of Sina *et al.*[15] was used. A sample of 5 mg of pyloric mucosa was lysed on a filter (Fluorinert, 1.0 μm pore size, 25 mm, Japan Millipore Ltd., Tokyo) with 3.0 ml of lysis solution, pH 9.7, in the presence of proteinase K (1 mg/ml, Sigma Chemical Co.) for 1 h in the dark, and then DNA was eluted in the dark at 20–25°C with 30 ml of solution of pH 12.1 at a flow rate of 0.05 ml/min. Fractions of 3.0 ml were collected and the DNA contents of the fractions and on the filters were determined with 3,5-diaminobenzoic acid.[14] The elution rate constant (ml^{-1}) of DNA was calculated from a plot (log scale) from the start to the 5th fraction. The results were analyzed by means of Student's *t* test.

RESULTS

Induction of ODC activity Figure 1 shows the induction of ODC activity in the pyloric mucosa of rat stomach after administration of catechol at a dose of 75 mg/kg body weight. The ODC activity in the pyloric mucosa of control rat stomach was about the lowest level detectable by the present assay method (3.7±4.0 pmol CO_2/30 min/mg protein). The activity was increased between 4 and 24 h after administration of catechol with a maximum after 8 h and returned to the control level within 48 h. Table I shows the dose-dependence of induction of ODC activity in the pyloric mucosa 8 h after administration of catechol at doses of 10 to 80 mg/kg body weight. A dose of 40 mg/kg body weight induced a 19-fold increase in ODC activity.

Stimulation of RDS Figure 2 shows the increase of DNA synthesis in the absence of HU in the pyloric mucosa of rat stomach after administration of catechol at a dose of 75 mg/kg body weight. In normal stomach, RDS is always observed in cells of the proliferative zone of the pyloric mucosa where the cells are renewed. DNA synthesis at time 0 in Fig. 2 represents the control level of DNA synthesis in the pyloric mucosa (153±75 dpm [^{3}H]dThd/μg DNA). DNA synthesis increased with a maximum after 24 h of 8 times the initial value. Table II shows the dose-dependent stimulation of RDS in the

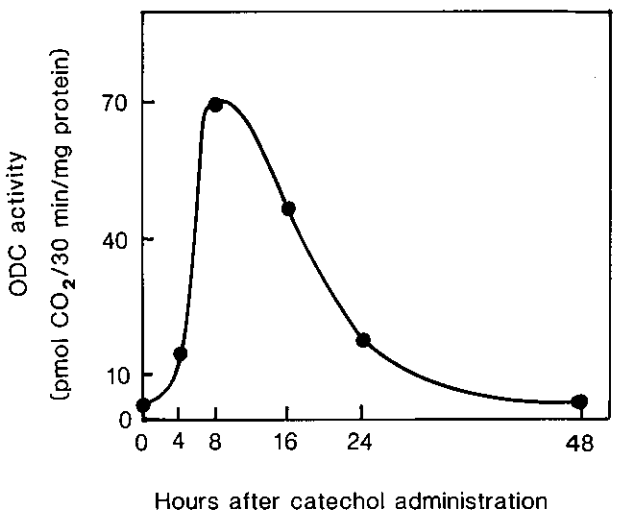

Fig. 1. Induction of ODC activity in the pyloric mucosa of rat stomach after administration of catechol at a dose of 75 mg/kg body weight. Results are means for duplicate assays on pooled materials from four rats.

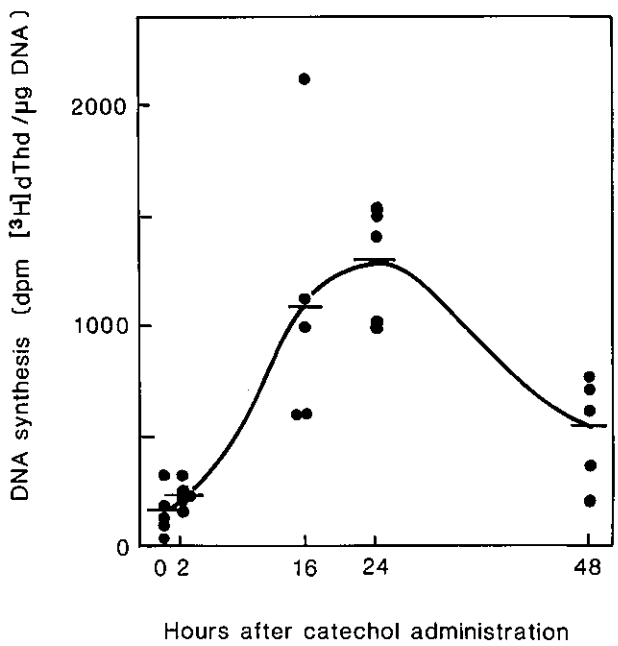

Fig. 2. Stimulation of RDS in the pyloric mucosa of rat stomach after administration of catechol at a dose of 75 mg/kg body weight. Results are for five individual rats at each time, and horizontal lines are means of the five values. Values at 24 h were significantly different from that at time 0 by Student's *t* test ($P<0.001$).

Table I. Dose-dependent Induction of ODC Activity in the Pyloric Mucosa of the Stomach of Rats Treated with Catechol

Dose (mg/kg body weight)	ODC activity (pmol CO_2/30 min/ mg protein)
0	3.72±4.04[a]
10	16.6
40	71.2
80	59.7

a) Results are means±SD of values in 18 experiments.

Table II. Dose-dependent Stimulation of Replicative DNA Synthesis in the Pyloric Mucosa of the Stomach of Rats Treated with Catechol

Dose (mg/kg body weight)	DNA synthesis (dpm[^{3}H]dThd/ μg DNA)[a]	*P* value by Student's *t* test
0	163±61	
10	315±162	
20	628±124	<0.01
90	745±336	<0.05

a) Results are means±SD of values in five rats 20 h after administration of catechol.

Table III. Absence of Induction of Unscheduled DNA Synthesis in the Pyloric Mucosa of the Stomach of Rats Treated with Catechol

Experiment	Dose (mg/kg body weight)	Time after treatment (h)	DNA synthesis (dpm[^{3}H]dThd/ μg DNA)[a]
I	0	2	21.7±4.0
	37.5	2	28.1±9.9
II	0	2	25.8±3.9
	75	2	35.2±9.1
III	0	6	15.6±5.44
	37.5	6	12.7±3.55
	75	6	14.0±2.60

a) Results are means±SD of values in five rats 2 h after administration of catechol.

pyloric mucosa 20 h after administration of catechol at doses of 10 to 90 mg/kg body weight.

Non-induction of UDS DNA synthesis in the pyloric mucosa of rat stomach was measured in the presence of HU, an inhibitor of RDS, in *in vitro* cultures 2 and 6 h after administration of catechol to rats. The results in Table III show that UDS was not induced in the pyloric mucosa of rat stomach at either time after administration of catechol at a dose of 37.5 or 75 mg/kg body weight.

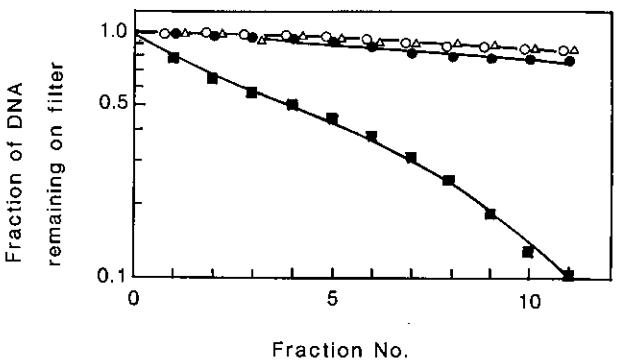

Fig. 3. Alkaline elution of DNA from stomach pyloric mucosa of rats 2 (○) and 12 (△) h after administration of catechol at a dose of 75 mg/kg body weight, 2 h after administration of MNNG at 10 mg/kg body weight (■) and 2 h after administration of 1 ml of H_2O (●).

Table IV. Alkaline Elution of DNA from Stomach Pyloric Mucosa of Rats Treated with Catechol

Dose (mg/kg body weight)	Time after treatment (h)	No. of experiments	Elution rate constant[a] ($ml^{-1}\times10^3$)
0	2/12	7	2.07±0.80
37.5	2	3	2.78±1.93
75	2	3	1.30±1.07
75	12	3	2.55±1.93
90	2	4	1.71±0.19

a) Mean±SD.

Addition of 10 m*M* HU to the culture medium inhibited RDS in the pyloric mucosa about 95%, and the DNA synthesis at zero dose in Table III corresponds to that not inhibited by 10 m*M* HU. DNA synthesis may have been lower 6 h after catechol administration than 2 h after its administration because the rats were starved for a longer period for measurement of UDS after 6 h. Compounds such as MNNG and 3-diazo-N-nitrosobamethan induced UDS, detected as an increase in DNA synthesis in the presence of HU, in the pyloric mucosa of rat stomach 2 h after their administration.[9, 10, 16, 17]

Non-induction of DNA single strand scission Figure 3 shows typical results of alkaline elution of DNA from the pyloric mucosa of rats 2 and 12 h after administration of catechol at a dose of 75 mg/kg body weight, 2 h after administration of MNNG, as a positive control, at a dose of 10 mg/kg body weight and 2 h after administration of 1 ml of H_2O, as a negative control. Table IV shows the elution rate constants. The elution rate constant did not increase after administration of catechol, suggesting that

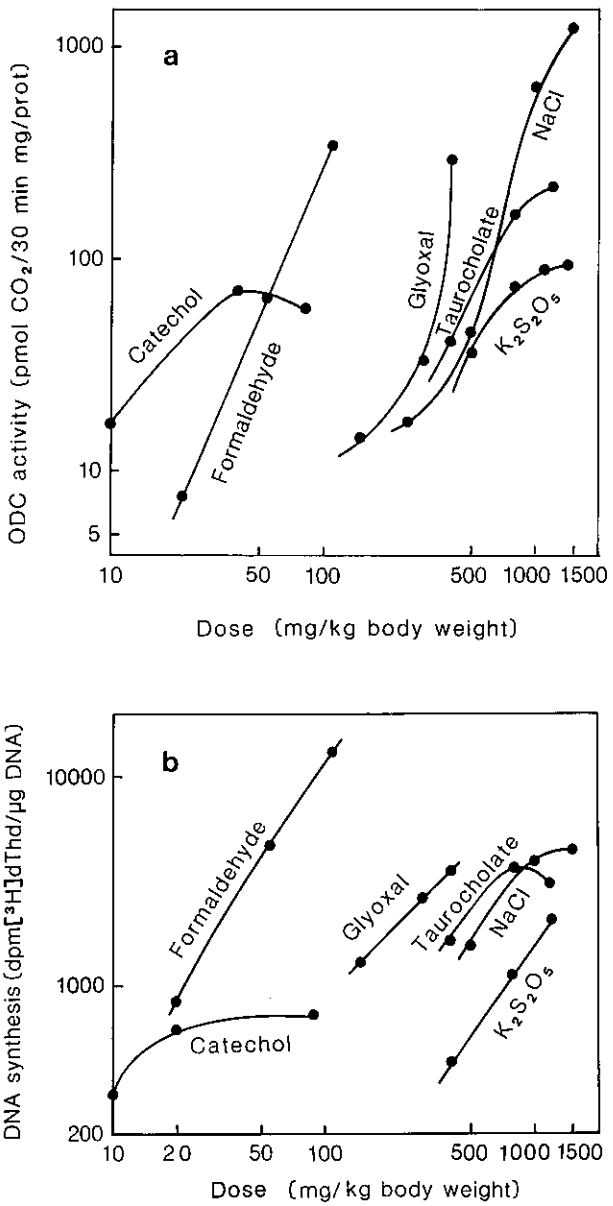

Fig. 4. Dose-dependent inductions of ODC (a) and RDS (b) in the pyloric mucosa of rat stomach by glandular stomach tumor promoters.

catechol did not induce single strand scission of DNA in the pyloric mucosa 2 or 12 h after its administration at a dose of 37.5, 75 or 90 mg/kg body weight. The positive controls, MNNG and glyoxal, increased the elution rate constant 2 h after their administration (MNNG at a dose of 10 mg/kg body weight, 28.8±12.6; glyoxal at a dose of 500 mg/kg body weight, 21.0±8.4).[11]

Comparison of catechol with other glandular stomach tumor promoters Figure 4-a, b shows the dose-dependent inductions of ODC activity and RDS by glandular stomach tumor promoters. Catechol has higher activity for induction of ODC activity and RDS than NaCl,[12] taurocholate,[18] $K_2S_2O_5$[19] and glyoxal[16] in the pyloric mucosa of rat stomach and catechol shows stronger tumor promotion than these compounds in glandular stomach carcinogenesis.[4, 5, 20-23]

DISCUSSION

Previously we found that five glandular stomach tumor promoters (NaCl,[12] glyoxal,[16] taurocholate,[18] $K_2S_2O_5$[19] and formaldehyde[24]) and five glandular stomach carcinogens [MNNG, N-ethyl-N′-nitro-N-nitrosoguanidine (ENNG), N-propyl-N′-nitro-N-nitrosoguanidine (PNNG), 4-nitroquinoline 1-oxide (4NQO) and N-nitroso-N-methylurethane (NMUT)[25]] all induced about 100-fold increase in ODC activity in the pyloric mucosa of F344 rats with maxima 4–24 h after their administrations, and also about 10-fold increase in RDS in the proliferative zone of the pyloric mucosa of F344 male rats with maxima 16–24 h after their administrations. However, ethanol did not induce ODC or RDS in the pyloric mucosa of the stomach of F344 rats[25] and did not enhance stomach carcinogenesis,[20] although it induces acute ulcers in the stomach mucosa of rats, as NaCl does. Moreover, compounds that are not glandular stomach carcinogens, such as 2-acetylaminofluorene (2AAF), dimethylnitrosamine (DMN), and 3-amino-1-mehtyl-5*H*-pyrido[4, 3-*b*]indole, did not stimulate ODC or RDS in the pyloric mucosa of rats.[25] The present work confirmed that catechol, a strong glandular stomach tumor promoter and a probable glandular stomach carcinogen, may have tumor-promoting activity (induction of ODC and RDS) in the pyloric mucosa of rat stomach.

Previously we reported that all five glandular stomach carcinogens examined (MNNG, ENNG, PNNG, 4NQO and NMUT) induced UDS in the pyloric mucosa of the stomach of F344 male rats[9] and that at least MNNG[11] and NMUT (Furihata *et al.*, unpublished) induced DNA single strand scissions in the pyloric mucosa of rat stomach. However, non-glandular stomach carcinogens, such as 2AAF and DMN did not induce UDS[9] or DNA single strand scissions[11] in the pyloric mucosa of rat stomach. In the present work, catechol did not induce DNA single strand scissions or UDS in the pyloric mucosa of rat stomach at doses of up to 90 mg/kg body weight.

Since catechol is not mutagenic to *Salmonella typhimurium*,[6] it is probably not genotoxic in the pyloric mucosa of rat stomach. No glandular stomach carcinogens that are not genotoxic have been reported before, but some nongenotoxic liver carcinogens such as CCl_4[26]

and di(2-ethylhexyl)phthalate[27] have been reported.[28, 29] These nongenotoxic liver carcinogens induced cell proliferation but not UDS in rat liver. On the other hand, various phenols, such as epinephrine, norepinephrine and catechol were reported to induce DNA double strand breaks in cultured rat fetal lung cells as shown by neutral sucrose gradient centrifugation.[8] Epinephrine was suggested to bind to DNA non-covalently.[30] Catechol also induces sister chromatid exchanges in human lymphocytes in culture. Catechol may induce DNA double strand breaks but not DNA single strand scissions in the pyloric mucosa of rat stomach. Further study of the DNA double strand breaks induced by catechol in the pyloric mucosa of rat stomach seems indicated.

In long-term experiments on stomach carcinogenesis, rats were given catechol at doses of 1.5% and 0.8% in their diet.[4] These daily doses are calculated to be about 750 and 400 mg/kg body weight/day. In the present study, rats received lower doses than in these long-term experiments, because catechol in aqueous solution was more toxic to rats that had been given a limited amount of diet overnight.

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REFERENCES

1) IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 38. "Tobacco Smoking," pp. 83–126 (1986). IARC, Lyon.
2) IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 15. "Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals," pp. 155–175 (1977). IARC, Lyon.
3) Rahn, W. and König, W. A. GC/MS investigations of the constituents in a dietary ether extract of an acidified roast coffee infusion. *J. High-Resolut. Chromatogr.*, **1**, 69–71 (1979).
4) Hirose, M., Kurata, Y., Tsuda, H., Fukushima, S. and Ito, N. Catechol strongly enhances rat stomach carcinogenesis: a possible new environmental stomach carcinogen. *Jpn. J. Cancer Res.*, **78**, 1144–1149 (1987).
5) Hirose, M., Fukushima, S., Kurata, Y., Tsuda, H., Tatematsu, M. and Ito, N. Modification of N-methyl-N′-nitro-N-nitrosoguanidine-induced forestomach and glandular stomach carcinogenesis by phenolic antioxidants in rats. *Cancer Res.*, **48**, 5310–5315 (1988).
6) Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. and Zeiger, E. *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutag.*, Suppl. 1, 3–142 (1983).
7) Morimoto, K. and Wolff, S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. *Cancer Res.*, **40**, 1189–1193 (1980).
8) Yamada, K., Shirahata, S., Murakami, H., Nishiyama, K., Shinohara, K. and Omura, H. DNA breakage by phenyl compounds. *Agric. Biol. Chem.*, **49**, 1423–1428 (1985).
9) Furihata, C., Yamawaki, Y., Jin, S-S., Moriya, H., Kodama, K., Matsushima, T., Ishikawa, T., Takayama, S. and Nakadate, M. Unscheduled DNA synthesis in rat stomach mucosa induced by glandular stomach carcinogens. *J. Natl. Cancer Inst.*, **72**, 1327–1334 (1984).
10) Furihata, C., Sato, Y., Matsushima, T. and Tatematsu, M. Induction of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by methylglyoxal. *Carcinogenesis*, **6**, 91–94 (1985).
11) Furihata, C., Hatta, A., Sato, Y. and Matsushima, T. Alkaline elution of DNA from stomach pyloric mucosa of rats treated with glyoxal. *Mutat. Res.*, **213**, 227–231 (1989).
12) Furihata, C., Sato, Y., Hosaka, M., Matsushima, T., Furukawa, F. and Takahashi, M. NaCl induced ornithine decarboxylase and DNA synthesis in rat stomach mucosa. *Biochem. Biophys. Res. Commun.*, **121**, 1027–1032 (1984).
13) Heil, A. and Zilling, W. Reconstitution of bacterial DNA-dependent RNA-polymerase from isolated subunits as a tool for the elucidation of the role of the subunits in transcription. *FEBS Lett.*, **11**, 165–168 (1970).
14) Kissane, J. M. and Robins, E. The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *J. Biol. Chem.*, **233**, 184–188 (1958).
15) Sina, J. F., Bean, C. L., Dysart, G. R., Taylor, V. I. and Bradley, M. O. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat. Res.*, **113**, 357–391 (1983).
16) Furihata, C., Yoshida, S. and Matsushima, T. Potential initiating and promoting activities of diacetyl and glyoxal in rat stomach mucosa. *Jpn. J. Cancer Res.*, **76**, 809–814 (1985).
17) Furihata, C., Yamakoshi, A., Matsushima, T., Kato, T. and Kikugawa, K. Possible tumour-initiating and -promoting activities of 3-diazo-N-nitrosobamethan in rat stomach mucosa. *Mutagenesis*, **3**, 299–301 (1988).
18) Furihata, C., Takezawa, R., Matsushima, T. and Tatematsu, M. Potential tumor-promoting activity of bile acids in rat glandular stomach. *Jpn. J. Cancer Res.*, **78**, 32–39 (1987).

19) Furihata, C., Yamakoshi, A., Takezawa, R. and Matsushima, T. Various sodium salts, potassium salts, a calcium salt and an ammonium salt induced ornithine decarboxylase and stimulated DNA synthesis in rat stomach mucosa. *Jpn. J. Cancer Res.*, **80**, 424–429 (1989).
20) Takahashi, M., Hasegawa, R., Furukawa, F., Toyoda, K., Sato, H. and Hayashi, Y. Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with N-methyl-N′-nitro-N-nitrosoguanidine. *Jpn. J. Cancer Res.*, **77**, 118–124 (1986).
21) Takahashi, M., Kokubo, T., Furukawa, F., Kurokawa, Y. and Hayashi, Y. Effects of sodium chloride, saccharin, phenobarbital and aspirin on gastric carcinogenesis in rats after initiation with N-methyl-N′-nitro-N-nitrosoguanidine. *Gann*, **75**, 494–501 (1984).
22) Takahashi, M., Okiyama, H., Furukawa, F., Toyoda, K., Sato, H., Imaida, K. and Hayashi, Y. Effects of glyoxal and methylglyoxal administration on gastric carcinogenesis in Wistar rats after initiation with N-methyl-N′-nitro-N-nitrosoguanidine. *Carcinogenesis*, **10**, 1925–1927 (1989).
23) Kobori, O., Shimizu, T., Maeda, M., Atomi, Y., Watanabe, J., Shoji, M. and Morioka, Y. Enhancing effect of bile and bile acid on stomach tumorigenesis induced by N-methyl-N′-nitro-N-nitrosoguanidine in Wistar rats. *J. Natl. Cancer Inst.*, **73**, 853–861 (1984).
24) Furihata, C., Yamakoshi, A. and Matsushima, T. Induction of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by formaldehyde. *Jpn. J. Cancer Res.*, **79**, 917–920 (1988).
25) Furihata, C., Yoshida, S., Sato, Y. and Matsushima, T. Inductions of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by glandular stomach carcinogens. *Jpn. J. Cancer Res.*, **78**, 1363–1369 (1987).
26) Mirsalis, J. C., Tyson, C. K. and Butterworth, B. E. Detection of genotoxic carcinogens in the *in vivo-in vitro* hepatocyte DNA repair assay. *Environ. Mutag.*, **4**, 553–562 (1982).
27) Butterworth, B. E., Bermudez, E., Smith-Oliver, T., Earle, L., Cattley, R., Martin, J., Popp, J. A., Strom, S., Jirtle, R. and Michalopoulos, G. Lack of genotoxicity of di(2-ethylhexyl)phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis*, **5**, 1329–1335 (1984).
28) Reddy, J. K., Azarroff, D. L. and Hignite, C. E. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature*, **283**, 397–398 (1980).
29) Bieri, F., Bentley, P., Wachter, F. and Staubli, W. Use of primary cultures of adult rat hepatocytes to investigate mechanisms of action of nafenopin, a hepatocarcinogenic peroxisome proliferator. *Carcinogenesis*, **5**, 1033–1039 (1984).
30) Yamada, K., Murakami, H. Shirahata, S., Shinohara, K. and Omura, H. Binding of epinephrin to chromatin components. *Agric. Biol. Chem.*, **48**, 2033–2038 (1984).