[CANCER RESEARCH 50, 381-384, January 15, 1990]

Effect of Flurbiprofen and 16,16-Dimethyl Prostaglandin E_2 on Gastrointestinal Tumorigenesis Induced by *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine in Rats: Glandular Epithelium of Stomach and Duodenum[1]

Thomas Lehnert,[2] Eleanor E. Deschner, Rashida A. Karmali, and Jerome J. DeCosse

Department of Surgery [T. L.] and Gastrointestinal Service, Department of Medicine [E. E. D.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021; Department of Nutrition, Rutgers University, New Brunswick, New Jersey 08903 [R. A. K.]; and Department of Surgery, Cornell University Medical College, New York, New York 10021 [J. J. D.]

ABSTRACT

The effect of an exogenous synthetic prostaglandin analogue, 16,16-dimethyl prostaglandin E_2 (16,16-dm-PGE_2), as well as the effect of endogenous prostaglandin synthesis inhibition by a cyclooxygenase inhibitor, flurbiprofen, on chemically induced gastric carcinogenesis has been investigated in rats. Carcinogenesis was induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; CAS:70-25-7). Animals were divided into six groups: Group I, treatment with MNNG alone; Group II, treatment with 16,16-dm-PGE_2 plus MNNG; Group III, treatment with flurbiprofen plus MNNG; Group IV, treatment with 16,16-dm-PGE_2 alone; Group V, treatment with flurbiprofen alone; and Group VI, controls.

Treatment with high doses of MNNG resulted in rapid development of malignant tumors originating from the glandular epithelium of the stomach and duodenum in animals of all groups receiving the carcinogen. The first gastric adenocarcinoma infiltrating the muscularis proper was detected after 139 days in an animal treated with a combination of MNNG and flurbiprofen. The incidence of infiltrating adenocarcinoma and the incidence of all neoplastic lesions of the glandular stomach were both significantly higher in animals treated with a combination of MNNG and flurbiprofen compared with treatment by MNNG alone or in combination with 16,16-dm-PGE_2 ($P < 0.05$ and $P < 0.001$). The difference in tumor incidence between the last two groups was not significant.

The first duodenal adenocarcinoma was detected on Day 114 in another animal of the group treated with MNNG plus flurbiprofen. When compared with the group treated with MNNG plus 16,16-dm-PGE_2, significantly more animals developed duodenal adenocarcinoma when treated with MNNG plus flurbiprofen ($P < 0.005$) or with MNNG alone ($P < 0.05$).

Results of this study indicate that inhibition of endogenous prostaglandin synthesis favors development of adenocarcinoma in the glandular stomach of rats. Vice versa, the addition of an exogenous prostaglandin analogue inhibits the development of duodenal adenocarcinoma. This protective effect of prostaglandins may be due to an increase of the thickness of the mucus gel covering the glandular epithelium, thereby preventing access of carcinogen to the mucosa.

INTRODUCTION

With an overall 5-yr survival of 20% at best, individuals with a malignancy originating from the glandular epithelium of the stomach have a poor prognosis. The only treatment offering a chance for cure is surgery, while chemotherapy is largely ineffective (1). Still, only 75% of patients presenting with gastric cancer are explored, and only 33% of all patients have procedures deemed curative. The 5-yr survival rate for these patients is 26% (2).

With such dismal therapeutic options at hand, much would be gained if we had a better understanding of the sequence of events leading to gastric cancer. Epidemiological studies have indicated that morphological changes, such as atrophic gastritis, are accompanied by an increased risk of developing gastric cancer (3–5). Other studies have demonstrated a reduction of prostaglandin E_2 tissue levels in gastric mucosa of patients with atrophic gastritis and gastric cancer (6). Synthesis of mucosal prostaglandins can be effectively reduced by administration of nonsteroidal antiinflammatory drugs such as aspirin, indomethacin, or flurbiprofen (7, 8). These compounds inhibit cyclooxygenase, the enzyme that controls conversion of arachidonic acid to endoperoxides and subsequently to various prostaglandins.

To evaluate the influence of gastric mucosal prostaglandin synthesis on gastric carcinogenesis, we studied the effect of flurbiprofen and 16,16-dm-PGE_2[3] on chemically induced gastric cancer in rats treated with MNNG.

MATERIALS AND METHODS

Briefly, 171 female Wistar rats (100 g) were divided into six groups and treated with MNNG alone ($n = 43$) (Group I), MNNG plus flurbiprofen ($n = 44$) (Group II), MNNG plus 16,16-dm-PGE_2 ($n = 43$) (Group III), flurbiprofen alone ($n = 15$) (Group IV), or 16,16-dm-PGE_2 alone ($n = 11$) (Group V). Group VI received no specific treatment and served as control. MNNG (10 mg), flurbiprofen (0.5 mg), and 16,16-dm-PGE_2 (0.2 μg) were given by orogastric gavage for repeated 3-day treatment cycles followed by rest periods. Six cycles to a total dose of 180 mg of MNNG were given during 40 days. A detailed description of the methodology has already been published (9).

The χ^2 test with Yates' correction where appropriate and Student's *t* test were applied for statistical analysis. Analysis of tumor incidence included only those animals that died after the first neoplastic lesion had been detected to allow for equal chances of tumor development. Glandular proliferative lesions restricted to the mucosa with atypia and proliferative lesions of the glandular mucosa with infiltration into the muscularis mucosae or submucosa were designated neoplastic lesions regardless of the degree of atypia. Lesions infiltrating the muscularis propria were designated adenocarcinoma.

RESULTS

Animals treated with either flurbiprofen alone or 16,16-dm-PGE_2 alone developed normally, and their weight gain was comparable to that of untreated controls. Final weight was 302 ± 7 g (mean ± SEM) for controls as compared with 311 ± 8 g for animals treated with flurbiprofen and 311 ± 7 g for animals treated with 16,16-dm-PGE_2 alone. Animals treated with MNNG alone or in combination with either flurbiprofen or 16,16-dm-PGE_2 gained less weight during the course of the study. Their final weights were 272 ± 12 g (MNNG alone), 263 ± 20 g (MNNG plus 16,16-dm-PGE_2), and 276 ± 6 g (MNNG plus flurbiprofen). Addition of either flurbiprofen or 16,16-dm-PGE_2 to treatment with MNNG did not result in a statistically

Received 8/30/88; revised 8/21/89; accepted 9/26/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This is the second paper in a series.

[2] Present address: Department of Surgery, University of Heidelberg, Im Neuenheimer Feld 110, D-6900 Heidelberg, Federal Republic of Germany. To whom requests for reprints should be addressed.

[3] The abbreviations used are: 16,16-dm-PGE_2, 16,16-dimethyl prostaglandin E_2; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

significant difference in final weight as compared with treatment with the carcinogen alone. During the treatment period (*i.e.*, up to Day 41) and during follow-up, the main cause of death was pneumonia. Later in the study moribund animals with severe cachexia were killed. All remaining animals were killed on Day 161.

Macroscopic polypoid and ulcerating tumors of the glandular stomach were noted in animals treated with MNNG alone or in combination (Fig. 1). Tumors were located in the antrum and in the fundus. Tumors of the duodenum were often shallow and ulcerating, but large tumors caused obstruction. Most duodenal tumors were located between the pylorus and the opening of the common bile duct.

Histological evaluation of gastric glandular mucosa revealed signs of gastritis in effective animals treated with MNNG alone (6 of 12) or in combination with flurbiprofen (7 of 18) or 16,16-dm-PGE_2 (3 of 5). These differences are not significant. Intestinal metaplasia or gastric ulceration was not found in these animals. No signs of gastritis or gastric ulceration were observed in control animals or animals treated with either flurbiprofen or 16,16-dm-PGE_2 alone.

Glandular atypia of gastric mucosa was observed in both antrum and fundus (Fig. 2). Changes were most frequent and severe in fundic mucosa bordering squamous epithelium at the forestomach ridge and in areas with ingrowth of squamous epithelium into the glandular stomach. Glandular atypia was observed in all treatment groups exposed to the carcinogen.

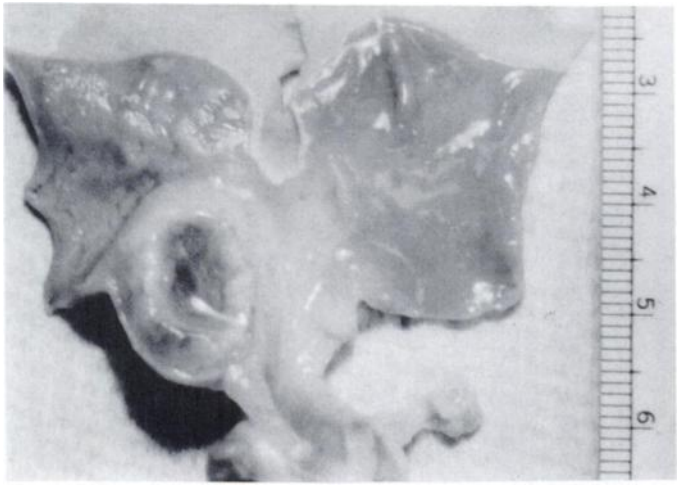

Fig. 1. Ulcerating tumor in the antrum of an animal treated with MNNG plus flurbiprofen.

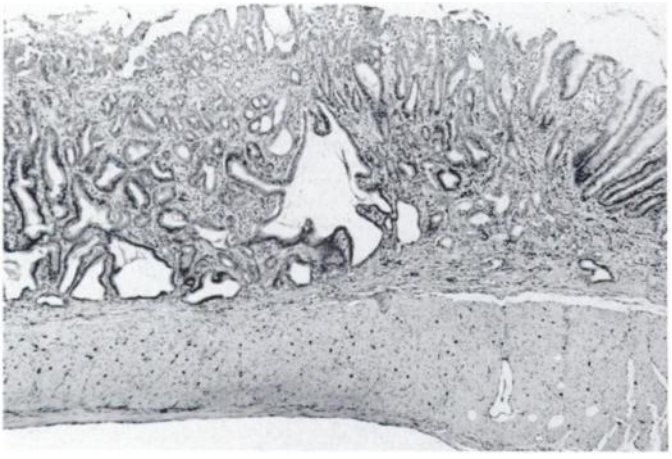

Fig. 2. Glandular atypia of the antrum following treatment with MNNG. H & E, × 100.

Atypia was first noted on Days 47 (MNNG alone), 51 (MNNG plus 16,16-dm-PGE_2), and 57 (MNNG plus flurbiprofen). Glandular atypia was not detected in animals treated with flurbiprofen or 16,16-dm-PGE_2 alone or in untreated controls.

Gastric adenocarcinoma developed without demonstrable site preference from both fundic (Fig. 3) and antral (Fig. 4) mucosa in all treatment groups receiving the carcinogen. The first neoplastic lesion with marked glandular proliferation and invasion of the muscularis mucosae was detected on Day 60 for MNNG plus flurbiprofen, Day 87 for MNNG alone, and Day 129 for MNNG plus 16,16-dm-PGE_2. The first gastric adenocarcinoma with infiltration of the muscularis propria was observed on Day 139 in an animal treated with MNNG plus flurbiprofen, Day 148 for treatment with MNNG alone, and Day 157 for MNNG plus 16,16-dm-PGE_2. The incidence of both neoplastic lesions and advanced gastric adenocarcinoma was highest in animals exposed to treatment with MNNG plus flurbiprofen (Table 1), whereas significantly fewer tumors developed in animals treated with MNNG either alone or in combination with 16,16-dm-PGE_2 ($\chi^2 = 18.62$, 2 *df*, $P < 0.001$ and $\chi^2 = 6.90$, 2 *df*, $P < 0.05$). No malignant tumors arising from glandular epithelium of the stomach were observed in animals treated with either flurbiprofen or 16,16-dm-PGE_2 alone or in untreated controls.

Malignant tumors originating from glandular epithelium were also observed in the duodenum and proximal small bowel (Fig. 5). The first such adenocarcinoma in the study was detected on Day 114 in an animal treated with MNNG plus flurbiprofen. Adenocarcinoma of the duodenum and small bowel also developed in animals treated with MNNG alone or in combination with 16,16-dm-PGE_2. In these groups the first adenocarcinoma was detected on Days 148 and 157, respectively. The tumor incidence was comparable in animals treated with MNNG either alone or in combination with flurbiprofen (75 and 83%, respectively), but was significantly lower in animals treated with MNNG plus 16,16-dm-PGE_2 (Table 2). In this treatment group the incidence of adenocarcinoma of the duodenum and small bowel was 14% ($\chi^2 = 11.95$, 2 *df*, $P < 0.005$).

Tumors of the duodenum and small bowel were often multiple in animals treated with MNNG either alone or combined with flurbiprofen. Both the number of tumors per tumor-bearing animal and the average tumor diameter were greater in animals treated with a combination of carcinogen plus flurbiprofen as compared with animals exposed to MNNG alone. These differences, however, were not statistically significant.

DISCUSSION

Treatment of female Wistar rats with high doses of MNNG resulted in the development of malignant tumors originating from glandular epithelium of the stomach and duodenum. Similarly rapid tumor development with gastric adenocarcinoma demonstrable after 120 days has been reported after treatment with high doses of MNNG (10). Concurrent treatment with flurbiprofen during the period of tumor initiation increased the incidence of gastric adenocarcinoma in animals exposed to MNNG, whereas the addition of 16,16-dm-PGE_2 did not alter the incidence of gastric adenocarcinoma significantly. Conversely, the addition of 16,16-dm-PGE_2 reduced the incidence of duodenal adenocarcinoma, but addition of flurbiprofen did not have any significant effect on the incidence of duodenal adenocarcinoma. Malignant epithelial tumors of the gastrointestinal tract were not found in animals treated with

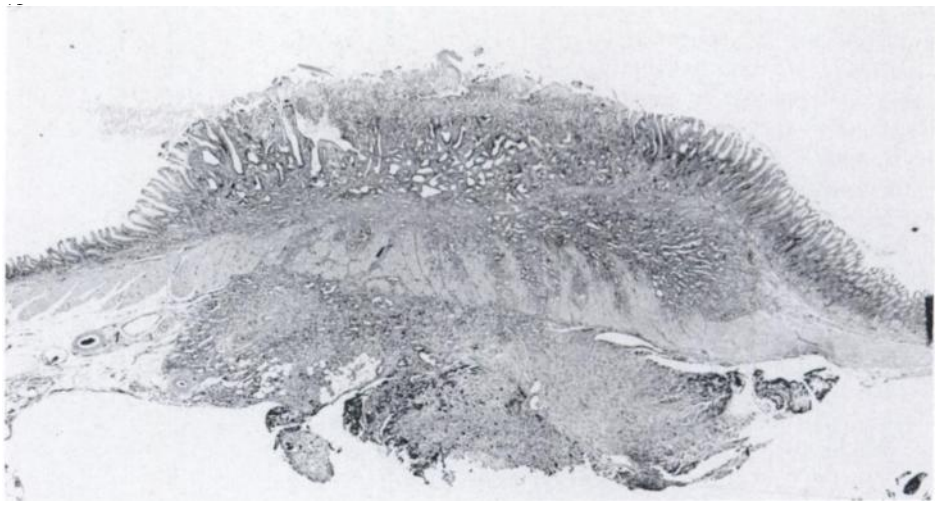

Fig. 3. Well-differentiated adenocarcinoma of the antrum with poorly differentiated portions infiltrating the muscularis. H & E, × 4.

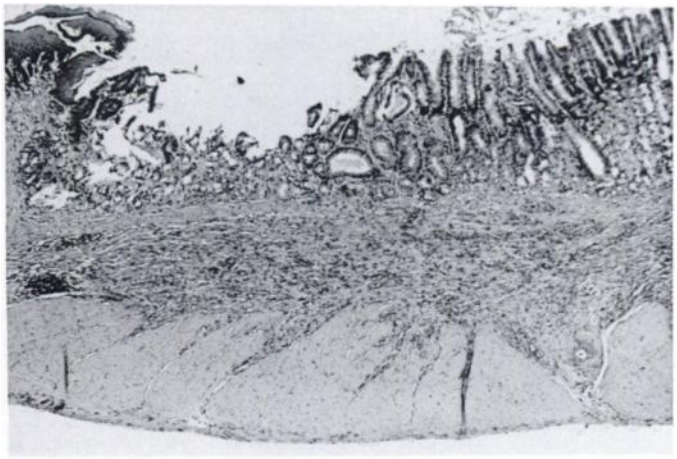

Fig. 4. Poorly differentiated adenocarcinoma originating from fundic mucosa near the forestomach border. H & E, × 100.

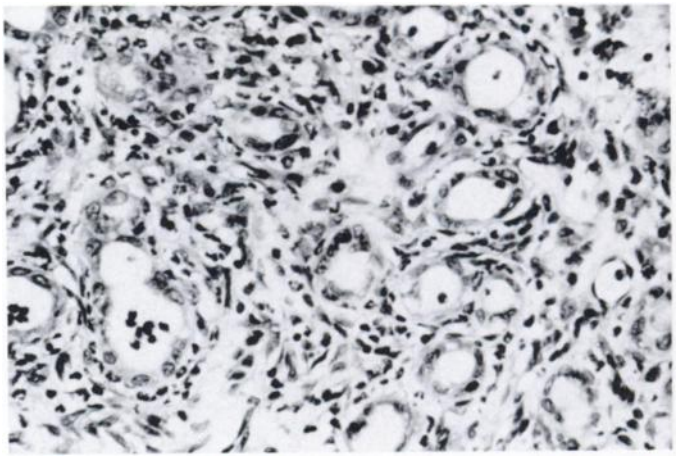

Fig. 5. Moderately differentiated duodenal adenocarcinoma. H & E, × 400.

Table 1 *Gastric adenocarcinoma following treatment with MNNG, flurbiprofen, and 16,16-dimethyl prostaglandin E_2*

Treatment group	n^a	Survival (days)	Animals with adenocarcinoma[b]	Tumor incidence (%)
MNNG alone	12	155.3 ± 1.3[c]	2	17
MNNG plus flurbiprofen	18	154.9 ± 2.0	11	61
MNNG plus 16,16-dm-PGE_2	5	157.0 ± 0	1	20

[a] Number of animals alive on the day the first adenocarcinoma was detected.
[b] $\chi^2 = 6.90$, 2 *df*, $P < 0.05$.
[c] Mean ± SEM.

Table 2 *Duodenal and small bowel adenocarcinoma following treatment with MNNG, flurbiprofen, and 16,16-dimethyl prostaglandin E_2*

Treatment group	n^a	Survival (days)	Animals with adenocarcinoma[b]	Tumor incidence (%)
MNNG alone	12	155.3 ± 1.3[c]	9	75
MNNG plus flurbiprofen	23	149.6 ± 2.8	19	83
MNNG plus 16,16-dm-PGE_2	7	148.4 ± 5.6	1	14

[a] Number of animals alive on the day the first adenocarcinoma was detected.
[b] $\chi^2 = 11.95$, 2 *df*, $P < 0.005$.
[c] Mean ± SEM.

either flurbiprofen or 16,16-dm-PGE_2 alone or in untreated control animals.

The incidence of gastric adenocarcinoma was also increased, when rats were treated with a combination of MNNG and aspirin, another potent inhibitor of prostaglandin synthesis (11), although this was not confirmed by another study (12). Such differences may be due to different treatment schedules.

Manipulation of prostaglandin metabolism, either by addition of exogenous synthetic prostaglandin or by inhibition of endogenous prostaglandin formation, could have influenced the development of gastric and duodenal adenocarcinoma by two separate mechanisms, which have also been considered to explain cytoprotection exerted by prostaglandins. This protection of gastric mucosa by prostaglandins against ulcerogenic agents may in part be due to reduction of gastric acid secretion at high doses of prostaglandins (13). Such reduction of gastric acid would have delayed decomposition of the carcinogen, and a higher rather than lower tumor incidence would have been expected in animals receiving additional prostaglandin. It would seem, therefore, that changes in tumor incidence were not mediated by changes in gastric acid secretion. Mucosal cytoprotection may also be related to the increase of gastric mucus gel thickness caused by topical prostaglandins (14), although other evidence indicates that prostaglandins are cytoprotective even at low doses not affecting acid secretion and irrespective of mucus gel thickness (15).

Still, gastric mucus may influence the development of chemically induced gastric cancer. Concurrent treatment of rats with MNNG and iodoacetamide, a substance that cleaves disulfide bridges between mucus glycoprotein subunits, changed the site of tumor development to the gastric fundus (16), whereas addition of mucin to the diet of rats treated with MNNG reduced the incidence of gastric carcinoma (17). It has also been

suggested that the particular resistance to MNNG-induced gastric cancer by some rat strains (18) may be related to increased gastric mucus (19). It would seem possible, therefore, that p.o. administered prostaglandins caused an increase of mucus gel thickness or intraluminal mucus content (20), thereby increasing the diffusion pathway or unspecific adsorption of the carcinogen. This would eventually reduce access of MNNG to gastric and duodenal epithelial cells.

Inhibition of prostaglandin synthesis by flurbiprofen and subsequent reduction of mucus thickness could in turn have facilitated access of the carcinogen to gastric epithelium. Indeed, both production and secretion of gastric mucus are reduced after treatment of gastric mucosa with salicylates (21, 22).

Although changes of prostaglandin metabolism may modify the incidence of malignant tumors by altering access of carcinogen to the mucosa, a direct effect of prostaglandins could render both gastric and duodenal epithelium susceptible to the carcinogen. There is evidence that long-term treatment with a prostaglandin E_1 analogue reduces proliferative activity of antral and fundic mucosa (23), although other prostaglandins did not significantly alter gastric mucosal proliferative activity (24–26). Consequently, treatment with inhibitors of prostaglandin synthesis, such as aspirin or indomethacin, has been shown to increase gastric and duodenal epithelial proliferation (27, 28). Chronic aspirin treatment has been shown to result in gastric mucosal dysplasia (29). These findings are also paralleled by observations on atrophic gastritis which is associated with a long-term risk of gastric cancer approximating 10% (3–5). Cell kinetic studies of atrophic gastritis have demonstrated both increased proliferative activity as compared with normal gastric mucosa (30, 31) as well as reduced mucosal prostaglandin synthesis (6). It may be, therefore, that inhibition of prostaglandin synthesis by flurbiprofen resulted in increased proliferative activity of gastric epithelium, thereby increasing the risk of malignant change.

REFERENCES

1. LeChevalier, T., Smith, F. P., Harter, W. K., and Schein, P. S. Chemotherapy and combined modality therapy for locally advanced and metastatic gastric carcinoma. Semin. Oncol., *12:* 46–63, 1985.
2. Douglass, H. O., and Nava, H. R. Gastric adenocarcinoma—management of the primary disease. Semin. Oncol., *12:* 32–45, 1985.
3. Cheli, R., Sano, L., Giancamerla, G., and Canciani, G. A clinical and statistical follow-up study of atrophic gastritis. Am. J. Dig. Dis., *18:* 1061–1066, 1973.
4. Siurala, M., Varis, K., and Wiljasalo, M. Atrophic gastritis: a ten to fifteen year follow-up. Scand. J. Gastroenterol., *1:* 40–48, 1966.
5. Walker, J. R., Strickland, R. G., Ungar, B., and McKay, R. Simple atrophic gastritis and gastric carcinoma. Gut, *12:* 906–913, 1972.
6. Wright, J. P., Young, G. O., Klaff, L. F., Weers, L. A., Price, S. K., and Marks, I. N. Gastric mucosal prostaglandin E levels in patients with gastric ulcer disease and carcinoma. Gastroenterology, *82:* 263–267, 1982.
7. Fitzpatrick, F. A., and Wyndala, M. A. *In vivo* suppression of prostaglandin biosynthesis by non-steroidal antiinflammatory agents. Prostaglandins, *12:* 1037–1051, 1976.
8. Higgs, G. A., Harvey, E. A., Ferreira, S. H., and Vane, J. R. The effects of antiinflammatory drugs on the production of prostaglandins *in vivo*. Adv. Prostaglandin Thromboxane Res., *1:* 105–110, 1976.
9. Lehnert, T., Deschner, E. E., Karmali, R. A., and DeCosse, J. J. Effect of flurbiprofen and 16,16-dimethyl-prostaglandin E_2 on gastrointestinal tumorigenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats. I. Squamous epithelium and mesenchymal tissues. J. Natl. Cancer Inst., *78:* 923–929, 1987.
10. Tabuchi, Y., Orino, T., Mitsuno, T., and Sugiyama, T. Possible role of mucosal damage in stomach carcinogenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in the rat. J. Natl. Cancer Inst., *52:* 1589–1594, 1974.
11. Chang, T. H., Lee, Y. C., Lee, K. Y., Sun, C. H., and Chang, Y. P. Cocarcinogenic action of aspirin on gastric tumors induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats. J. Natl. Cancer Inst., *70:* 1067–1075, 1983.
12. Takahashi, M., Kokubo, T., Furukawa, F., and Kurokawa, 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.
13. Robert, A. Antisecretory, antiulcer, cytoprotective, and diarrheogenic properties of prostaglandins. Adv. Thromboxane Prostaglandin Res., *2:* 507–520, 1976.
14. Kerss, S., Allen, A., and Garner, A. A simple method for measuring thickness of the mucus gel layer adherent to rat, frog, and human gastric mucosa: influence of feeding, prostaglandin, *N*-acetylcysteine, and other agents. Clin. Sci., *63:* 187–195, 1982.
15. Robert, A., Böttcher, W., Golonska, E., and Kauffmann, G. L. Lack of correlation between mucus gel thickness and gastric cytoprotection in rats. Gastroenterology, *86:* 670–674, 1984.
16. Takahashi, M., Shirai, T., Fukushima, S., Hananouchi, M., Hirose, M., and Ito, N. Effect of fundic ulcers induced by iodoacetamide on development of gastric tumors in rats treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Gann, *67:* 47–54, 1976.
17. Tatematsu, M., Takahashi, M., Hananouchi, M., Shirai, T., Hirose, M., Fukushima, S., and Ito, N. Protective effect of mucin on experimental gastric cancer induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine plus sodium chloride in rats. Gann, *67:* 223–229, 1976.
18. Bralow, S. P., Gruenstein, M., and Meranze, D. R. Host resistance to gastric adenocarcinomatosis in three strains of rats ingesting *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Oncology, *27:* 168–180, 1973.
19. Sugimura, T., and Kawachi, T. Experimental stomach carcinogenesis. *In:* M. Lipkin and R. A. Good (eds.), Gastrointestinal Tract Cancer, pp. 327–341. New York: Plenum Medical Book Co., 1978.
20. Bolton, J. P., Palmer, D., and Cohen, M. M. Stimulation of mucus and nonparietal cell secretion by the E_2 prostaglandins. Dig. Dis., *23:* 359–364, 1978.
21. Kent, P. W., and Allen, A. The biosynthesis of intestinal mucins: effect of salicylate on glycoprotein biosynthesis by sheep colonic and human gastric mucosal tissues *in vitro*. Biochem. J., *106:* 645–658, 1967.
22. Menguy, R., and Masters, B. S. The effects of aspirin on gastric mucus secretion. Surg. Gyn. Obstet., *120:* 92–98, 1965.
23. Fich, A., Arber, N., Sestieri, M., Zajicek, G., and Rachmilewitz, D. Effect of misoprostol and cimetidine on gastric cell labeling index. Gastroenterology, *89:* 57–61, 1985.
24. Johnson, L. R., and Guthrie, P. Effect of cholecystokinin and 16,16-dimethyl-prostaglandin E_2 on RNA and DNA of gastric and duodenal mucosa. Gastroenterology, *70:* 59–65, 1976.
25. Kawai, K., Murakami, M., Sasaki, S., and Misaki, F. Cell kinetics of superficial epithelial cells of mouse gastric mucosa. Gastroenterology, *84:* 1204, 1983.
26. Tytgat, G. N. J., Offerhaus, G. J. A., VanMinnen, A. J., Everts, V., Hensen-Logmans, S. C., and Samson, G. Influence of oral 15(*R*)-15-methyl-prostaglandin E_2 on human gastric mucosa. A light microscopic, cell kinetic, and ultrastructural study. Gastroenterology, *90:* 1111–1120, 1986.
27. Eastwood, G. L., and Quimby, G. F. Effect of chronic aspirin ingestion on epithelial proliferation in rat fundus, antrum, and duodenum. Gastroenterology, *82:* 852–856, 1982.
28. Kuwayama, H., and Eastwood, G. L. Effects of water immersion, restraint, stress, and chronic indomethacin ingestion on antral and fundic epithelial proliferation. Gastroenterology, *88:* 362–365, 1985.
29. St. John, D. J. B., Yeomans, N. D., Bourne, C. A. J., and deBoer, G. R. M. Aspirin-induced glandular dysplasia of the stomach. Arch. Pathol. Lab. Med., *101:* 44–48, 1977.
30. Hart, H. O., Johansen, A. A., Larsen, J. K., and Svendson, L. B. Cell proliferation in normal and diseased gastric mucosa. Acta Pathol. Microbiol. Scand. Sect. A, *87:* 217–222, 1979.
31. Lehnert, T., and Deschner, E. E. Cell kinetics of gastric cancer and precancer. *In:* M. Filipe and J. R. Jass (eds.), Gastric Carcinoma, pp. 45–67. Edinburgh: Churchill-Livingstone, 1986.