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Alterations in Gastric Mucus Secretion in Rhesus Monkeys After Exposure to Ionizing Radiation

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The aim of the present study was to evaluate the effect of γ-irradiation on soluble gastric mucus. Six conscious chair-adapted rhesus monkeys were studied once before and twice after exposure to ionizing irradiation (800 rads). Using a marker (^{99m}Tc-DTPA) dilution technique, acidic glycoprotein (AG), neutral glycoprotein (NG), ion, and fluid output were determined during a basal period and after the administration of an 80-ml water load. Irradiation significantly increased the outputs of both AG and NG during the basal period. After the water load, NG output remained elevated but irradiation abolished postload AG output thus inhibiting the normal rise in AG output stimulated by the load. Two days after irradiation NG output had returned to control levels whereas AG output was still suppressed. Sodium and potassium ion outputs were unaltered by irradiation. Chloride and fluid outputs were significantly inhibited on the day of irradiation but had returned to control levels within 3 days. These results indicate that irradiation produces significant changes in both the quantity and nature of the soluble mucus glycoproteins secreted into the gastric juice. It is suggested that these changes may compromise the protective ability of gastric mucus.

Exposure to high doses of radiation (>600 rads) produces diarrhea, infection, and fluid loss within 1 wk as a result of damage to epithelial cells. Moreover, a recent study has demonstrated that gastric mucosal biopsy specimens obtained on the day of radiation exposure (800 rads) produce superficial ulceration which fail to heal within 7–9 days (1). This observation could be related to many factors including the secretion of mucus, the presence of a mucosal gel layer, the intragastric hydrogen ion concentration, as well as the integrity and rate of renewal of epithelial cells. In the present study, the effect of irradiation on changes in gastric soluble mucus and ion secretion were evaluated in primates. Gastric secretion of glycoproteins was used as an index of both soluble and insoluble mucus production (2).

Methods

Six male unanesthetized monkeys (*Macaca mulatta*) weighing 3–4 kg were adapted to primate restraining chairs and housed in closed, ventilated, lighted booths between 9 AM and 12 PM. The monkeys were trained to accept a 12F double-lumen nasogastric ventrol Levin tube (National Catheter, Mallencrodt, Argyle, N.Y.; bore, 4 mm; wall thickness, 1 mm). The experiments were conducted after an overnight fast and were started 45 min after the tube had been placed. Proper positioning of the tube in the most dependent part of the stomach was verified by demonstrating that, after injecting 15 ml of water into a

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Animal Resources, National Research Council, DHEW Publication No. (NIH) 78-23.

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Abbreviations used in this paper: AB, Alcian blue; AG, acidic glycoprotein; NG, neutral glycoprotein.

previously emptied stomach, the total volume could be recovered.

A marker (^{99m}Tc-DTPA) dilution technique previously described and validated in monkeys and humans (3–6) was used to determine gastric ion and mucus output. The animals were studied during a 40-min basal period and for 60 min after the intragastric administration of an 80-ml water load (pH 7.4, 37°C). Samples of gastric juice were centrifuged and samples of the clear supernatant were counted in a auto-γ-counter (Ultragamma, LKB Instruments Inc., Gaithersburg, Md.). Intragastric volumes of fluid (V_1, V_2, . . .) and amounts of ^{99m}Tc-DTPA were determined using the marker dilution principle (4,7,8). Net rate of fluid output was determined for each 10-min interval (t) between two dilutions assuming that it remained constant over the given interval and using the equation

$$R_v = [V_2 - V_1 \times \exp(-gt)] \times g/[1 - \exp(-gt)],$$

where g is the fractional emptying rate.

The concentration of soluble mucus in each sample was estimated using two methods; the Alcian blue (AB) dye binding and the periodic acid–Schiff reaction. Alcian blue is a cationic dye that forms an irreversible complex with acidic glycoproteins and other negatively charged macromolecules. A modification of the method of Piper et al. (9) was used for the determination of AB binding to acidic glycoproteins. Briefly 0.1 ml of gastric juice was mixed with 4.2 ml of McIlvaine's citrate phosphate buffer (pH 5.8, 0.12 M $NaHPO_4$ and 0.4 M citric acid) and 0.2 ml of AB (10 mg/ml). The volume was then increased to 5 ml by adding distilled water. The concentration of AB in the reaction mixture was 0.4 mg/ml and the pH was 5.8. The reaction mixture was incubated at 22°C for 24 h and centrifuged at 2500 rpm for 10 min. The concentrations of AB in the supernatant fraction were estimated spectrophotometrically at 615 mm (Gilford Microsample, Oberlin, Ohio) and compared with a standard curve constructed using porcine gastric mucin (Sigma Chemical Co., St. Louis, Mo.).

The periodic acid–Schiff method described by Mantle and Allen (10) was used to estimate neutral glycoproteins. The reaction mixture consisted of 0.2 ml of gastric juice and 1.8 ml of isotonic saline to make a volume of 2 ml. Then, 0.2 ml of fresh periodic acid solution (10 μl of 50% periodic acid in 10 ml of 7% acetic acid) was added to the reaction mixture and was incubated at 37°C for 2 h. Subsequently, 0.2 ml of active Schiff solution was added and all tubes were vortexed immediately. The specimens stood for 30 min at room temperature after which the optical density was determined spectrophotometrically at 555 nm using porcine gastric mucin as a standard.

The intragastric concentration of soluble mucus at the start of the interval (M_1) and at the end of the interval (M_2) are expressed as milligrams of mucin per milliliter. The net rate of soluble mucus output (R_M) expressed in milligrams of mucin equivalents was then calculated using the equation

$$R_M = [M_2 - M_1 \times \exp(-gt)] \times g/[1 - \exp(-gt)].$$

The concentration and output of neutral glycoprotein (NG) and acidic glycoprotein (AG) were determined separately. Sodium (Na^+) and potassium (K^+) ion concentrations were measured using a flame photometer (Instrumentation Laboratory, Inc., Model 443, Lexington, Mass.) and chloride (Cl^-) ion concentration was determined using an amperometric titration method (Corning 920 M, Medfield, Mass.). The intragastric mass of each ion (I_1, I_2, . . .) was determined by multiplying the intragastric ion concentration by the corresponding intragastric volume. The net rate of each ion output (R_I) was then calculated using the equation

$$R_I = [I_2 - I_1 \times \exp(-gt)] \times g/[1 - \exp(-gt)].$$

The calculations were performed using a locally developed program and PDP-10 computer (Division of Computer Research Technology, National Institutes of Health, Bethesda, Md.). The assumptions involved have been described and discussed elsewhere (3–6) and are based on the original contribution of Hildes and Dunlop (8).

In this study each monkey was studied on three separate days: once before (preirradiation), on the day of (irradiation), and 2 days after (2 days postirradiation) irradiation; 800 rads were delivered bilaterally to the whole body using a large 10^5 Ci ^{60}Co irradiation at 500 rads/min. Values for gastric secretory parameters obtained during the first two 10-min intervals of each study were discarded in order to allow for the establishment of a steady state. Those obtained during the third and fourth 10-min fasting intervals were averaged for each study to obtain one fasting (basal) value per animal, and the mean (±SE) was calculated for each day of the study. Values obtained during seven 5- or 10-min intervals after the 80-ml water load (postload) were also averaged for each study to determine one postload value per animal. The mean (±SE) postload value was then calculated for each type of study. The statistical significance of differences observed for each measurement of gastric function (e.g., Na^+ output, mucus output) was evaluated using a three-factor (treatment, time, and monkey) analysis of variance with repeated measures on the last two factors (11), the program LDU-040 (K.L. Dorn), and an IBM 370 computer (Division of Computer Research and Technology, National Institutes of Health).

Results

Before irradiation, the basal output and concentration of AGs is approximately twice that of NGs (Figures 1 and 2). In addition, the output and concentration of AG are significantly ($p < 0.05$) increased by 270% and 88%, respectively, in response to the water load (Figures 3 and 4 vs. Figures 1 and 2). In contrast, NG output and concentration are relatively unchanged by the administration of the water load. As a result of this difference, postload AG output is ~7 times postload NG output and postload AG concentration is 5–6 times that of postload NG concentration.

Exposure to irradiation significantly stimulated both the basal (Figure 1) and postload (Figure 2) output of NG by 150%. In addition, NG concentra-

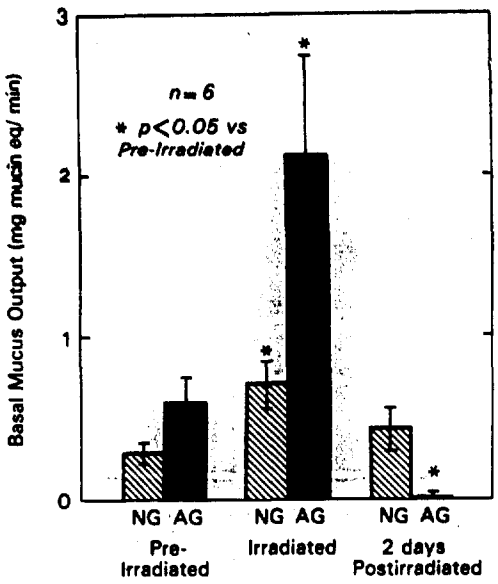

Figure 1. Basal soluble mucin output before, on the day of, and 2 days after exposure to irradiation. Each bar represents the mean ± SE of values obtained in 6 monkeys.

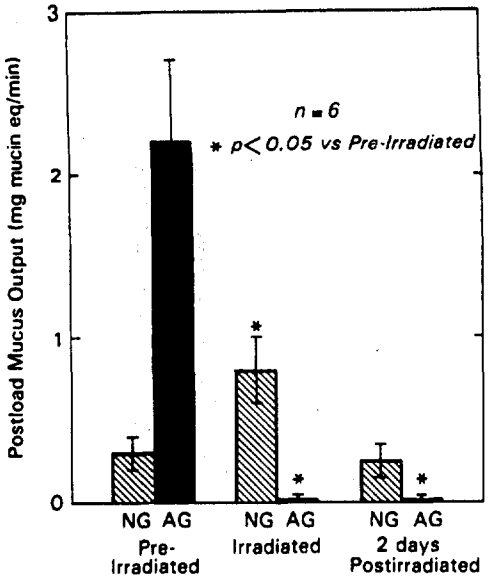

Figure 3. Postload soluble mucin output before, on the day of, and 2 days after exposure to irradiation. Each bar represents the mean ± SE of values obtained in 6 monkeys.

tion in the gastric juice was increased by 61% basally (Figure 3) and was significantly enhanced by 270% after the water load (Figure 4). However, both basal and postload NG output and concentration had returned to preirradiation levels within 2 days after irradiation. Like NG output, the basal secretion of AG was increased significantly by 250% after exposure to radiation (Figure 1). In addition, basal AG concentration was elevated by 137% (Figure 2). However, in contrast to NG, postload AG output (Figure 3) and concentration in the gastric juice (Figure 4) were significantly suppressed by irradiation and remained suppressed after 2 days. Thus, the increase in AG output and concentration stimulated by the water load in the control state was abolished after irradiation.

Na^+ and K^+ output and concentration were unaltered by irradiation (Table 1) but Cl^- concentration was significantly reduced after irradiation during the basal period. Fluid and Cl^- secretion were signifi-

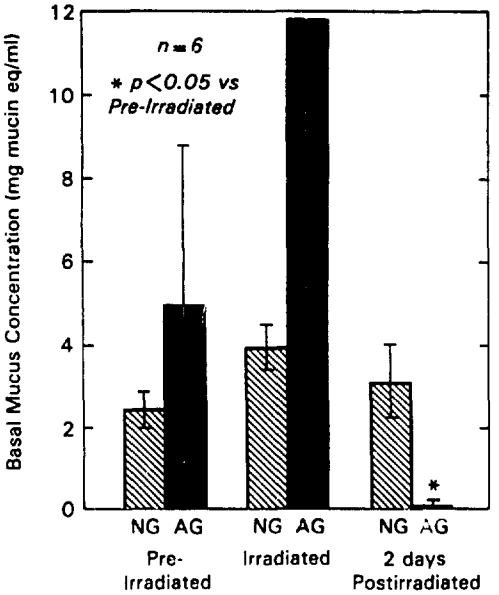

Figure 2. Basal soluble mucin concentration in the secreted juice before, on the day of, and 2 days after exposure to irradiation. Each bar represents the mean ± SE of values obtained in 6 monkeys.

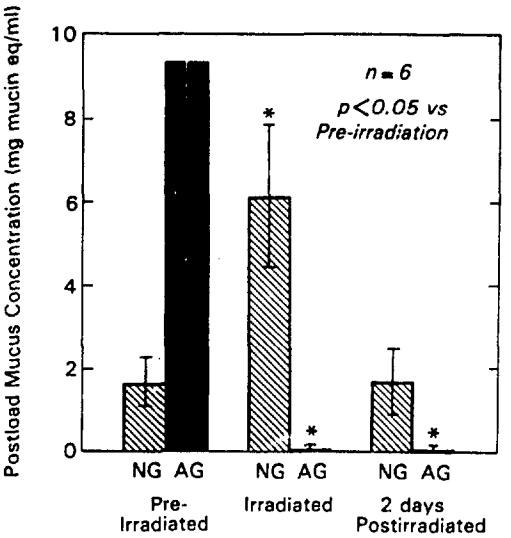

Figure 4. Postload soluble mucin concentration in the secreted juice before, on the day of, and 2 days after exposure to irradiation. Each bar represents the mean ± SE of values obtained in 6 monkeys.

Table 1. Effect of Irradiation on Basal and Postload Fluid and Ions

Treatment	Fluid output (ml/min)	Na^+ output (μEq/min)	Na^+ conc (μEq/ml)	K^+ output (μEq/min)	K^+ conc (μEq/min)	Cl^- output (μEq/min)	Cl^- conc (μEq/ml)
Basal							
Preirradiation	0.12 ± 0.04	12.2 ± 3.1	122 ± 33	2.8 ± 0.6	27 ± 5	18.3 ± 3.5	195 ± 40
Irradiation	0.18 ± 0.04	18.6 ± 3.6	110 ± 10	4.3 ± 1.3	20 ± 3	16.9 ± 3.2	119 ± 31[a]
2 days postirradiation	0.14 ± 0.02	13.4 ± 4.2	93 ± 18	2.9 ± 0.8	20 ± 4	25.5 ± 5.0	221 ± 44
Postload							
Preirradiation	0.19 ± 0.03	10.2 ± 1.9	85 ± 24	3.3 ± 0.6	24 ± 7	24.6 ± 6.6	174 ± 58
Irradiation	0.12 ± 0.02[b]	10.8 ± 2.2	153 ± 35	2.3 ± 0.6	19 ± 6	11.0 ± 1.5[a]	228 ± 89
2 days postirradiation	0.15 ± 0.03	9.1 ± 1.4	111 ± 36	2.7 ± 0.7	25 ± 6	26.4 ± 6.3	311 ± 143

Values are mean ± SE of measurements in 6 monkeys. [a] $p < 0.05$ and [b] $p < 0.01$ when compared with preirradiation using a three-factor (treatment, time, and monkey) analysis of variance with repeated measures on the last two factors.

cantly inhibited by irradiation only after the water load. However, 2 days after irradiation, fluid and ion output and ion concentration were not significantly different from preirradiation levels.

Discussion

The human gastric mucosa has several histologically distinct cell types which secrete various mucins. These cells include the mucus neck cells in the fundus, the mucus cells of the cardiac and pyloric glands, and the surface epithelia. The normal gastric mucosa is covered with a continuous thin layer of adherent mucus. This film is not only a product of the surface epithelial cells and mucus neck cells of the adjacent area but also of the mucosa higher in the stomach. Decreases in the quantity and quality of mucus glycoproteins have been associated with gastric mucosal injury and may reflect alterations in the integrity of the surface epithelial cells which produce the mucosal gel layer as well as the cellular ability to synthesize glycoproteins and secrete mucus. Studies correlating changes in soluble and insoluble mucus are inconsistent (2,12). However, Lamont et al. (2) recently found that increases in the AG concentrations of soluble mucus were associated with increases in the AG concentration of the adherent mucus gel. In addition, AG in both soluble and insoluble mucus was inversely correlated with gastric mucosal damage.

The present study demonstrates that, immediately after irradiation, both basal and postload NG output are significantly increased. This, however, is a transient effect as 2 days after irradiation, NG output is not significantly different from control output. In contrast, AG output follows a biphasic time course after irradiation. Immediately after total body exposure, AG output, like NG output, is significantly stimulated; this early elevation of glycoprotein output does not appear to result from an increased acid-pepsin digestion of the mucus layer, as it coincides with an inhibition of gastric acid secretion (13). After the water load, however, AG output is completely abolished. This absence of the normal rise in postload AG output can be attributed to either a prolonged effect of irradiation at that time or to a complete radiation-induced depletion of available AG material during the preceding basal period. Unlike NG output, AG output remains suppressed 2 days after irradiation. As AG output returns to normal within 2 days in the absence of radiation, this much longer lasting effect on both basal and postload AG output can be attributed directly to the effects of irradiation on the gastric mucosa.

The radiation-induced response in soluble mucus output may represent the secretory counterpart of morphologic changes described in the same model (1). In this study, using different monkeys, no gross mucosal damage (endoscopic view) was apparent 3 h after irradiation. Furthermore, light microscopic evaluation of biopsy specimens taken at this same time revealed no disruption of the lining epithelial cells or mucus-containing cells for up to 3 days after irradiation (1). However, SEM showed a marked hypertrophy of the microvilli of some surface epithelial cells. This is similar to the appearance of surface cells before the apical expulsion of mucus. Although earlier biopsy specimens were not obtained, this hypertrophy may represent the final stages of an initial extrusion of mucus from cells, which is reflected in the initial increase in NG and AG output observed immediately after irradiation in the present study. Interestingly, release of mucus by apical expulsion is often followed by cell degeneration and may be characteristic of a response to injury (14).

Alcian blue binds not only to acidic glycoproteins but to other negatively charged macromolecules as well. Thus, an increase in AG output could be due to an increase in cell shedding immediately after irradiation. Scanning electron microscopy and light microscopy, however, do not reveal significant increases in cell shedding at the approximate time of the rise in AG output observed after irradiation in the present study. Moreover, if significant cell shed-

ding occurred later on, one would expect an increase in AG output rather than the observed decrease.

The differences between AG and NG output observed 2 days after irradiation may be related to a differential effect of irradiation on mucus cells producing biochemically distinct mucins. Zalewsky and Moody (14) reported that in dogs, mucus cells located on the surface and in gastric pits contain a highly sulfated mucin, as well as neutral glycoproteins, whereas neck cells in gastric glands contain predominantly neutral glycoproteins. Although the presence or function of sulfated mucin in normal gastric mucosa of humans has been questioned (15), increased levels of sulfated glycoproteins have been found in humans following stress (16). The results of the present study support the distribution of AG and NG proposed by Zalewsky and Moody (14). The surface epithelial cells extend into the gastric pits and are renewed approximately every 3 days. The mucus neck cells which lie deeper in the mucosa near the parietal cells are renewed in about 1 wk. Cells with a high turnover rate are particularly susceptible to radiation injury (17). Thus, the AG-containing suface epithelial cells in this model exhibit a markedly greater vulnerability to radiation damage than do the NG-containing mucus neck cells. This is further supported by the fact that both NG output and acid secretion, but not AG output, have returned to normal within 3 days of radiation exposure. The significance of this observation is illustrated by SEM studies of gastric biopsy specimens. At 2 days postirradiation, there is damage or absence of numerous surface cells, the presence of exposed lamina propria in some areas, and a persistence of ulceration at the site where biopsy specimens were obtained on the day of irradiation (1).

The increase in soluble mucus output in the present study may be part of a response to stress. An increase in the release of β-endorphin from the pituitary has been shown to accompany other types of experimental stress (18,19). That irradiation is a stress is supported by the fact that total body irradiation has been shown to significantly enhance plasma β-endorphin (20). The initial rise in soluble mucus output observed immediately after irradiation in the present study is not reported in earlier studies of experimental stress. This may be attributed to differences in methodology. In previous studies, the stress was applied for a longer period of time and gastric samples were usually not obtained until several hours after the stress was initiated. In the current study, the monkeys were exposed to irradiation for <2 min and samples of gastric juice were obtained within 10 min. Thus, this initial rise in soluble mucus glycoprotein secretion may not have been observed previously. In contrast, the significant inhibition of AG output observed after the water load is similar to the previous findings of a reduction in glycoprotein content of the gastric juice within the first 24 h (16,21,22). Earlier studies have shown that AGs are the major type of glycoproteins secreted in the rat (2). Although purified monkey mucin was not used as a standard in the present study, AGs also appear to be the major type of glycoproteins in the present model. Thus, even in the presence of an elevated postload NG output, the potent suppression of postload AG output results in a net reduction of the mucus glycoprotein content of the gastric juice.

After the first 24 h, radiation-induced changes in soluble mucus do not parallel those reported after other types of stress. Much of this difference can be attributed to the more disruptive or permanent changes in the gastric mucosal barrier that arise from radiation exposure. Studies of experimental stress show a gradual increase in soluble mucus glycoproteins and acid secretion during the 5–6 days post-trauma (16). After irradiation, acid output was also found to return to control levels within 3 days (13). In the present study, however, although NG output returns to normal, AG output is still deficient at this time.

Irradiation produces significant changes in mucus secretion, but does not appear to be accompanied by a "break" of the gastric mucosal barrier. Such a breakdown has been associated with the appearance of Na^+ in the lumen and a back diffusion of H^+ ions. In the present study, however, irradiation did not significantly alter Na^+ or K^+ output or concentration, suggesting a lack of significant mucosal damage for 3 days after irradiation. The significant decrease in fasting Cl^- concentration and in postload Cl^- and fluid output on the day of irradiation reflect the reported suppression of acid secretion (13). Two days after irradiation the observed return of Cl^- and fluid output to control values accompanies the previously reported restoration of acid secretion to normal (13).

These studies demonstrate that irradiation produces significant alterations in the amount and type of mucus glycoprotein secreted into the gastric juice. It is not likely that these changes are a response to gastric injury because the gastric mucosal barrier remains intact. However, they may seriously affect the protective ability of gastric mucus.

References

1. Dubois A, Danquechin-Dorval E, Wood L, et al. Effect of γ-irradiation on the healing of gastric biopsy sites in monkeys. An experimental model for peptic ulcer disease and gastric protection. Gastroenterology 1985;88:375–81.
2. Lamont JT, Ventola AS, Maull EA, Szabo S. Cysteamine and

prostaglandin $F_{2\beta}$ stimulate rat gastric mucin release. Gastroenterology 1983;84:306–13.
3. Dubois A, Natelson B, Van Eerdewegh P, Gardner J. Gastric emptying and secretion in the rhesus monkey. Am J Physiol 1977; 232:E186–92.
4. Dubois A, Van Eerdewegh P, Gardner J. Gastric emptying and secretion in Zollinger-Ellison syndrome. J Clin Invest 1977;59:255–63.
5. Shea-Donohue PT, Myers L, Castell DO, Dubois A. Effect of prostacyclin on gastric emptying and secretion in rhesus monkeys. Gastroenterology 1980;78:1476–9.
6. Shea-Donohue PT, Adams N, Arnold J, Dubois A. Effects of met-enkephalin and naloxone on gastric emptying and secretion in rhesus monkeys. Am J Physiol 1983;245:G196–200.
7. George JD. New clinical method for measuring the rate of gastric emptying: the double sampling test meal. Gut 1968;9:237–42.
8. Hildes HA, Dunlop KL. A method for estimating the ratio of gastric secretion and emptying. Can J Med Sci 1951;29:83–9.
9. Piper DW, Whitecross D, Leonard P, Clarke A. Alcian blue dye binding properties of gastric juice. Gastroenterology 1970;59:534–8.
10. Mantle M, Allen A. A colorimetric assay for glycoproteins based on the periodic acid Schiff stain. Biochem Soc Trans 1978;6:607–9.
11. Kirk RE. In: Experimental design: procedures for the behavioral sciences. Monterey, California: Brooks Cole, 1968:110–2.
12. McQueen S, Hutton D, Allen A, Garner A. Gastric and duodenal surface mucus gel thickness in rat: effects of prostaglandins and damaging agents. Am J Physiol 1983;8:G388–93.
13. Danquechin-Dorval E, Eng R, Columbaro P, Durakovic A, Conklin J, Dubois A. Radiation-induced vomiting and altered gastric emptying and secretion. Gastroenterology 1983; 84:1183.
14. Zalewsky CA, Moody EF. Mechanisms of mucus release in exposed canine gastric mucosa. Gastroenterology 1979; 77:719–29.
15. Lambert R, Andre C, Bernard A. Origin of sulphated glycoproteins in human gastric secretions. Digestion 1971;4:234–9.
16. Stremple JF, Mori H, Lev R, Glass GBJ. In: Ravitch M, ed. Current problems in surgery. Chicago: Yearbook Medical Publishers, Inc., 1973:1–64.
17. Lipkin M, Shenock P, Bell B. Cell proliferation kinetics in the gastrointestinal tract of man. II. Cell renewal in the stomach, ileum, colon, and rectum. Gastroenterology 1963;45:721–9.
18. Guillemin R, Vargo T, Rossier J, et al. β-Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. Science 1977;197:1367–9.
19. Rossier J, French E, Rivier C, Ling N, Guillemin R, Bloom F. Foot-shock induced stress increases β-endorphin in rat blood but not brain. Nature 1977;270:618–20.
20. Danquechin-Dorval E, Mueller GP, Eng R, Durakovic A, Conklin J, Dubois A. Involvement of endogenous opiates in radiation-induced suppression of gastric emptying. Gastroenterol Clin Biol 1983;7:712.
21. Dekanski JB, MacDonald M, Sucra P. Effects of fasting, stress and drugs on gastric glycoprotein synthesis in the rat. Br J Pharmacol 1975;55:385–92.
22. Ludwig W, Lipnin M. Biochemical and cytological alterations in gastric mucosa of guinea pigs under restraint stress. Gastroenterology 1969;56:895–902.