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Effect of 3,4-Toluenediamine on Output From *In Situ* Rat Brunner's Glands Pouches(38941)

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(Introduced by A. R. Imondi)

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Selye (1) has recently reported that 3,4-toluenediamine, administered either parenterally or orally, causes duodenal ulceration in fed, unrestrained rats. Discrete, often perforated, lesions were typically observed just distal to the pylorus. This is the region of the mammalian proximal duodenum which contains Brunner's glands, and is generally resistant to the corrosive action of gastric juice. Brunner's glands secrete an alkaline mucoid material and Florey and Harding (2) have suggested that malfunction of these glands may result in the formation of duodenal ulcers. Florey *et al.* (3) found that pig duodenum containing Brunner's glands exhibited greater resistance to acid-induced ulceration than other regions of the small intestine which lack these glands. This observation supports the conclusion that secretions from this area have a protective function. Therefore, it may be that 3,4-toluenediamine, by reducing the secretion of protective fluids from the proximal duodenum, causes duodenal ulceration in the rat. Data presented in this communication indicate that 3,4-toluenediamine inhibits secretion from Brunner's glands pouches in the rat and suggest a correlation between the duodenal ulcerogenic and duodenal antisecretory activities of this compound.

Materials and Methods. Fed, female Sprague–Dawley strain rats were used in all experiments.

The subcutaneous ulcerogenic activity of single doses of 3,4-toluenediamine ("3,4-TDA," 97% pure, Aldrich Chemical Co.) was determined in animals weighing 200–300g 3,4-TDA, dissolved in 10% ethanol by warming with tap water, was administered at 62.5, 125, 250, 300, 350. 400, 450, and 500 mg/kg; concentrations were adjusted to standardize injection volume at 1.0 ml/250 g body wt. The animals were stunned and exsanguinated 24 hr after drug administration and the stomachs and duodena were removed and examined for mucosal damage.

A technique similar to that described by Florey and Harding (2) for collecting secretions from rabbit duodenal pouches was employed to collect secretions from *in situ* Brunner's glands pouches in rats weighing 240–290 g. After positioning each rat in a flat bottomed, wedge-shaped wire cage fashioned from $\frac{1}{2}$ in. mesh hardware cloth, the animals were anesthetized with pentobarbital (Nembutal®; 45 mg/kg ip). When anesthetized, each animal was removed from its cage, and the abdomen was clipped and opened along the linea alba. The pylorus and proximal duodenum were exposed and the pylorus was ligated. A second 4-0 silk ligature was passed around the duodenum 4–5 mm distal to the pyloric ligature; histological examination indicated that Brunner's glands are located in the first 3–4 mm of the proximal duodenum in these rats. A 1-mm stab wound was made on the anti-mesenteric border approximately 5 mm distal to the second ligature with a hypodermic needle. The heat flared end of a 9-cm PE 190 polyethylene tubing catheter was passed into the duodenum and then forward just under the second ligature. The second ligature was then tied securely around the duodenum and catheter, thus isolating the 4–5 mm duodenal segment. The intestine was returned to the abdominal cavity, the cannula was positioned in the incision so that it would drain freely, and the incision was closed. Drug (125, 350, or 500 mg/kg) or carrier (10%

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TABLE I. ULCEROGENIC EFFECTS OF SINGLE SUBCUTANEOUS DOSES OF 3,4-TOLUENEDIAMINE IN UNANESTHETIZED FED RATS.

Dose (mg/kg)	No. of animals	Mortality (%)	% Total no. of animals with lesions[a] in Glandular stomach	% Total no. of animals with lesions[a] in Proximal duodenum
62.5	7	0	0	0
125	16	0	0	19
250	16	0	19	50
300	10	0	10	70
350	20	5	30	80
400	10	30	10	80
450	10	50	100	20
500	15	94	87	27

[a] Erosions or ulcers.

ETOH) was administered subcutaneously; injection volume did not exceed 1.5 cc per animal. The animal was returned to its wedge-shaped cage and the cage was positioned on wooden rails raised 5 in. above the bench top. With the caged rat in the prone position, the catheter was allowed to drain into a vented B-D Plastipak tuberculin syringe which was positioned horizontally beneath the restraining cage. Secretions were collected for 3 hr. At the end of this period, the abdominal incision was opened, residual pouch secretions were squeezed into the cannula, and the cannula was removed. Material remaining in the cannula was then aspirated into the syringe, all the air bubbles were expelled, and the volume was recorded. Student's nonpaired *t* test was used to determine the significance of differences in volume output.

Results. Subcutaneous ulcerogenic activity of 3,4-TDA. Results of experiments to determine the incidence of gastric and duodenal damage in rats following administration of single subcutaneous doses of 3,4-TDA are summarized in Table I. Duodenal lesions were found only in the proximal duodenum immediately distal to the gastroduodenal junction. The incidence of duodenal damage increased progressively with dose up to 80%, which was observed with both the 350 and 400 mg/kg doses, and then dropped to 20% at 450 mg/kg and 27% at 500 mg/kg. The incidence of gastric glandular mucosal damage ranged from 0 to 30% with doses from 62.5 to 400 mg/kg; it increased to 100% at 450 mg/kg and was 87% at 500 mg/kg. No deaths were observed within 24 hr following administration of 3,4-TDA at doses from 62.5 to 300 mg/kg. However, the mortality increased progressively from 5% at 350 mg/kg to 94% at 500 mg/kg. Perforated lesions were not observed following subcutaneous administration of single doses of this material.

Effect of 3,4-TDA on output from rat Brunner's glands pouches. Three doses of 3,4-TDA, 125 mg/kg which caused minimal gastroduodenal damage, 350 mg/kg which caused maximal duodenal damage and minimal mortality, and 500 mg/kg which caused a low incidence of duodenal and a high incidence of gastric damage, were evaluated for effects on volume output from Brunner's glands pouches in pentobarbital anesthetized rats. Each dose of 3,4-TDA tested significantly reduced the output of fluid from Brunner's glands pouches (Table II). However, the 350 and 500 mg/kg doses caused a greater decrease in secretion than did the 125 mg/kg dose. A 25% reduction in output was observed following administration of 125 mg/kg, while 350 and 500 mg/kg reduced output by 61 and 57%, respectively.

Discussion. Our results support Selye's (1) data indicating that 3,4-TDA will cause duodenal damage in fed, unrestrained rats. Discrete, nonperforated duodenal lesions were observed immediately distal to the gastroduodenal junction in rats 24 hr following subcutaneous administration of single doses of 3,4-TDA ranging from 125 to 500 mg/kg. The optimal single duodenal ulcerogenic dose of this compound in our hands, i.e., that dose causing a low mortality and a maximal incidence of duodenal damage within 24 hr, was 350 mg/kg. Additionally, gastric glandular mucosal damage was observed following administration of 3,4-TDA, although the incidence was relatively low (0–30%) with doses up to 400 mg/kg. The two highest doses of 3,4-TDA tested, 450 and 500 mg/kg, both caused a higher incidence of gastric damage (100 and 87 vs 30%) and a lower incidence of duodenal damage (20 and 27% vs 80%) than was observed with the optimal 350 mg/kg dose.

TABLE II. EFFECT OF 3,4-TDA ON SECRETION FROM BRUNNER'S GLANDS POUCHES IN FED PENTOBARBITAL ANESTHETIZED RATS.

Dose (mg/kg sc)	Volume[a]		P
	Control	Treated	
125	0.24 ± 0.08 (29)	0.18 ± 0.06 (29)	0.002
350	0.28 ± 0.12 (18)	0.11 ± 0.04 (20)	0.001
500	0.28 ± 0.11 (11)	0.12 ± 0.04 (9)	0.001

[a] Mean ml/3 hr ± SD; number of rats in parentheses.

This shift from duodenal to gastric damage cannot be explained at this time. The observation that the mortality at 450 mg/kg was approximately half that observed at 500 mg/kg suggests that the shift in ulcer incidence most probably is not an expression of the toxicity of this material.

Only a few compounds are reported to inhibit output from Brunner's glands pouches. Hartiala *et al.* (4) found that cinchophen, an agent which causes ulceration in the region of the pyloric and Brunner's glands in the dog, reduces volume, base, and mucin output from Brunner's glands pouches in this animal. Aspirin, a known gastrointestinal irritant, has recently been reported to reduce meal stimulated output from dog duodenal pouches containing Brunner's glands (6). Our results indicate that 3,4-TDA, a gastroduodenal ulcerogen in the rat, is an inhibitor of secretion from *in situ* rat Brunner's glands pouches. The ability to reduce the secretion of protective neutralizing fluids from the proximal duodenum may be a common characteristic of agents which disrupt the mucosal integrity of the upper gastrointestinal tract.

The mechanism by which 3,4-TDA causes duodenal ulceration in rats is unknown. Data reported in this communication indicate that the duodenal ulcerogenic activity of this compound may be related to its ability to reduce the secretion of protective fluids in the duodenum. Both the minimal (125 mg/kg) and optimal (350 mg/kg) duodenal ulcerogenic doses significantly decreased secretion from rat Brunner's glands pouches. However, the reduction observed following the higher, definitely duodenal damaging dose was more than twice that observed following the lower, minimally ulcerogenic dose of this drug. Interestingly, 500 mg/kg 3,4-TDA, a dose which caused a low incidence of duodenal damage and a high incidence of gastric damage, also significantly reduced output from Brunner's glands pouches. The magnitude of the effect following this dose was similar to that observed following 350 mg/kg, the dose which caused a high incidence of duodenal damage. Apparently processes necessary for duodenal ulcer formation, but against gastric ulcer formation, are also inhibited by high doses of this compound. Further experimentation is necessary to completely define the gastrointestinal actions of this compound.

Florey and Harding (2) described tying off sections of proximal duodenum to determine if they could expect to collect Brunner's glands secretions from cannulated segments in the rabbit, guinea pig, cat, and rat. They reported that only very small and apparently nonusable quantities of juice collected in the noncannulated loops of cat or rat intestine. Subsequently, and possibly because of this report, rats have been neglected as an animal for studying output from Brunner's glands pouches. The model described in the present communication was developed after observing that *in situ* loops of rat duodenum containing Brunner's glands were distended with fluid within 3 hr after placement of the ligatures. The data reported here indicate that measurable quantities of fluid can indeed be collected from cannulated *in situ* pouches of proximal duodenum in pentobarbital anesthetized rats and indicate the utility of this model for determining the antisecretory effect of compounds on output of protective fluids from this region of the gastrointestinal tract.

Summary. Duodenal and gastric glandular mucosal damage have been observed 24 hr following single subcutaneous doses of 3,4-TDA in fed, unrestrained rats. 3,4-TDA significantly reduced secretion from *in situ* Brunner's glands pouches in pentobarbital anesthetized rats. The reduction in volume

output with a definitely duodenal ulcerogenic dose of this compound was more than twice that observed with a minimally ulcerogenic dose, suggesting a correlation between the duodenal ulcerogenic and duodenal antisecretory activities of this compound. The animal model described in this communication should facilitate experimentation to establish the inhibitory effect of compounds on the output of protective fluids from the proximal duodenum.

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