Physiol. Res. 41: 381 - 386, 1992

Effect of Sialoadenectomy on Stomach Lesions Induced by Indomethacin and Ethanol in Relation to Gastric Vascular Permeability, the Gastrin Level and HCl Secretion in Rats

A. KOHÚT, O. MAHEĽOVÁ, J. MOJŽÍŠ, L. MIROSSAY

Department of Pharmacology, Faculty of Medicine, Šafárik University, Košice

Received February 3, 1992
Accepted April 4, 1992

Summary

Stomach lesions induced by indomethacin (20 $mg.kg^{-1}$ i.p.) and ethanol (1 ml 95 % intragastrically) were studied after a 24 hour fast in rats which had undergone sialoadenectomy. The size of the lesions was correlated with gastric HCl secretion, with gastric vascular permeability (determined from the Evans blue concentration in the stomach tissue after its i.v. administration) and with the serum gastrin level. These parameters were also studied in sialoadenectomized rats and in animals given epidermal growth factor (EGF) (50 $lg.kg^{-1}$). It was found that sialoadenectomy significantly ($p<0.01$) raised the incidence of stomach lesions after the administration of indomethacin and also after ethanol ($p<0.05$). A significant increase in both basal and stimulated HCl secretion was found after sialoadenectomy. Both indomethacin and ethanol also increased gastric vascular permeability in rats not subjected to sialoadenectomy, but sialoadenectomy raised it significantly compared with the non-sialoadenectomized group. The serum gastrin levels fell after sialoadenectomy and the decrease was significant after the subsequent administration of indomethacin or ethanol.The administration of EGF to sialoadenectomized rats lowered the incidence of stomach lesions, inhibited HCl secretion and reduced vascular permeability. The lowered susceptibility of the gastric mucosa to the formation of lesions in sialoadenectomized rats given indomethacin or ethanol can be regarded as the outcome of the uptake of EGF.

Key words

Stomach lesions – Sialoadenectomy – Indomethacin – Ethanol – Vascular Permeability – Epidermal Growth Factor (EGF)

Introduction

The administration of certain extracts from the saliva or the salivary glands, like the stimulation of saliva secretion in mice, for instance, has a trophic effect on the mucosa of the gastrointestinal tract (GIT) (Skinner *et al.* 1984) and stimulates proliferation processes in the GIT (Li *et al.* 1980). Conversely, extirpation of the salivary glands inhibits proliferation processes, reduce DNA synthesis in the gastric mucosa (Bucker and Schemken 1964, Skinner *et al.* 1984) and increases the sensitivity of the gastric mucosa to different noxae (Skinner and Tepperman 1981, Olsen *et al.* 1984). The substance responsible for the above changes, which is produced mainly in the submandibular glands and is transported to the stomach in the saliva, is the epidermal growth factor - EGF (Skinner *et al.* 1984, Joh *et al.* 1987). Evidence that the EGF plays an important role in maintaining the integrity of the gastric mucosa is its protective effect in the presence of various types of injury to the mucosa (Skinner and Tepperman 1981, Turek *et al.* 1981, 1988, 1989, Olsen *et al.* 1984, Imai *et al.* 1987). It is not clear, however, which changes take place in the gastric mucosa in connection with raised sensitivity to noxae after sialoadenectomy, or with the protective effect after the administration of EGF.

The aim of this study was to analyse the changes in HCl secretion, gastric vascular permeability and serum gastrin levels during the development of stomach lesions induced by indomethacin or ethanol in rats subjected to sialoadenectomy, with or without subsequent administration of EGF.

Material and Methods

The experimental animals were male rats (Wistar strain) weighing 260 ± 30 g, fed on a standard (Larsen) diet. Sialoadenectomy was performed under pentobarbital anaesthesia (60 $mg.kg^{-1}$). The submandibular glands were removed bilaterally and the ducts were ligated. These rats and animals subjected to

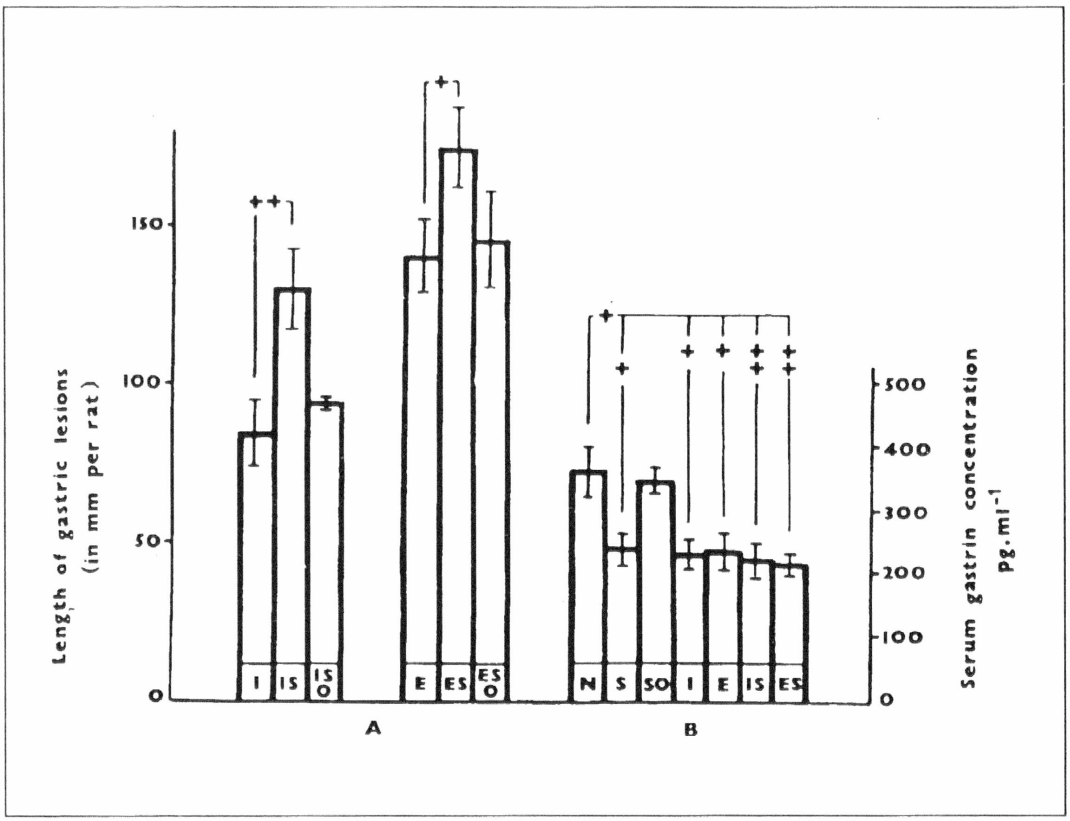

Fig. 1

Gastric lesions (A) induced by indomethacin (I) and ethanol (E) in rats with sialoadenectomy (IS, ES) and after a sham-operation (ISO, ESO). Serum gastrin concentration (B) in intact control rats (N), in rats with sialoadenectomy (S), after a sham-operation (SO), after indomethacin (I), after ethanol (E), and in rats after sialoadenectomy given indomethacin (IS) or ethanol (ES). Mean values ± S.E.M are presented (+ $p<0.05$, + + $p<0.01$).

a sham operation without removal of the submandibular glands were used for further experiments 20 days later. Gastrin was determined before the operation and on the 20th day after it, 4 h after administering indomethacin or 1 h after administering ethanol. Blood was taken from the tail vein and the serum gastrin level was determined by means of commercial RIA gastrin test kits (Institute of Radioecology and Utilization of Nuclear Techniques, Košice).

Stomach lesions were induced 20 days after the operation, following a 24 h fast, by an intraperitoneal injection of indomethacin in a dose of 20 $mg.kg^{-1}$, dissolved in 2 % $NaHCO_3$ solution (Sigma Chemical Co), or by intragastric administration of 1 ml 95 % ethanol. The resultant values were compared with the values in intact rats and in controls with a sham operation. The lesions were evaluated 4 h after injecting indomethacin or 1 h after administering ethanol. The damage was expressed as the length of mucosal lesions in mm. For the determination of gastric HCl, we ligated the pylorus under mild ether anaesthesia, opened the stomach 4 h later, filtered the contents, measured the pH and determined the total amount of HCl by titration with 0.1 M NaOH. EGF (Sigma) was administered intragastrically in a dose of 50 $mg.kg^{-1}$ 30 min before injecting indomethacin or determining HCl, gastrin or Evans blue. The stimulant effect of pentagastrin (Acignost, VEB Berlin-Chemie) (0.25 $mg.kg^{-1}$) was determined by subcutaneous administration 30 min before determining HCl.

For the determination of gastric vascular permeability we used Evans blue (Fluka Chemia AG) and the method described by Udaka *et al.* (1970) and Szabo *et al.* (1985). The Evans blue was injected into the tail vein in a dose of 20 $mg.kg^{-1}$ as a 0.5 % solution and 50 min later, after extraction (Lacy *et al.* 1990), its concentration in the glandular part of the stomach was determined spectrometrically and converted to the amount per 1 g tissue. The animals were killed under ether anaesthesia.

Groups of 10 animals each were used for the experiments. The results were evaluated by Student's t-test for paired values.

Results

It can be seen from Fig. 1 that damage of the gastric mucosa after indomethacin was significantly greater ($p < 0.01$) in rats subjected to sialoadenectomy than in the controls without sialoadenectomy; damage to the mucosa after administration of ethanol was also greater ($p < 0.05$). The serum gastrin levels fell in sialoadenectomized rats ($p<0.05$). The administration of indomethacin and ethanol reduced gastrin levels significantly ($p<0.01$) in non-sialoadenectomized animals compared with normal gastrin values. Significantly lower ($p<0.01$) gastrin levels were observed after indomethacin or ethanol in sialoadenectomized rats than under normal conditions, but they were not significantly different from those with sham operation, while the corresponding values after a sham operation did not differ significantly from those in the groups of intact rats.

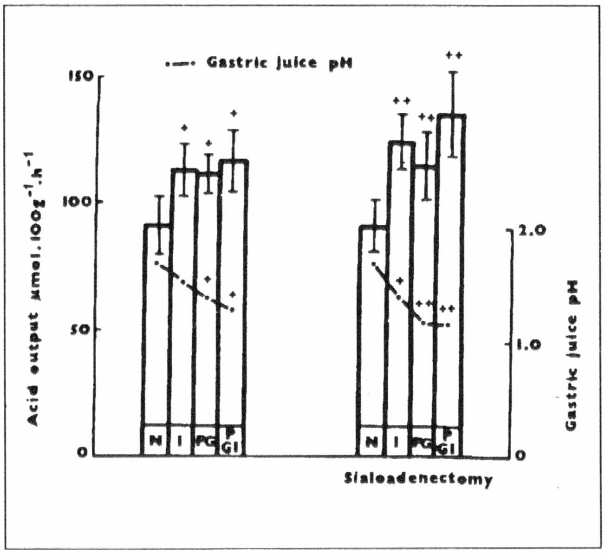

Fig 2

Acid output in intact and sialoadenectomized rats. Control rats (N), after indomethacin (I), after pentagastrine (PG) and after a combination of pentagastrine + indomethacin (PGI). Gastric juice pH in the above mentioned groups. Mean values ± S.E.M. are presented (+ $p<0.05$, ++ $p<0.01$) when compared with the corresponding value in the saline-treated control group.

When studying gastric secretion (Fig. 2), we found that indomethacin stimulated HCl output in sialoadenectomized rats ($p<0.01$), whereas the increase in non-sialoadenectomized rats was less pronounced ($p<0.05$). The pH in the stomach contents of sialoadenectomized rats fell significantly ($p<0.05$) after indomethacin, but no such decrease was observed in rats not subjected to sialoadenectomy. Pentagastrin stimulation was followed by a significant increase in HCl output in sialoadenectomized rats ($p<0.01$) and also when sialoadenectomy was combined with indomethacin and in non-sialoadenectomized animals ($p<0.05$).

Fig. 3 shows that in a study concerning the Evans blue concentration in the stomach tissue (as the criterion of permeability of the gastric blood vessels), we observed, after indomethacin and ethanol, an increase in its concentration compared with normal animals ($p<0.01$), but when these substances were administered to sialoadenectomized rats, its concentration rose significantly more $p<0.01$) than in the group merely given indomethacin or ethanol.

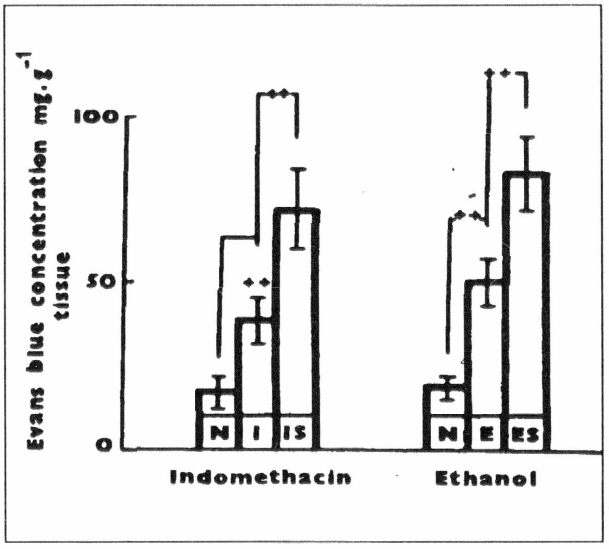

Fig. 3

Evans blue gastric concentration in control rats (N) rats given indomethacin (I), ethanol (E) and in rats after sialoadenectomy given indomethacin (IS) or ethanol (ES). Mean values ± S.E.M. are presented (+ + $p<0.01$).

Fig. 4 illustrates the results after the administration of EGF to rats subjected to sialoadenectomy. EGF reduced the incidence of stomach lesions after indomethacin ($p<0.01$), lowered HCl secretion ($p<0.05$), inhibited pentagastrin stimulation of gastric secretion and lowered the Evans blue concentration ($p<0.01$). It did not alter the serum gastrin levels significantly.

Discussion

Our results have shown that sialoadenectomy increases the sensitivity of the gastric mucosa to both indomethacin and ethanol. This confirms the findings made by other authors, showing that the sensitivity of the rat's gastric mucosa to the action of various other harmful stimuli is raised after sialoadenectomy

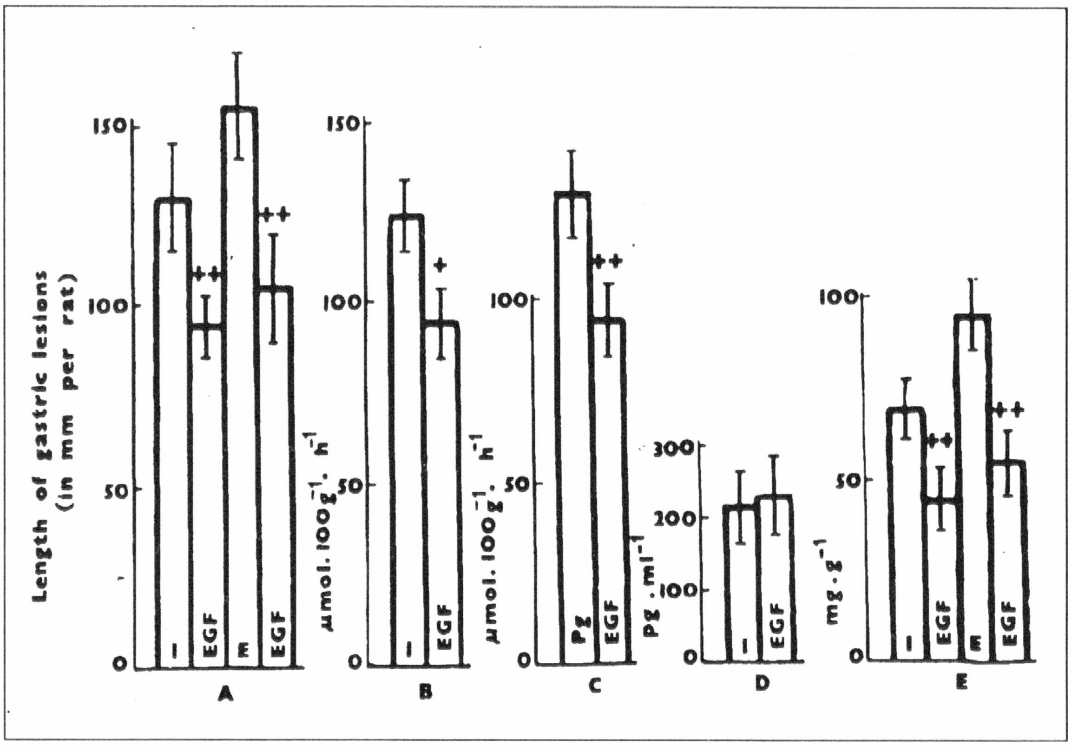

Fig. 4

Gastric lesions (A), basal acid output (B), pentagastrine (PG) stimulated acid output (C), serum gastrin concentration (D), gastric Evans blue concentration (E) in sialoadenectomized rats given indomethacin (I) or ethanol (E), or after combination with epidermal growth factor (EGF). Mean values are presented ± S.E.M. (+ $p<0.05$, + + $p<0.01$) when compared with the corresponding value in the group without EGF.

(Konturek *et al.* 1981, 1990, Poulsen *et al.* 1985). The hypersensitivity of the gastric mucosa after sialoadenectomy could be associated with a reduced production of saliva, whose bicarbonates, under normal conditions, can neutralize gastric HCl (Helm 1989). As we already mentioned, however, the increase in the sensitivity of the gastric mucosa of sialoadenectomized rats to harmful substances is undoubtedly due to the absence of EGF, which is produced mainly in the salivary glands and has a cytoprotective effect. This has been demonstrated by the finding that its administration to sialoadenectomized rats reduced damage of the gastric mucosa and speeded up the healing process (Konturek 1981, Poulsen *et al.* 1985). EGF was also observed to have a cytoprotective effect when administered intragastrically (Poulsen 1988), indicating that its action on the gastric mucosa requuires actual contact with the mucosa.

Considerable interest is evidenced in the mechanism of this cytoprotective effect. Since it is speculated that EGF may have a bearing on gastric HCl secretion, we studied the effect of sialoadenectomy on HCl secretion and the pH. In sialoadenectomized rats given indomethacin, we found a more marked increase in gastric HCl secretion and a greater drop in the pH than in rats not subjected to sialoadenectomy. At the same time we found an increased response to pentagastrin stimulation of gastric HCl secretion.

Some authors found that intragastrically administered EGF did not inhibit HCl secretion (Olsen *et al.* 1986). Others found, in the contrary, that its systemic administration in large doses reduced HCl secretion (Gregory 1975, Konturek *et al.* 1984, Olsen 1988). Our findings in sialoadenectomized rats given indomethacin showed a higher gastric HCl output and a raised response to the administration of pentagastrin. These changes were not observed in sialoadenectomized rats given EGF, however, possibly indicating that the absence of EGF stimulates the gastric HCl output and increases the sensitivity to pentagastrin. Interesting in this context is our further finding that sialoadenectomy reduces the serum gastrin level and that the drop is particularly manifested after the administration of indomethacin or ethanol. The administration of EGF had no effect on the serum gastrin levels in rats subjected to sialoadenectomy. The decrease in gastrin levels could be associated with the increased sensitivity of the gastric mucosa, especially in connection with data demonstrating that the administration of exogenous gastrin has a protective effect, in the presence of damage to the gastric mucosa by certain noxae (Takeuchi and Johnson 1982). Other authors found no changes in the serum gastrin levels of

sialoadenectomized rats (Skinner 1984). We also found that gastric vascular permeability rose markedly after both indomethacin and ethanol. In sialoadenectomized rats, the vascular permeability rose significantly compared with animals whose glands were not removed - possibly in association with the absence of EGF. In the literature, an increase in the permeability of gastric blood vessels is known chiefly in connection with the action of ethanol (Szabo *et al.* 1985, Lacy *et al.* 1990) and of indomethacin, we therefore assume that the increase in vascular permeability could contribute to the mechanisms involved in the hypersensitivity to indomethacin and ethanol.

In conclusion, we can claim that sialoadenectomy increases the susceptibility of the gastric mucosa to damage induced by indomethacin and ethanol. The increase is accompanied by increased production of gastric HCl, by a decrease in the serum gastrin level and an increase in gastric vascular permeability. These changes are probably correlated to extirpation of the salivary glands, i.e. to the absence of EGF, and are a part of the chain leading to increased damage to the gastric mucosa in sialoadenectomized rats. This is borne out by our finding that the administration of EGF rectifies these changes (apart from gastrin levels) in sialoadenectomized rats, in which we also found diminution of gastric mucosal damage caused by the administration of ethanol or indomethacin.

Acknowledgment
We thank Mrs. A. Lacková for technical assistance.

References

BUECKER E.D., SCHEMKEIN I.: Effects of daily subcutaneous injections of nerve growth stimulating protein fractions on mice during postnatal to adult stage. *Ann. N. Y. Acad. Sci.* **118**: 183–205, 1964.

GREGORY H.: Isolation and structure of urogastrone and its relationship to epidermal growth factor. *Nature* **257**: 325–327, 1975.

HELM J.F.: Role of saliva in esophageal function and disease. *Dysphagia* **4**: 76–84, 1989.

IMAI S., ITOH M., KATSUMI K.: Effects of endogenous epidermal growth factor (EGF) on acid secretion, intramucosal mucus and HCl-induced mucosal injury in the stomach in submandibular gland removed rats. *Jpn. J. Gastroenterol.* **84**: 1573–1578, 1987.

JOH T., ITOH M., KATSUMI K.: Immunoreactive epidermal growth factor in mouse digestive organs. Use of a sensitive enzyme immunoassay. *Acta Endocrinol.* **115**: 203–210, 1987.

KONTUREK S.J., BRZOZOWSKI T., DEMBINSKY A., WARZECHA Z., YAMAZAKI I.: Gastric protective and ulcer healing action of epidermal growth factor. In: *Advances in Drug Therapy of Gastrointestinal Ulceration*. GARNER A., WHITTLE B.J.R. (eds), John Wiley and Sons, Chichester, 1989, pp. 269–271.

KONTUREK P.K., BRZOZOWSKI T., KONTUREK S.J., DEMBI SKI A.: Role of epidermal growth factor, prostaglandin, and sulphydryls in stress-induced gastric lesions. *Gastroenterology* **99**: 1607–1615, 1990.

KONTUREK S.J., CIESZKOWSKI J., JAWOREK J., KONTUREK T., BRZOZOWSKI T., GREGORY H.: Effect of epidermal growth factor on gastrointestinal secretions. *Am. J. Physiol.* **246**: G580–G586, 1984.

KONTUREK S.J., DEMBINSKI A., BRZOZOWSKI T., GREGORY H.: Role of epidermal growth factor in healing of chronic gastroduodenal ulcers in rats. *Gastroenterology* **94**: 1300–1307, 1988.

KONTUREK S.J., RADECKI T., BRZOZOWSKI T., PIASTUCKI I., DEMBINSKI A., DEMBINSKA-KIEC A., ZMUDA A., GRYGLEWSKI R., GREGORY H.: Gastric cytoprotection by epidermal growth factor. Role of endogenous prostaglandins and DNA synthesis. *Gastroenterology* **81**: 438–443, 1981.

LACY E.R., LMUND P., TIETGE J.: Effect of misoprostol, cimetidine and ethanol on rat gastric plasma volume and morphology. *J. Clin. Gastroenterol.* **12** (Suppl. 1): S158–S169, 1990.

LI A.K.C., SCHATENKERK M.E., HUFFMAN R.G., ROSS J.S., MALT R.A.: Hypertrophy of submandibular saliva in male mice: trophic response in small intestine. *Gastroenterology* **84**: 949–955, 1980.

OLSEN P.S.: Epidermal growth factor in gastroduodenal mucosal protection. *J. Clin. Gastroenterol.* **10** (Suppl.1): S146–S151, 1988.

OLSEN P.S., POULSEN S.S., KIRKEGAARD P., NEXO E.: Role of submandibular saliva and epidermal growth factor in gastric cytoprotection. *Gastroenterology* **87**: 103–108, 1984.

OLSEN P.S., POULSEN S.S., THERKELSEN K., NEXO E.: Effect of sialoadenectomy and synthetic human urogastrone on healing of chronic gastric ulcers in rat. *Gut* **27**: 1443–1449, 1986.

POULSEN S.S., OLSEN P.S., KIRKEGARD P.: Healing of cysteamine-induced duodenal ulcers in the rat. *Dig. Dis. Sci.* **30**: 161–167, 1985.

SKINNER K.A., SOPER B.D., TEPPERMAN B.L.: Effect of sialoadenectomy and salivary gland extracts on gastrointestinal mucosal growth and gastrin levels in the rat. *J. Physiol.* **351**: 1–12, 1984.

SKINNER K.A., TEPPERMAN B.L.: Influence of desalivation on acid secretory output and gastric mucosal integrity in the rat. *Gastroenterology* **81**: 335–339, 1981.

SZABO S., TRIER J.S., BROWN A., SCHNOOR J.: Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology* **88:** 228–236, 1985.

TAKEUCHI K., JOHNSON L.R.: Effect of cell proliferation and loss on aspirin-induced gastric damage in the rat. *Am. J. Physiol.* **243:** G463–G468, 1982.

TAKEUCHI K., OKADA M., EBARA S., OSANO H.: Increased microvascular permeability and lesion formation during gastric hypermotility caused by indomethacin in the rat. *J. Clin. Gastroenterology* **12** (Suppl.1): S76–S84, 1990.

UDAKA K., TAKEUCHI N., MOVAT H.Z.: Simple method for quantitation of enhanced vascular permeability. *Proc. Soc. Exp. Biol. Med.* **133:** 1384–1387, 1970.

Reprint Requests

Prof. A. Kohút, Department of Pharmacology, Faculty of Medicine, Šafárik University, CS-040 66 Košice, Tr. SNP 1.