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Effect of Gastroduodenal Juice and Dietary Fat on the Development of Barrett's Esophagus and Esophageal Neoplasia: An Experimental Rat Model

Geoffrey W.B. Clark, FRCS(Ed), Thomas C. Smyrk, MD, Sidney S. Mirvish, PhD, Marco Anselmino, MD, Yoshinori Yamashita, MD, Ronald A. Hinder, MD, PhD, Tom R. DeMeester, MD, and Diane F. Birt, PhD

Background: Reflux of duodenal content into the lower esophagus of rats enhances the formation of nitrosamine-induced esophageal cancer and results in the induction of adenocarcinoma. We investigated the extent of the mucosal injury that was produced when the lower esophagus of rats was exposed to the reflux of gastroduodenal juice in the presence or absence of a carcinogen and tested the hypothesis that induction of esophageal cancer in this model would be influenced by the intake of dietary fat.

Methods: Esophagoduodenostomy with gastric preservation was performed in 165 Sprague-Dawley rats in order to expose the lower esophagus to the reflux of gastroduodenal juice. Postoperatively selected groups of rats were treated with the carcinogen methyl-*n*-amylnitrosamine (MNAN). Subsequently, rats were fed diets of differing fat and calorie content for 20 weeks until they were put to death.

Results: Refluxed gastroduodenal juice, in the absence of MNAN, induced esophageal inflammatory changes (diffuse papillomatosis and hyperkeratosis) in 38 of 39 rats (97%), specialized columnar metaplasia (Barrett's esophagus) in four of 39 (10%), dysplasia in three of 39 (8%), and squamous cell carcinoma in one of 39 (3%). Diet did not influence the incidence of neoplasia in the absence of MNAN treatment. In rats treated with MNAN, refluxed gastroduodenal juice induced inflammation in 110 of 111 rats (99%), columnar metaplasia in 14 of 111 (13%), and cancer in 63 of 111 (57%). Fifty-eight percent of esophageal tumors were squamous cell carcinoma and 42% were adenocarcinoma. The highest incidence of tumors was observed in rats fed the semipurified high-fat diet (24 of 29; 83%) compared with rats fed the semipurified control diet (13 of 29; 45%), semipurified, calorie-restricted diet (15 of 27; 55%), and chow diet (11 of 26; 42%), $p < 0.05$.

Conclusions: Reflux of gastroduodenal content into the lower esophagus of rats can induce both Barrett's metaplasia and neoplasia. Addition of a carcinogen increases the tumor yield and results in a proportion of the lesions being adenocarcinoma. This carcinogenic process is promoted by a diet with a high fat content.

Key Words: Reflux—Dietary fat—Barrett's esophagus—Esophageal cancer.

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From the Department of Surgery, University of Southern California School of Medicine, Los Angeles, California (G.W.B.C., T.R.D.); Department of Pathology, Clarkson Memorial Hospital, Omaha, Nebraska (T.C.S.); The Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska (S.S.M., D.F.B.); and Department of Surgery, Creighton University, Omaha, Nebraska (M.A., Y.Y., R.A.H.), USA.

Address correspondence and reprint requests to Dr. Tom R. DeMeester, University of Southern California School of Medicine, Department of Surgery, 1510 San Pablo Street, Suite 514, Los Angeles, CA 90033-4612, USA.

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The incidence of esophageal adenocarcinoma has been increasing in the United States during the past 20 years (1,2). The reasons for this change are unclear. The tumor predominantly affects elderly white men, and the most significant risk factor is the presence of the premalignant condition of Barrett's esophagus (3), a metaplasia from the normal stratified squamous epithelial lining of the distal esophagus to columnar mucosa. Barrett's esophagus is an acquired condition that is associated with profound gastroesophageal reflux (4,5). Malignant degeneration in Barrett's esophagus may be related to specific components of the refluxate because previous studies in rats have shown that duodenal juice enhances the expression of nitrosamine-induced esophageal cancer (6,7).

Adenocarcinoma of the colon, breast, and pancreas, which are common tumors in Western countries, have been linked epidemiologically (8–14) and experimentally (15–17) to a high intake of dietary fat. The question arises whether the "Western" style diet in combination with Barrett's esophagus might encourage the development of adenocarcinoma, because both Barrett's esophagus and esophageal adenocarcinoma are uncommon and rarely reported in Asian and African countries.

The N-nitroso compounds methyl-*n*-amylnitrosamine (MNAN) and 2,6-dimethylnitrosomorpholine (DMNM) are carcinogens that specifically affect the esophageal epithelium and induce squamous cell carcinoma in rats (18–20). These carcinogens are thought to be activated by specific cytochrome P450 isoenzymes of the esophageal mucosal cells and exert their effect through the alkylation of DNA. Pera et al. exposed the distal esophageal mucosa of rats to duodenal contents by surgically diverting pancreaticobiliary secretions into the lower esophagus (6). When these rats were treated with DMNM, esophageal cancers were produced in a shorter time period, and in greater numbers, than in rats treated with the carcinogen alone. Approximately half of the tumors were adenocarcinomas. Attwood et al. confirmed the induction of adenocarcinomas in carcinogen-treated rats subjected to esophagoduodenal anastomosis and showed that no adenocarcinomas developed when the esophagus of carcinogen-treated rats was exposed to "pure" acid reflux, induced by the surgical operation of cardioplasty (7). These findings support the hypothesis that duodenal juice may be involved in the induction of esophageal adenocarcinoma in rats.

The aim of this study was (a) to investigate the extent of the mucosal injury that was produced when the lower esophagus of rats was exposed to the reflux of gastroduodenal juice in the presence or absence of a carcinogen and (b) to test the hypothesis that induction of esophageal cancer in this model would be influenced by the intake of dietary fat.

MATERIALS AND METHODS

One hundred ninety male Sprague-Dawley rats were obtained from Sasco (Omaha, NE) at 8 weeks of age. Rats were randomly assigned to one of nine groups, as shown in Table 1. Twenty-five rats served as nonoperated controls and 165 underwent the operation of end-to-side esophagoduodenostomy with gastric preservation to induce profound reflux of gastroduodenal juice into the lower esophagus (Fig. 1). Carcinogen was administered in selected groups, and the tumor-promoting effect of different diets was evaluated. The study was approved by the Animal Review Committee of the University of Nebraska Medical Center.

Rats were housed five per cage, 50 × 40 × 20–cm in size, and bedded on corn cob bedding (Bed-O-Cobs, Anderson Cob Division, Maumee, OH). The rats fed the calorie-restricted diet were housed individually in 35 × 20 × 20–cm cages. Rats were

TABLE 1. *Experimental design*

Group	No. starting	No. finishing	Operation	Carcinogen	Diet
1	10	10	−	−	Chow
2	15	15	−	+	Chow
3	15	13	+	−	Chow
4	15	14	+	−	Semipurified, control
5	15	12	+	−	Semipurified, high fat
6	30	26	+	+	Chow
7	30	27	+	+	Semipurified, calorie restricted
8	30	29	+	+	Semipurified, control
9	30	29	+	+	Semipurified, high fat

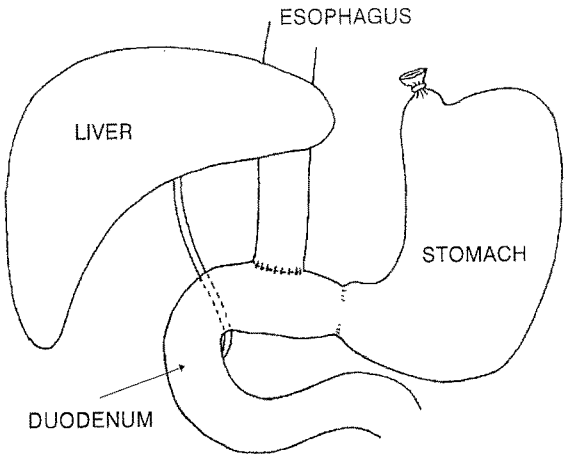

FIG. 1. Esophagoduodenostomy with gastric preservation.

kept for the entire experiment in an animal care facility under the following conditions: 12-h light/dark cycle at a temperature of 68°F, humidity 40 ± 5%, and Ketamine 10 air changes per hour.

Surgical Technique

Before surgery rats were housed in hanging cages for 48 h to prevent coprophagia. Solid food was withdrawn for 24 h and water for 12 h before surgery. Anesthesia was administered by the intramuscular injection of a mixture of xylazine 100 mg/ml and ketamine 10 mg/ml in doses of 0.4 mg/kg, and additional doses were given intraoperatively into the peritoneal cavity as necessary. Esophagoduodenal anastomosis was performed in a sterile field through an upper midline incision. The distal esophagus was exposed, and two stay sutures were placed laterally in the wall. The gastroesophageal junction was ligated flush with the stomach. The distal esophagus was transected proximal to the ligature and anastomosed to a 4-mm transverse enterotomy on the antimesenteric border of the duodenum 1 cm distal to the pylorus. The anastomosis was fashioned with eight equally spaced interrupted 7/0 proline sutures tied on the outside of the bowel with accurate mucosal-to-mucosal apposition. The abdominal incision was closed and postoperatively the rats were allowed to drink water after 6 h and were fed the following day.

Carcinogen Treatment

At 10 weeks of age the rats were transferred to a chemical fume hood to exhaust the volatile carcinogen. This allowed a 2-week recovery period for the operated rats. Rats were injected weekly for 4 weeks with MNAN via the intraperitoneal route at a dose of 25 mg/kg (one fifth of the LD_{50}) (21). MNAN was synthesized by slow addition of sodium nitrite solution to an aqueous solution of methyl-*n*-amylamine and hydrochloric acid, followed by separation of the organic layer. The purity was shown to be >95% by the ultraviolet absorption of aqueous solutions. Before use MNAN was dissolved in 0.9% saline and diluted to a concentration of 2.5 mg/ml.

Diets

All rats were initially fed commercial chow diet (Wayne Lab Blox, Allied Mills, Chicago, IL), which was replaced by respective semipurified diets at 14 weeks of age. The diets were continued for 20 weeks, until the rats were put to death. In MNAN-treated rats this step allowed the separation of the induction period (carcinogen treatment) from the promotion phase (diet) and eliminated the possibility that the diets may have affected the metabolism of MNAN. The semipurified diets were formulated as recommended by the American Institute of Nutrition (22,23), except that glucose/dextrin was used in place of sucrose as the carbohydrate. Diets were made up in bulk every 3 weeks, pelleted (California Pellet Mill, Crawfordsville, IN) without heat or steam, and stored at 4°C. The semipurified, high-fat diet contained 24.6% corn oil; the semipurified control diet contained 5% corn oil; and the semipurified, calorie-restricted diet contained 3.8% corn oil (Table 2). The high-fat diet provided 420 Kcal/100 g and the control diet 380 Kcal/100 g. Previous studies have shown that rats fed the high-fat diet consume only 85% of the weight consumed by rats fed the control diet, so that these two diets provided similar caloric intake and a constant intake of vitamins, minerals, fiber, and protein per calorie (24). The calorie-restricted diet was formulated so that the rats received 40% fewer calories (with calories removed from fat and carbohydrate) than did rats fed the control diet but had the same daily intake of protein, fiber, vitamins, and minerals.

The chow diet and the semipurified, high-fat and control diets were given ad libitum. The calorie-restricted diet was given once daily in controlled amounts. Groups of five rats fed the semipurified control diet were paired with five rats fed the calorie-restricted diet throughout the experiment. The total amount of food consumed by rats fed the control diet was recorded at the end of each week and the average amount consumed per rat per day was calculated. The following week, the rats in the calorie-restricted group were fed each day 0.64 times

TABLE 2. *Semipurified AIN diets*

Diet	High fat (%)	Control (%)	Calorie restricted (%)
Corn oil	24.6	5.0	3.8
Casein	24.6	20.0	31.1
Glucose	9.0	15.0	11.5
Dextrin	29.5	50.0	38.1
Fiber[a]	6.2	5.0	7.8
AIN mineral mix[b]	4.3	3.5	5.4
AIN vitamin mix[c]	1.2	1.0	1.5
DL methionine	0.5	0.3	0.5
Choline bitartrate	0.3	0.2	0.3
	100	100	100

All dietary components were supplied by Teklad Test Diets, Madison, WI.

[a] Teklad, nonnutritive fiber.

[b] AIN mineral mix composed of 207 g $CaCO_3$/kg, 323 g $CaHPO_4$/kg, 66 g $MgSO_4$/kg, 209 g KCl/kg, 186 g Na_2HPO_4/kg, 0.37 g $CuSO_4$/kg, 4.3 g ferric citrate/kg, 4.4 g $MnSO_4 \cdot H_2O$/kg, 0.03 g KIO_3/kg, and 0.6 g $ZnCO_3$/kg.

[c] AIN vitamin mix at 1.5% supplies in the final diet (in mg/100 g) p-aminobenzoic acid (16), ascorbic acid (149), biotin (0.07), vitamin B_{12} (0.0045), calcium pantothenate (10), choline (214), folic acid (0.3), inositol (16), menadione (7.4), niacin (15), pyridoxine HCl (3), riboflavin (3), and thiamine HCl (3). In addition, it supplies (in U/100 g) vitamin A palmate (29.74), vitamin D_2 (3.3), and vitamin E acetate (18).

the average daily weight consumed the previous week by the rats in the paired control group. This allowed a 40% reduction in calorie intake of animals in the calorie restricted group compared with that of the control group (25). Body weights were recorded monthly.

Sacrifice and Handling of Specimens

The rats were killed at 34 weeks of age by an overdose of phenobarbital. The thoracic and abdominal cavities were inspected for the presence of tumors, and the esophagus, anastomosis, proximal duodenum, and stomach were excised en bloc. The small and large intestines were removed and opened along their full length on the antimesenteric border. The lumen was visually inspected, but, because no tumors were seen in any animal, no further examination was undertaken.

The specimen was opened and photographed. The esophageal length and maximum diameter were recorded. We noted the presence of mucosal abnormality and the number of papillomas and macroscopic tumors. The location of each tumor was recorded. Two strips of the length of the esophagus, each with a segment of duodenum, were taken from each rat, Swiss rolled, and held in position with a pin. The strips were taken to include all macroscopic tumors and were fixed in 10% buffered formalin. Specimens were embedded in paraffin wax, and 5-μm sections were made. Sections were stained with hematoxylin and eosin and, when tumors were present, with mucicarmine.

Microscopic evaluation was performed by a histopathologist (TCS) who was blinded to experimental conditions. Each strip was evaluated for signs of esophageal inflammation. Barrett's esophagus was identified when specialized columnar epithelium with goblet cells extended at least 5 mm above the site of the surgical anastomosis (the site of anastomosis was evident in sections by the presence of the nonabsorbable blue suture material). Columnar cells lining the esophagus were not identified in any of the 10 control rats, where the squamous epithelium of the esophagus is continuous with that of the squamous forestomach; therefore, the presence of intestinal metaplasia lining any length of the esophagus was considered abnormal. However, because the specialized intestinal epithelium lining the distal esophagus was continuous with that of the duodenum, it was necessary to select a minimum distance of 5 mm of length to be confident that this mucosa was lining the esophagus and not distorted duodenal mucosa at the site of anastomosis. The diagnosis of a columnar-lined esophagus, therefore, also could be made when squamous epithelium was present distal to the location of the area of columnar change even when it measured <5 mm in length.

The diagnosis of dysplasia was made according to standard criteria (26) and included dysplasia arising in either glandular or squamous epithelium. The presence of benign pedunculated squamous cell papillomas was recorded. Carcinomas were diagnosed as either squamous cell carcinomas or adenocarcinomas. All adenocarcinomas were confirmed when the glandular elements stained magenta with mucicarmine. Tumors classed as adenocarcinomas generally had both squamous elements located toward the luminal aspect of the lesion, comprising about half the tumor mass, and glandular elements located more deeply, comprising the other half. The number of tumors found in each rat was counted from the histological specimens.

Statistical Tests

Statistical tests were performed using the Fisher's exact test for comparisons of proportions. Analysis of variance (ANOVA) was used for comparison of animal weights and esophageal measurements, with Bonferroni corrected multiple *t* tests to

identify differences between each of the groups. Comparison of total number of tumors and tumor histology was performed with the Kruskal-Wallis test, and comparisons between groups performed with the Wilcoxon rank sum test. Significance was taken at the 0.05 level.

RESULTS

Early Deaths

There was no mortality in the 25 nonoperated rats. Fifteen of 165 operated rats died before the end of the experiment. Four rats died during the operation from the anesthesia and the remaining deaths occurred between days 11 and 43 postoperation. The cause of death in these rats was acute gastric dilatation (n = 2), peritonitis (n = 2), anastomotic obstruction with mega-esophagus (n = 2), MNAN toxicity (n = 3), and unknown (n = 2). All deaths occurred before conversion to the semipurified diets.

Animal Weights

Preoperative weights were similar in all rats. Operated rats that did not receive MNAN gained weight at a significantly slower rate than did nonoperated controls. No difference was observed between the rate of weight gain in operated rats fed ad libitum the semipurified high-fat, semipurified control, or chow diets. Operated animals that received MNAN treatment gained weight at a slower rate than did MNAN-treated controls (Fig. 2). Weight gain in operated rats receiving MNAN and fed ad libitum was similar, whereas rats fed the calorie-restricted diet gained weight at a significantly impaired rate compared with all the other groups.

Reflux of Gastroduodenal Juice

Ten untreated control rats fed chow were compared with 13 rats fed chow who underwent esophagoduodenostomy. No abnormality was detected in the 10 untreated controls. The reflux of gastroduodenal juice into the lower esophagus, induced by the operation, resulted in severe esophageal inflammation. Macroscopically there was significant shortening of the esophagus and an increase in esophageal width compared with that of controls (Table 3). The esophageal shortening can be explained in part because the esophagus had been divided and anastomosed. However, the esophagus was divided as close to the stomach as possible, and in no animal was there an esophageal stump of >2–3 mm. Esophageal shortening of an average of 1.5 cm compared with that of controls is taken to be the effect of distal esophageal inflammatory changes. After the operation the distal esophageal mucosa became abnormal, with multiple longitudinally running white plaques and intervening small superficial ulcers. Microscopically there was severe squamous basal cell hyperplasia in all rats, throwing the

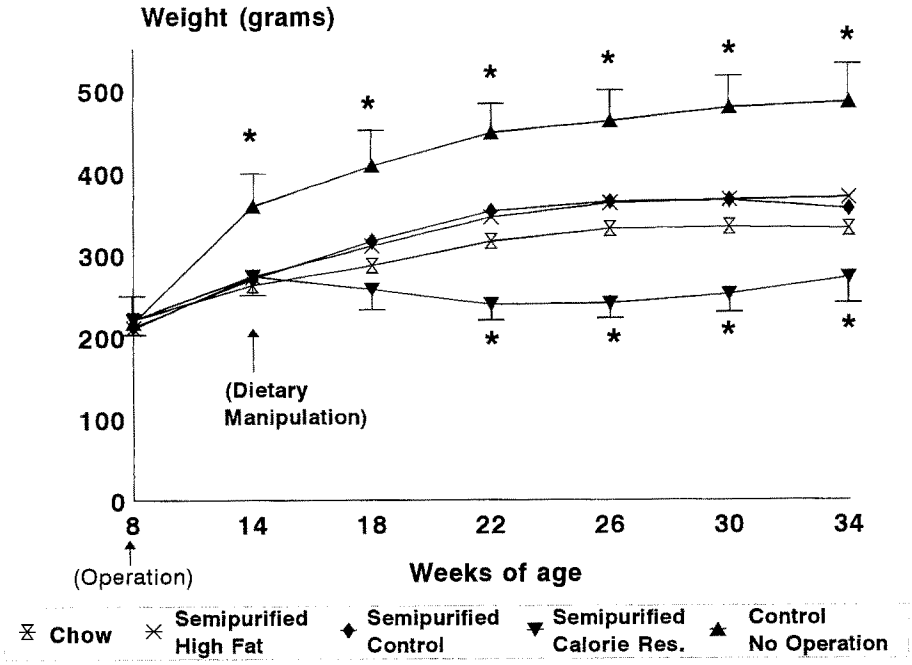

FIG. 2. Weight gain in rats undergoing esophagoduodenostomy followed by MNAN treatment. Values are expressed as means (standard deviations are only shown for two of the groups for clarity). There was a significant difference between the groups: $p < 0.01$, analysis of variance, F value 55.8, (697,29 df). *Significant difference versus each of the other groups, $p < 0.05$, with multiple t tests corrected with the Bonferroni technique.

TABLE 3. *Esophageal characteristics in rats not receiving MNAN*

No. of animals	No operation and chow (n = 10)	Operation and chow (n = 13)	Semipurified diets: Operation and control diet (n = 14)	Semipurified diets: Operation and high fat (n = 12)
Esophageal length (cm)[a]	8.6 ± 0.6	7.1 ± 0.7[b]	7.3 ± 0.6[b]	6.6 ± 0.7[c]
Maximal esophageal width (cm)[d]	0.5 ± 0.1	1.1 ± 0.2[e]	1.1 ± 0.2[e]	1.2 ± 0.2[e]
Diffuse papillomatosis	0	13[f]	14[f]	11[f]
Columnar lining	0	1	0	3
Dysplasia	0	0	1	2
Papilloma	0	1	0	1
Cancer	0	0	0	1

Measurements are means ± SD.

[a] Significant difference between the groups, $p < 0.01$, ANOVA, F value 16.0 (45,3 df).

[b] Significant difference versus nonoperated controls, $p < 0.05$, with multiple *t* tests corrected with the Bonferroni technique.

[c] Significant difference versus operated rats fed semipurified control diet, $p < 0.05$, with *t* test corrected with the Bonferroni technique.

[d] Significant difference between the groups, $p < 0.01$, ANOVA, F value 28.3, (45,3 df).

[e] Significant difference versus nonoperated controls, $p < 0.05$, with multiple *t* tests corrected with the Bonferroni technique.

[f] Significant difference versus nonoperated controls $p < 0.01$, Fisher's exact test.

esophageal mucosa up into multiple folds, accompanied by hyperkeratosis. We termed these changes diffuse papillomatosis (Table 3). One operated rat developed a segment of columnar-lined esophagus.

Diet and Reflux of Gastroduodenal Juice (no MNAN)

The operated rats fed the semipurified, high-fat and semipurified control diets showed significant esophageal shortening and dilatation compared with nonoperated animals fed chow (Table 3). Diffuse papillomatosis after the operation was seen with a similar frequency as in the operated rats fed chow. Operated rats fed the high-fat diet showed significantly more esophageal shortening than was seen in the rats fed the semipurified control diet (Table 3). In operated rats fed the high-fat diet, three developed specialized columnar epithelium proximal to the anastomosis and two developed dysplasia, one of which had a squamous cell carcinoma. One operated rat fed the semipurified control diet developed dysplasia (Table 3).

Overall, in the three groups of rats with surgically induced gastroduodenal reflux, the incidence of a Barrett's esophagus was 10% (four of 39), dysplasia 8% (three of 39), and squamous cell carcinoma 3% (one of 39) in the absence of MNAN treatment.

Reflux of Gastroduodenal Juice and MNAN Treatment (Animals Fed Chow)

In 15 rats that were not operated but received MNAN alone, esophageal inflammation and diffuse papillomatosis were not seen (Table 4). In 26 operated rats treated with MNAN, the reflux of gastroduodenal juice induced diffuse papillomatosis in all rats. In addition, a columnar lining was identified proximal to the anastomosis in 27% of animals (Fig. 3) as compared with none in MNAN-treated controls ($p < 0.05$, Fisher's exact test). The combination of MNAN treatment and reflux of gastroduodenal juice induced dysplasia with a significantly higher frequency than in rats treated with MNAN alone (Table 4). No esophageal cancers were seen with MNAN treatment alone, whereas the combination of operation and MNAN resulted in a 42% incidence of esophageal cancer, ($p < 0.01$, Fisher's exact test).

Effect of Diet on MNAN-Treated Rats with Reflux of Gastroduodenal Juice

The inflammatory response in the lower esophagus in the operated groups fed the semipurified diets was similar to that of the operated groups fed chow (Table 4). These findings were significantly different from those in rats that received MNAN treat-

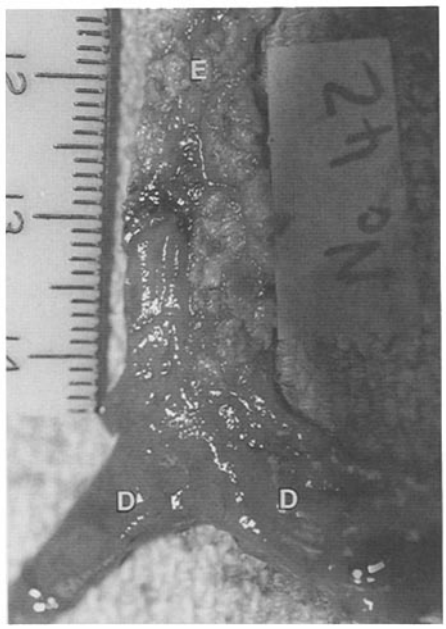

FIG. 3. The distal esophagus (E) and part of the duodenum (D) of a rat is shown. The site of the esophagoduodenal anastomosis is in-line with the end of the ruler. The distal esophagus on the right side is lined by papillomatous squamous epithelium, whereas on the left side the distal esophagus is lined by columnar epithelium extending for >1 cm.

TABLE 4. *Esophageal characteristics in rats receiving MNAN*

No. of animals	No operation and chow (n = 15)	Operation and chow (n = 26)	Semipurified diets: Operation and calorie restricted (n = 27)	Semipurified diets: Operation and control diet (n = 29)	Semipurified diets: Operation and high fat (n = 29)
Esophageal length (cm)[a]	8.3 ± 0.6	6.3 ± 0.7[b]	6.6 ± 0.6[b]	6.6 ± 0.6[b]	6.3 ± 0.6[b]
Maximal esophageal diameter (cm)[c]	0.5 ± 0.1	1.1 ± 0.2[b]	1.0 ± 0.2[b]	1.1 ± 0.2[b]	1.1 ± 0.3[b]
Diffuse papillomatosis	0	25[d]	27[d]	29[d]	29[d]
Columnar lining	0	7[e]	0	4	3
Dysplasia	0	23[d]	17[d]	22[d]	22[d]
Papilloma	2	5	6	10	12
Cancer	0	11[d]	15[d]	13[d]	24[df]

Measurements (cm) are means ± SD.
[a] Significant difference between groups, $p < 0.01$, ANOVA, F value 31.0, (121,4 df).
[b] Significant difference versus nonoperated controls, $p < 0.05$ with multiple Student's *t* tests corrected with the Bonferroni technique.
[c] Significant difference between the groups, $p < 0.01$, ANOVA, F value 32.9, (121,4 df).
[d] Significant difference versus nonoperated controls, $p < 0.01$, Fisher's exact test.
[e] Significant difference versus nonoperated controls and operated rats fed calorie-restricted diet, $p < 0.05$, Fisher's exact test.
[f] Significant difference compared with all operated groups, $p < 0.05$, Fisher's exact test.

ment alone. Three rats fed the high-fat diet and four fed the control diet showed evidence of a segment of columnar-lined esophagus, whereas none of the rats fed a calorie-restricted diet developed this change.

The incidence of dysplasia was similar in all operated rats after MNAN treatment. More rats fed the high-fat diet had papillomas than did any of the other groups, but the differences were not significant (Table 4). The effect of diet was most pronounced when the incidence of malignant tumors was considered. The operation plus MNAN followed by administration of a high-fat diet produced significantly more rats with cancer than was seen with any of the other diets (Fig. 4). A similar incidence of tumors was seen in animals fed both the semipurified control and the calorie-restricted diets. The total number of tumors was also significantly increased in rats fed the high-fat diet (Fig. 5). The histology of the tumors was adenocarcinoma and squamous cell carcinoma in approximately equal proportions in the different dietary groups. The incidence of adenocarcinomas in rats fed the high-fat diet was double that found in any of the other groups, and a similar situation was seen for squamous carcinomas. However, statistical differences were not identified for the individual histological tumor types (Fig. 5). Overall, in the operated rats treated with MNAN the incidence of Barrett's change was 14 of 111 (13%), and the incidence of cancer was 63 of 111 (57%). Of all the tumors induced, 58% were squamous carcinoma and 42% were adenocarcinoma.

The site of origin of the tumors was most commonly in the lower third of the esophagus, usually at the region of the anastomosis. Squamous cell carcinomas were more often seen in the mid and proximal esophagus than were adenocarcinomas (Table 5). We saw two examples where adenocarcinomas arose directly from segments of columnar metaplasia (Figs. 6 and 7).

DISCUSSION

These studies have demonstrated the damaging effects of free reflux of gastroduodenal juice on the

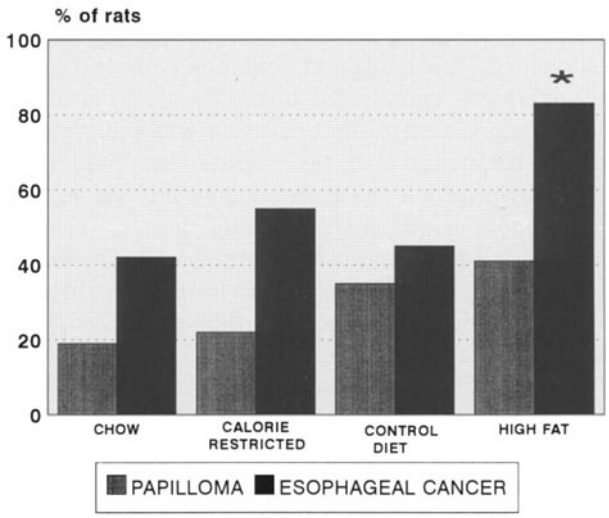

FIG. 4. Percentage of operated and MNAN-treated rats that developed esophageal papillomas and esophageal cancer in each of the dietary groups. (*$p < 0.05$ vs. each of the other groups, Fisher's exact test).

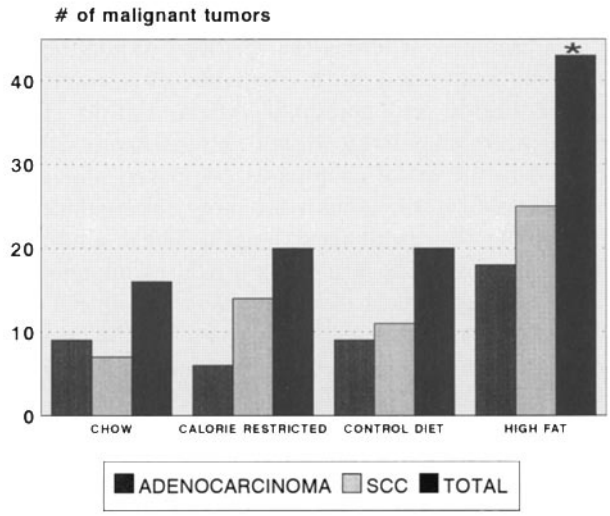

FIG. 5. The number of tumors and histological classification of tumor morphology in operated rats treated with MNAN and fed different diets. The total number of tumors was significantly different between the groups: $p < 0.01$, Kruskal-Wallis test, ($\chi^2 = 13.5$, 3 df). *Significantly different versus each of the other groups, $p < 0.01$, Wilcoxon rank sum test.

esophagus of rats. Macroscopically, esophageal shortening and distal esophageal dilatation were observed, postulated to be the result of transmural esophageal damage with loss of normal muscular tone. Microscopic changes included a marked squamous hyperplastic response of the esophageal lining combined with the production of a thickened keratin layer. Added to this, we identified a small number of animals with segments of columnar-lined esophagus, dysplasia, and even neoplasia. When MNAN was combined with the reflux of gastroduodenal juice, >50% of animals developed esophageal cancer located most commonly in the distal esophagus. Histological examination confirmed that these tumors included both squamous cell carcinomas and adenocarcinomas in approximately equal proportions.

The major focus of this study was to assess the effects of diet on the extent of inflammatory changes and on the production of esophageal neoplasia in rats with reflux of gastroduodenal juice into the esophagus. After esophagoduodenostomy, the reflux of gastroduodenal juice produced severe esophageal inflammation with diffuse papillomatosis and hyperkeratosis, which affected all rats and was not influenced by the different diets given. Increased esophageal shortening was noted in rats fed the high-fat diet, but the significance of this finding is unclear because after MNAN administration esophageal shortening was seen to the same extent in each of the different dietary groups. A columnar-lined esophagus, dysplasia, and neoplastic change were most common in the rats fed the high-fat diet, but in the absence of MNAN these differences were not statistically significant. When the effects of diet were evaluated in rats treated with MNAN, we identified a significant increase in the number of tumor-bearing animals that were fed the high-fat diet. The findings of a lower incidence of tumors in both the group fed the control diet and the calorie-restricted diet suggest that the increased tumor incidence in the group fed a high-fat diet was the effect of the fat rather than of the calorie intake of these animals. In this model calorie restriction did not protect against esophageal cancer.

The effects of refluxed gastroduodenal juice on the esophagus of rats has been previously reported in noncarcinogen- and carcinogen-treated animals (6,7). However, in this study we also reported areas of columnar metaplastic change in the esophageal lining of 18 of 150 rats (12%). Because the esophagus was anastomosed directly to the duodenum, there were difficulties distinguishing normal duodenal mucosa from an abnormal specialized columnar

TABLE 5. *Site of origin of esophageal cancer by histological type*

	Upper third	Middle third	Distal third
Squamous cell carcinoma	4	16	37
Adenocarcinoma	0	4	37

$p < 0.01$ ($\chi^2 = 8.8$, 2 df), distribution of adenocarcinomas versus squamous cell carcinomas.

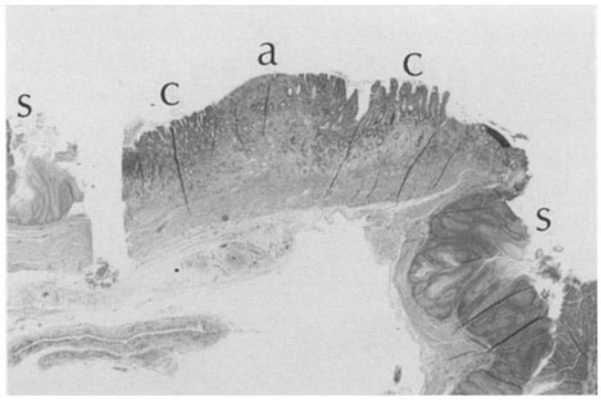

FIG. 6. An example of a rat with columnar epithelium (c) lining part of the esophagus. The columnar segment is surrounded by squamous epithelium (s). An adenocarcinoma (a) is seen in the middle of the field arising within the Barrett's segment (hematoxylin and eosin, original magnification ×14).

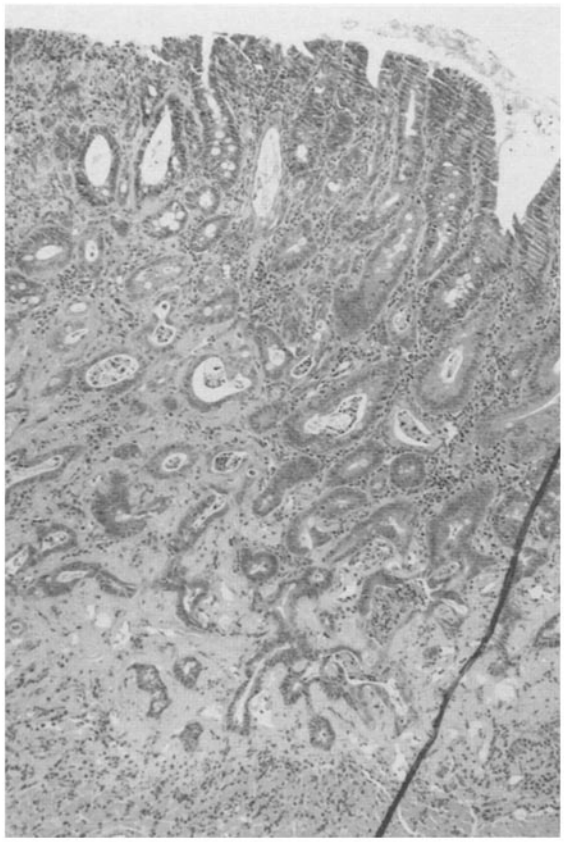

FIG. 7. Higher power view of the adenocarcinoma shown in Fig. 5 (hematoxylin and eosin, origninal magnification ×45). The tumor has arisen from specialized columnar epithelium and is invading the submucosa.

esophageal lining. We therefore only certified a columnar metaplasia when the length of affected esophagus extended ≥5 mm proximal to the anastomosis, which was easily identified by the blue suture material. We also made the diagnosis when there were segments of squamous epithelium situated between the area of columnar metaplasia and the site of the anastomosis to the duodenum. In most animals, the diagnosis was based on segments of change <5 mm in length with intervening areas of squamous epithelium; however, in two animals the specialized mucosa extended for 8 and 10 mm proximal to the anastomosis. Whether these short segments of change contributed to the development of adenocarcinomas in our animals requires further investigation.

This study demonstrated, as Pera and Attwood previously showed (6,7), that when gastroduodenal reflux is combined with an esophageal carcinogen, esophageal cancers are successfully induced and that approximately half of these are adenocarcinomas. In this study a few tumors were pure adenocarcinomas, whereas the majority of adenocarcinomas contained both elements of squamous differentiation and glandular differentiation. The adenocarcinomas induced were almost exclusively located in the distal esophagus compared with the squamous cancers, which were more evenly distributed along the esophageal length. These findings suggested that exposure of the distal esophagus to the combined effects of gastric acid and duodenal juice (bile and pancreatic secretions) may be important in the development of esophageal adenocarcinoma.

An increased incidence of breast, pancreas, and colon tumors has been reported in rodents fed diets with a high fat content (17,27). Results of previous studies indicate that polyunsaturated vegetable fats such as corn oil exert their effect primarily during the postinitiation phase of carcinogenesis, whereas saturated fats exert their action during both the induction and postinduction periods (27,28). The consumption of polyunsaturated fats in humans has increased in the United States (29), largely through the consumption of margarine, and might help to explain the increasing incidence of esophageal adenocarcinoma in the past 20 years. We chose to study only the postinitiation effects of dietary corn oil (polyunsaturated fat) and achieved this by separating the induction period (MNAN treatment), when all animals were fed commercial chow, from postinitiation dietary effects, the diets commencing 7 days after the final MNAN injection. To differentiate the effects of fat from those of calories, we included two diets with 5% and 25% corn oil but with similar high-calorie content and a third diet with 3.8% corn oil that was restricted in calories. A significant tumor-promoting effect of the high-fat diet was observed. However, the control and calorie-restricted diets resulted in a lower but similar incidence of tumors. These findings indicate that the tumor-promoting effect was associated with the high fat content and was largely independent of calorie intake. An interesting question which remains to be answered is whether the type of fat, polyunsaturates or saturates, would enhance tumor promotion to different degrees.

Our aim was to evaluate the effect of dietary fat on adenocarcinomas; however, the high-fat diet promoted the production of both squamous cell carcinomas and adenocarcinomas. The increased tumor promotion by dietary fat may be mediated through modification of biliary and pancreatic se-

cretions exerting a direct effect on lower esophageal injury. Indirect mechanisms whereby dietary fat promotes carcinogenesis that have been proposed include increased production of prostaglandins that can be tumor promoters (30).

In summary, this study confirms the damaging effects of gastroduodenal secretions on the esophageal mucosa of rats. We have shown that these effects include induction of columnar epithelium, dysplastic change, and even the development of carcinoma, in the absence of MNAN treatment. The combination of refluxed gastroduodenal juice and MNAN resulted in the formation of both squamous cell carcinomas and adenocarcinomas. The induction of esophageal tumors in this model was promoted by a diet with a high polyunsaturated fat content. This animal model has wide potential for the study of factors that contribute to esophageal carcinogenesis and specifically esophageal adenocarcinoma.

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