Jpn. J. Cancer Res. (Gann), 79, 917–920; August, 1988

# Inductions of Ornithine Decarboxylase and DNA Synthesis in Rat Stomach Mucosa by Formaldehyde

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**Administration of formaldehyde at doses of 11 to 110 mg/kg body weight by gastric intubation to male F344 rats induced up to 100-fold increase in ornithine decarboxylase activity with a maximum after 16 hr and up to 49-fold increase in DNA synthesis with a maximum after 16 hr in the pyloric mucosa of the stomach. These results suggest that formaldehyde has tumor-promoting activity in carcinogenesis in the glandular stomach.**

Key words: Formaldehyde — Ornithine decarboxylase — DNA synthesis — Rat glandular stomach

Takahashi *et al.* reported that formaldehyde (FA), which is a ubiquitous aldehyde in our environment, enhanced two-stage gastric carcinogenesis in rat stomach induced by N - methyl - N′ - nitro - N - nitrosoguanidine (MNNG).[1] We have developed a method for screening for possible stomach tumor promoters using induction of ornithine decarboxylase (ODC) activity and DNA synthesis as markers.[2-6] In this study we examined the induction of ODC activity and DNA synthesis in the glandular stomach mucosa of rats after gastric intubation of FA. The results showed the possible tumor-promoting activity of FA in the glandular stomach. Part of this work has been published previously.[6]

## MATERIALS AND METHODS

**Animals** Male Fischer rats (F344/Du Crj: Charles River Japan, Inc., Kanagawa), 7 weeks old, were kept in individual cages and given a restricted amount of diet overnight.[3] The following day they were given 1 ml of FA (reagent grade, Wako Pure Chemical Industries, Ltd., Osaka) in water (0.185–1.85%) by gastric intubation between 8 and 10 a.m. except that in a 16-hr experiment, they were given FA at 6 p.m. Control animals were given the same volume of water.

**ODC Activity** ODC activity in the crude extracts from the pyloric mucosa of the stomach was determined with L-[1-$^{14}$C]ornithine (54.3 mCi/mmol, New England Nuclear, Boston, MA) as a substrate, as described previously.[2,3] Results are expressed as means of duplicate assays in pooled materials from four rats.

**DNA Synthesis** DNA synthesis in the pyloric mucosa of the stomach was determined in *in vitro* organ culture in the presence of tritiated thymidine ([$^3$H]dThd, 80.9 Ci/mmol, New England Nuclear) after administration of FA *in vivo* as described previously.[3,7] Incorporation of [$^3$H]dThd into DNA was determined with a liquid scintillation counter. Results in each experiment are given as means of values in five individual rats.

## RESULTS

**Induction of ODC Activity** Figure 1 shows the induction of ODC activity in the pyloric mucosa of rat stomach after administration of FA at a dose of 110 mg/kg body weight. ODC activity in the pyloric mucosa of control rat stomach ($3.7 \pm 4.0$ pmol $CO_2$/30 min/mg protein) was at about the lowest limit measurable by the present assay method. ODC activity was increased 4 to 48 hr after FA administration, with a maximum after 16 hr. Figure 2 shows the dose-dependence of induction of ODC activity in the pyloric mucosa 16 hr after administration of FA at doses of 11 to 110 mg/kg body weight. A dose of 110 mg/kg body weight induced more than 100-fold increase in ODC activity, resulting in release of 350 pmol $CO_2$/30 min/mg protein. ODC activity in the forestomach epithelium of control rats ($1.6 \pm 1.6$ pmol $CO_2$/30 min/mg protein) was at about the lowest limit measurable by the present assay method. ODC activity was not induced in the forestomach epithelium for up to 48 hr (data were not shown). These

results show the tissue-specific induction of ODC activity in the pyloric mucosa by FA.

**Stimulation of DNA Synthesis** Figure 3 shows the increase of DNA synthesis in the pyloric mucosa of rat stomach after administration of FA at a dose of 110 mg/kg body weight. DNA synthesis is always observed in the cells in the proliferative zone of the pyloric mucosa of normal rat stomach, where the cells are renewed. DNA synthesis at time 0 in

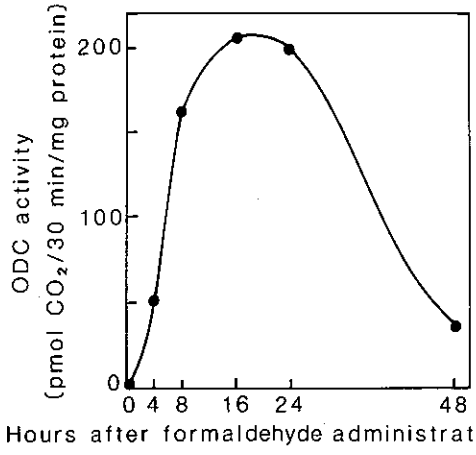


Fig. 1. Induction of ODC activity in the pyloric mucosa of rat stomach by FA at a dose of 110 mg/kg body weight. Results are means of duplicate assays on pooled materials from four rats.

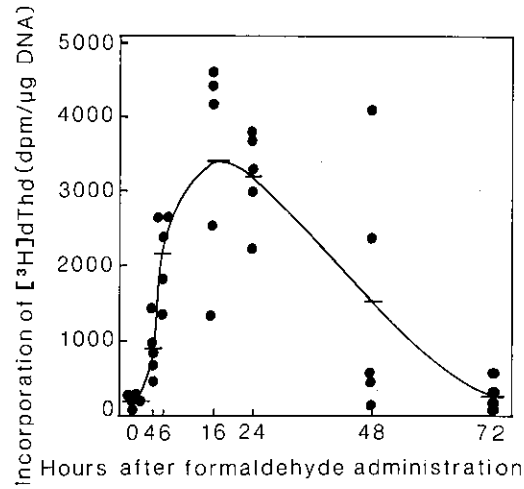


Fig. 3. Increase of DNA synthesis in the pyloric mucosa induced by FA at a dose of 110 mg/kg body weight. Results at each time are for five individual rats, and horizontal lines are means of the five values. Values are significantly different from that at time 0 by Student's *t*-test at 4 hr ($P<0.02$), 6 and 16 hr ($P<0.01$) and 24 hr ($P<0.001$).

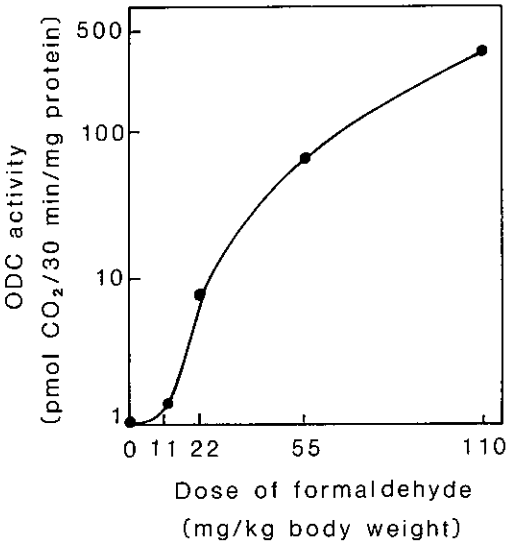


Fig. 2. Dose-dependent induction of ODC activity in the pyloric mucosa 16 hr after administration of FA.

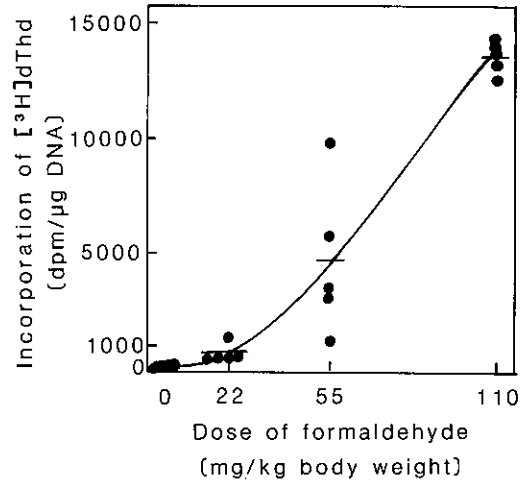


Fig. 4. Dose-dependent increase in DNA synthesis in the pyloric mucosa 16 hr after administration of FA. Values are significantly different from that without treatment by Student's *t*-test for 22 mg ($P<0.02$), 55 mg ($P<0.05$) and 110 mg ($P<0.001$) of FA.

Fig. 3 represents the level of DNA synthesis in control pyloric mucosa (168±71 dpm [$^3$H]-dThd/$\mu$g DNA). DNA synthesis was increased 4 to 48 hr after FA administration, with a maximum after 16 hr. Figure 4 shows the dose-dependence of stimulation of DNA synthesis in the pyloric mucosa 16 hr after administration of FA at doses of 22 to 110 mg/kg body weight. A dose of 110 mg/kg body weight induced a 49-fold increase in DNA synthesis with incorporation of 13600 dpm of [$^3$H]dThd into 1 $\mu$g of DNA.

## Discussion

Rats were given 0.185% FA in their drinking water in two-stage gastric carcinogenesis initiated by MNNG.[1] This daily dose of FA is calculated to be about 75 mg/kg body weight. Even lower doses of FA induced ODC activity and DNA synthesis in the pyloric mucosa in the present experiment. Reagent-grade FA contains 7–13% methyl alcohol as a stabilizer. Even the same amount of methyl alcohol as in the highest dose of FA used did not increase ODC activity or DNA synthesis in the pyloric mucosa of the stomach (data not shown).

We have found that sodium chloride, sodium taurocholate and potassium metabisulfite, which enhance or promote carcinogenesis in the glandular stomach of rats initiated by MNNG,[1,8,9] induced ODC activity and stimulated DNA synthesis in rat stomach mucosa.[2,5,6] We have also reported that glyoxal, which is present in various fermented foods and heated foods containing carbohydrate, induces ODC activity and DNA synthesis in rat stomach mucosa and we have suggested its possible tumor-promoting activity.[4] In fact Takahashi *et al.*[10] recently showed that glyoxal enhanced two-stage gastric carcinogenesis in the glandular stomach of rats initiated by MNNG. We have also reported that glandular stomach carcinogens such as MNNG, N-ethyl-N′-nitro-N-nitrosoguanidine, N-propyl-N′-nitro-N-nitrosoguanidine, 4-nitroquinoline 1-oxide (4NQO) and N-nitroso-N-methylurethane (NMUT) induced ODC activity and stimulated DNA synthesis in rat stomach mucosa.[11] MNNG, 4NQO and NMUT induced ODC activity and DNA synthesis in the pyloric mucosa at similar doses in long-term experiments on stomach carcinogenesis. Ethyl alcohol at toxic doses to the stomach and nongastric carcinogens such as 2-acetylaminofluorene, dimethylnitrosamine and Trp-P-2 did not induce ODC activity or stimulate DNA synthesis in the glandular stomach.[11] At present, all the glandular stomach carcinogens and tumor promoters examined have been found to induce ODC activity and stimulate DNA synthesis in the glandular stomach mucosa. These results suggest that the inductions of ODC activity and DNA synthesis are useful markers of possible tumor-promoting activity in the glandular stomach mucosa. Possible stomach-tumor promoters can be predicted by this *in vivo* short-term assay.

Of the five glandular stomach tumor promoters examined (FA, glyoxal, $K_2S_2O_5$, NaCl and taurocholate), FA induced ODC activity and stimulated DNA synthesis at the lowest doses. FA also promoted two-stage gastric carcinogenesis at the lowest dose (0.185% FA in drinking water).[1] The potencies of the glandular stomach tumor promoters in inducing ODC activity and in stimulating DNA synthesis in the pyloric mucosa of rat stomach were generally related to their tumor-promoting potencies in two-stage gastric carcinogenesis. As only one dose of tumor promoter was examined in each long-term two-step experimental stomach carcinogenesis, relative potencies of these five glandular tumor promoters in tumor-promoting activities can not be estimated precisely at present.

## Acknowledgments

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

*(Received May 13, 1988/Accepted June 29, 1988)*

## References

1) 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. (Gann)*, **77**, 118–124 (1986).

2) 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).
3) 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).
4) Furihata, C., Yoshida, S. and Matsushima, T. Potential initiating and promoting activities of diacetyl and glyoxal in rat stomach mucosa. *Jpn. J. Cancer Res. (Gann)*, **76**, 809–814 (1985).
5) Furihata, C., Takezawa, R., Matsushima, T. and Tatematsu, M. Potential tumor-promoting activity of bile acids in rat glandular stomach. *Jpn. J. Cancer Res. (Gann)*, **78**, 32–39 (1987).
6) Furihata, C., Sato, Y., Yamakoshi, A., Takimoto, M. and Matsushima, T. Inductions of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by 1-nitrosoindole-3-acetonitrile. *Jpn. J. Cancer Res. (Gann)*, **78**, 432–435 (1987).
7) Furihata, C., Yamawaki, Y., Jin, S-S., Moriya, H., Kodama, K., Matsushima, T., Ishikawa, T., Takayama, S. and Nakadate, M. Induction of unscheduled DNA synthesis in rat stomach mucosa by glandular stomach carcinogens. *J. Natl. Cancer Inst.*, **72**, 1327–1334 (1984).
8) 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).
9) 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).
10) Takahashi, M., Furukawa, F., Hasegawa, R., Sato, H., Jang, J. J. and Hayashi, Y. Promoting effect of glyoxal in two-stage gastric carcinogenesis in the rat. *Proc. Jpn. Cancer Assoc., 45th Annu. Meet.*, 43 (1986) (in Japanese).
11) 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. (Gann)*, **78**, 1363–1369 (1987).