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HISTOLOGY OF EXPERIMENTAL STRESS ULCER: THE EFFECT OF CIMETIDINE ON ADRENALINE-INDUCED GASTRIC LESIONS IN THE RABBIT

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Summary.—The gastric mucosal injury produced by i.p. instillation of adrenaline in the rabbit was examined and assessed histologically. Mucosal lesions were classified by microscopy into two types bearing distinct histological features. In Type A oedema only was seen and in Type B erosion and/or haemorrhage were added. Statistical analysis revealed that mucosal lesions were related to adrenaline dose. Cimetidine was ineffective in protecting against the stress-related gastric lesion in the present rabbit model. Biochemical studies demonstrated that severe lesions were associated with depleted mucosal histamine.

INTRAPERITONEAL INJECTION of adrenaline produces acute gastrointestinal erosion in several laboratory animals (Penner and Bernheim, 1939). In the rabbit, the morphology and the distribution of the observed gastric lesions are similar to those seen in stress states in man (Beraha *et al.*, 1980). However, in the latter communication, a poor correlation was found between macroscopically observed lesions on the one hand, and histological changes on the other. The discrepancy might be due to artefacts induced in the process of clearing the stomach of food residue and thus could be avoided by gentle handling of the stomach.

The present study describes: (a) some histological features of the stress lesion produced in the rabbit; (b) histological assessment of the association between lesions and dose of adrenaline; (c) the effect of cimetidine, an H_2-receptor antagonist, on the production of lesions; and (d) the correlation between the severity of the lesion and the change in histamine concentration in the gastric mucosa.

MATERIALS AND METHODS

Histological changes.—Details of a model for the production of stress ulcer in the rabbit by the i.p. instillation of adrenaline have been described previously (Beraha *et al.*, 1980). Briefly, New Zealand white rabbits weighing 3–4 kg were given water alone *ad libitum* for 24 h before each experiment. The animals were anaesthetized with pentobarbitone (Sagatal, May & Baker Ltd, 15 mg/kg i.v.). For each rabbit, the trachea was intubated and anaesthesia maintained throughout the experiment with fluothane (1%), nitrous oxide (1 l/min) and oxygen (1·5 l/min). An abdominal catheter was inserted for the i.p. injection of adrenaline.

Twenty-five rabbits were divided into 5 groups, which were given adrenaline according to different schedules (Table I). The order of the experiments followed a randomized Latin square design. Three hours after the first instillation of adrenaline, a lethal dose of pentobarbitone was given to the rabbit. The stomach was removed *en bloc*, opened, pinned flat on a cork board and fixed in 10% neutral buffered formalin for at least 24 h. The stomach was then cleared of food residue by gently brushing with a fine, artist's brush, recoloured in absolute alcohol and photographed at a standard distance with a scale. A freehand sketch was then made marking the distribution of the macroscopically identified lesions. All the observed

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lesions and one section of apparently unaffected tissue were processed for histological examination. For stomachs without macroscopic lesions, up to 9 randomly chosen specimens (for histological examination) were collected from sites evenly distributed over the fundus and corpus.

Effect of cimetidine.—Preparation and experimental procedures were identical to those described above. Twenty-five rabbits were divided into 4 groups, which were given cimetidine i.v. *via* an ear vein, according to different schedules (Table II), 10 min before the first i.p. injection of adrenaline. A total of 1·2 mg/kg body wt of adrenaline was instilled in 2 doses of 0·6 mg/kg at 30-min intervals. Three hours after the first adrenaline instillation the stomach was opened and inspected macroscopically. Three corpus mucosa samples, each weighing about 50 mg and with no apparent lesions, were taken for histamine determination based on the method of Lorenz, Benesch and Barth (1970). The stomach was then fixed in formalin for histological examination.

The corpus mucosal histamine concentration in 6 control rabbits which had not been treated with cimetidine or adrenaline was also determined.

Biochemical assays and histological examinations were performed by 2 investigators independently and the results were compared only at the conclusion of the experiments.

Statistical analysis.—Statistic comparisons were made using the Mann–Whitney U test and the Fisher test. P values $< 0{\cdot}05$ are recorded as statistically significant. Measurement of association were made using Spearman's rank correlation coefficient.

RESULTS

Gastric mucosal injuries

Macroscopic inspection revealed no significant gastric mucosal changes in any of the control rabbits. In the adrenaline-treated rabbits, gross mucosal lesions were observed. The appearance of the lesions ranged from oedema to petechial haemorrhages or haemorrhagic erosions.

Histological examination confirmed that mucosal injuries were observed only in the sections of specimens obtained from the adrenaline-treated rabbits. The lesions identified were oedema, erosion (loss of epithelium with distinctive pallor and hyalinization of oxyntic cells (or haemorrhage (Figs 1, 2, 3 and 4). All the observed mucosal changes were confined to the lamina propria. Occasional eosinophil clusters were seen in the surface mucus and in the lamina propria, but too infrequently for analysis. There was no evidence of thrombosis, inflammation or disease deeper than the muscularis mucosae.

In many specimens microscopy revealed unsuspected small haemorrhages in oedematous mucosa or mild oedema in macroscopically normal mucosa; hence histological assessment provided more sensitive discrimination of the lesions than macroscopic inspection. For comparison, all the histologically identified lesions were classified into 2 types:

Type A.—Oedema alone (Fig. 2); and

Type B.—Erosions and/or haemorrhage (Figs 3 and 4).

The numbers of specimens collected from each stomach showing the 2 types of lesions were listed in Table I.

Group A.—None of the 4 rabbits in the control group developed either Type A or Type B gastric lesions.

Group B.—Two of the 5 rabbits given 0·3 mg/kg dose of adrenaline developed Type A lesions. No Type B lesions were observed. The proportion of rabbits with gastric lesions in this group did not differ significantly from that in the control group.

Group C.—All the 5 rabbits given 0·6 mg/kg dose of adrenaline developed either Type A or Type B lesions. Thus the overall proportion of rabbits with gastric lesions was significantly greater than in the control group. However, the proportion of rabbits with either individual type of lesion did not differ from the controls.

Group D.—Five of the 6 rabbits given twp 0·6 mg/kg doses of adrenaline developed Type A lesions. In two rabbits both Type A and Type B lesions were identified. If Type A and B lesions are analysed separately, the proportion of animals developing Type A was significantly higher than in controls. This was not true of Type B lesions alone.

Group E.—All the 5 rabbits given three 0·6 mg/kg doses of adrenaline developed the more severe Type B lesions. Four

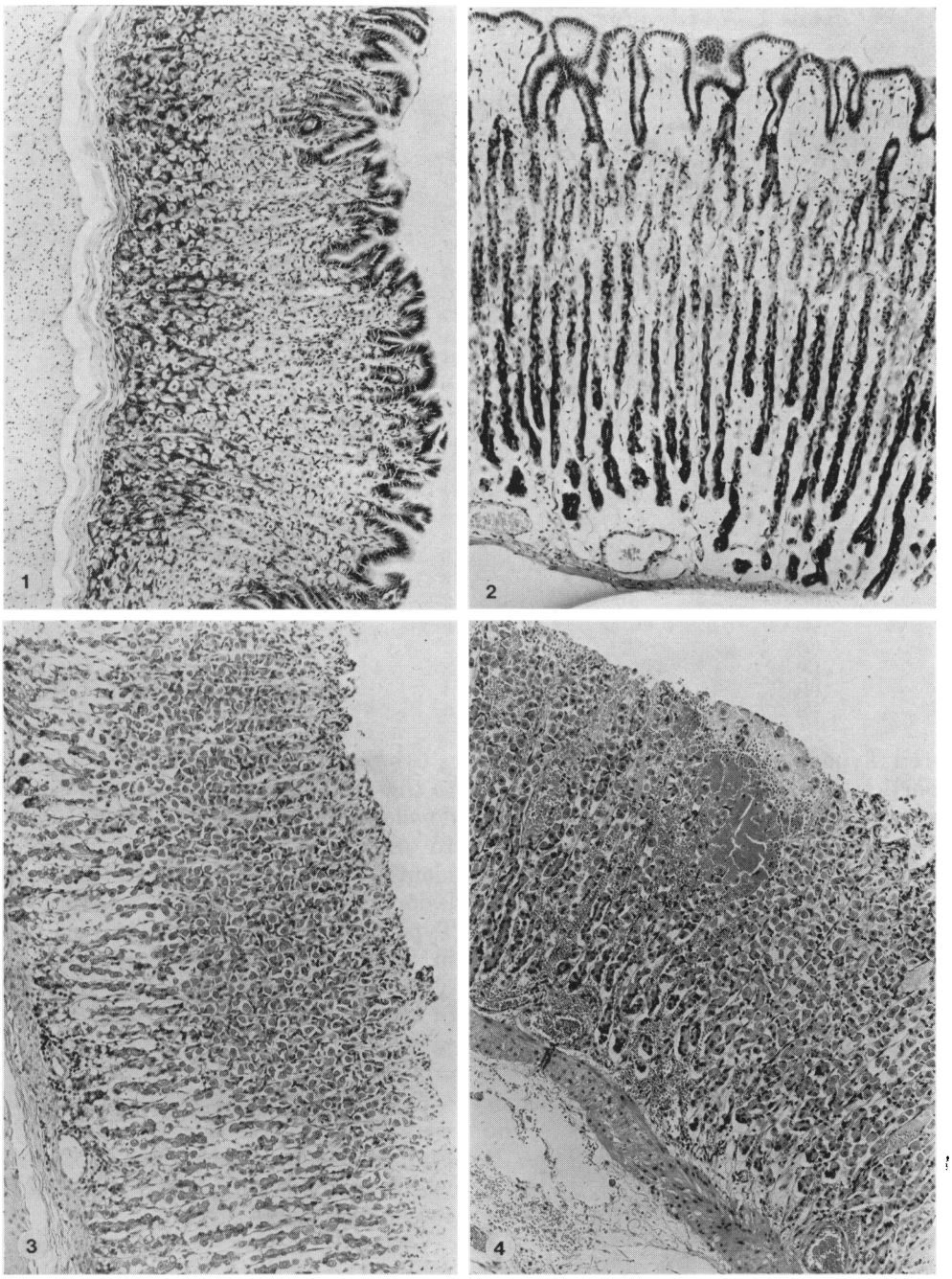

FIG. 1.—Normal corpus mucosa. H. & E. × 75.
FIG. 2.—Oedema; Type A lesion. H. & E. × 75.
FIG. 3.—Erosion; Type B. H. & E. × 75.
FIG. 4.—Erosion and haemorrhage; Type B. H. & E. × 75.

TABLE I.—*Adrenaline and histological assessment of gastric lesions*

		Adrenaline administration regimens			Histological assessment of lesions					
Group	Rabbit No.	Dose (mg/kg)	Time injected (min)	Total dose (mg/kg)	No. of Type A lesions	(Mean)	No. of Type B lesions	(Mean)	Total Type (A + B) lesions	(Mean)
A (Control)	(1)	0	–	0	0		0		0	
	(2)				0		0		0	
	(3)				0	(0)	0	(0)	0	(0)
	(4)				0		0		0	
B	(1)	0·3	0	0·3	0		0		0	
	(2)				0		0		0	
	(3)				0	(1·0)	0	(0)	0	(1·0)
	(4)				3		0		3	
	(5)				2		0		2	
C	(1)	0·6	0	0·6	1		0		1	
	(2)				0		2		2	
	(3)				5	(1·8)	2	(0·8)	5	(2·6)
	(4)				0		2		2	
	(5)				3		0		3	
D	(1)	0·6	0, 30	1·2	2		0		2	
	(2)				0		0		0	
	(3)				4	(2·8)	0	(1·7)	4	
	(4)				5		0		5	(4·5)
	(5)				2		3		5	
	(6)				2		7		9	
E	(1)	0·6	0, 30, 60	1·8	0		7		7	
	(2)				2		2		4	
	(3)				8	(2·8)	2	(4·0)	10	(6·8)
	(4)				1		7		8	
	(5)				3		2		5	

showed Type A lesions as well. Thus the proportion of rabbits with lesions in this group was significantly higher than in the control group. Each type of lesion developed in significantly more rabbits than in the control group ($P < 0{\cdot}02$).

The dose of adrenaline administered to the rabbits in each group was associated closely with the mean number of lesions developed (Fig. 5). These were a close correlation between the adrenaline dose and (a) the mean number of Type A lesions ($r = 0{\cdot}93$), (b) the mean number of Type B lesions ($r = 0{\cdot}97$), as well as (c) the mean number of Type A and Type B lesions ($r = 1{\cdot}00$).

Effect of cimetidine pretreatment

In this experiment all rabbits were given two 0·6 mg/kg doses of adrenaline. Ten minutes before the first injection of adrenaline, cimetidine was injected i.v. into the rabbits. The numbers of histologically identified lesions developed in each rabbit are listed in Table II.

Group CA.—No cimetidine was given to the rabbits in this group. All 6 rabbits developed either Type A or Type B lesions. In one rabbit, both types of lesions were identified. The proportion of rabbits with lesions and the numbers of Type A or Type B lesions developed in each rabbit in this group were not statistically significantly different from those in Group D of the previous experiment in which the rabbit was given the identical dose of adrenaline.

Group CB.—3mg/kg doses of cimetidine were given to the rabbits in this group. Five of the 6 rabbits developed lesions, which were not statistically different from the control group, CA.

Group CC.—9mg/kg doses of cimetidine were given to the 7 rabbits in this group. Six developed lesions. Thus this group was not significantly different when compared to the control group.

Group CD.—18mg/kg doses of cimetidine were given to the 6 rabbits in this group. All developed gastric lesions. No signifi-

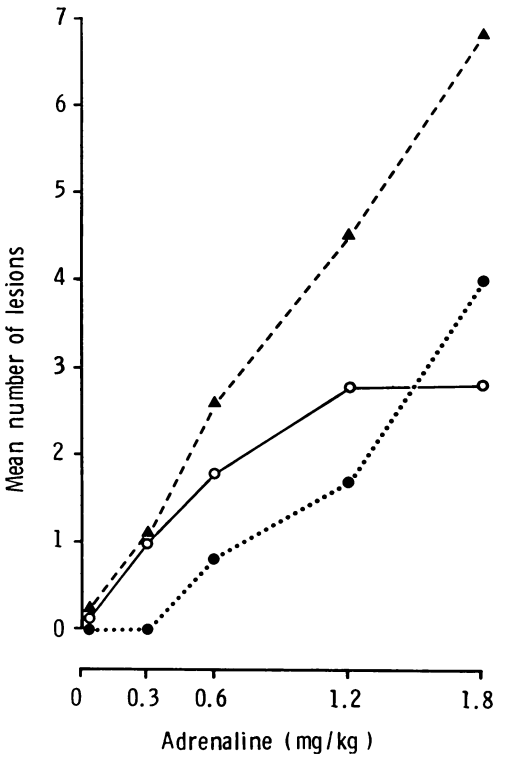

FIG. 5.—Mean number of lesions produced in response to adrenaline. ○—○, Type A lesions; ●—●, Type B lesions; ▲—▲, Type A and B lesions.

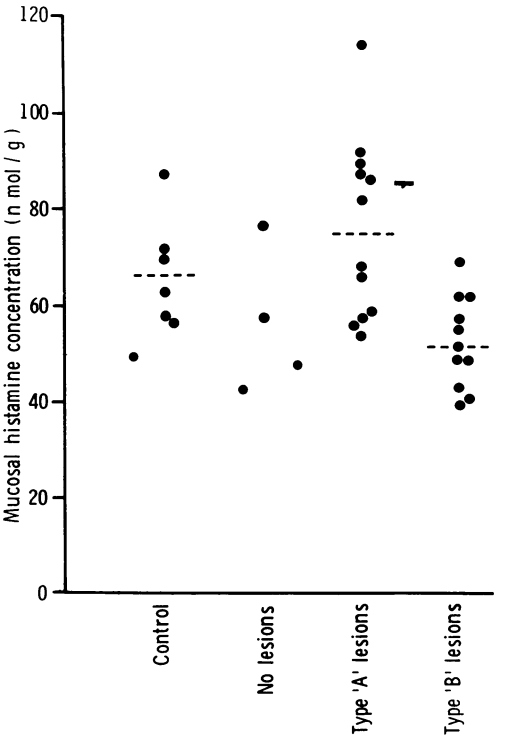

FIG. 6.—Mucosal histamine and gastric lesion. Lesions were induced by i.p. adrenaline (1·2 mg/kg) with and without i.v. cimetidine pretreatment. Control rabbits were given only i.p. saline.

cant difference was found between this group and Group CA.

No association was observed between the dose of cimetidine and the mean number of lesions developed in each group whether Type A and B lesions were analysed separately or together.

Mucosal histamine

The gastric mucosal histamine concentration in each rabbit at the end of the experiment is listed in Table II. No significant difference was found between the mean values of the groups with and without cimetidine pretreatment. However, when the data were assessed independently of groups, as in Fig. 6, it was found that severe (Type B) lesions were associated with a significantly lower mucosal histamine ($P < 0{\cdot}02$). No significant difference was found in the mucosal histamine concentration between Type A lesion stomachs and controls.

DISCUSSION

Intraperitoneal injection of adrenaline produced stress lesions in the rabbit. The gastric mucosal changes observed in the present study were oedema, erosion and haemorrhage, as previously reported (Beraha *et al.*, 1980).

Microscopy revealed that the earliest mucosal disorder induced by adrenaline was oedema. As the adrenaline dose increased, acute superficial lesions developed and eventually resulted in a more widespread mucosal disorder, manifested by multiple haemorrhages and erosions. The histological features were similar to the acute gastric erosions and haemor-

TABLE II.—*Cimetidine and adrenaline induced gastric lesions (2 doses adrenaline 0·6 mg/kg at 0 min and 30 min)*

Group	Rabbit No.	Cimetidine pretreatment dose i.v. (mg/kg)	Histological assessment of lesions: No. of Type A lesions	(Mean)	No. of Type B lesions	(Mean)	No. of A & B lesions	(Mean)	Mucosal histamine nmol/g wet
CA	(1)	0	2		0		2		57·6
	(2)		0		1		1		48·9
	(3)		1	(1·1)	0	(0·7)	1	(1·8)	82·0
	(4)		3		0		3		66·3
	(5)		1		2		3		39·6
	(6)		0		1		1		69·0
CB	(1)	3	1		0		1		85·8
	(2)		0		0		0		77·1
	(3)		0	(1·1)	2	(0·3)	2	(1·5)	57·0
	(4)		2		0		2		53·8
	(5)		3		0		3		87·5
	(6)		1		0		1		58·7
CC	(1)	9	0		0		0		58·7
	(2)		0		2		2		48·9
	(3)		1		1		2		42·9
	(4)		1	(0·7)	0	(0·6)	1	(1·3)	67·9
	(5)		1		0		1		91·8
	(6)		2		0		2		56·5
	(7)		0		1		1		51·6
CD	(1)	18	1		2		3		55·4
	(2)		1		3		4		61·9
	(3)		0		2		2		61·9
	(4)		1	(1·0)	1	(1·3)	2	(2·3)	40·7
	(5)		2		0		2		114·1
	(6)		1		0		1		89·1

rhagic gastritis seen in man, as illustrated in Whitehead's *Mucosal Biopsy of the Gastrointestinal Tract* (Whitehead, 1980).

In man, bleeding stress ulcer occurred after sudden massive trauma (Kunzman, 1970). The earliest lesions are seen as petechial haemorrhages in the gastric mucosa. Microscopic examination reveals a pinpoint loss of surface epithelium. Thereafter, the petechiae develops into discrete ulcers. Very low levels of intraluminal gastric acid are found at the time ulcers develop. The histological changes are comparable with those seen in the present rabbit model.

In the present study, microscopic examination facilitated the study of the dose–response correlation between adrenaline and the gastric mucosal disorder, because microscopy often showed lesions not visible to the naked eye. As the prominent features of the gastric mucosal damage seen in microscopy were oedema, erosion and haemorrhage, all instances of identified mucosal damage were classified into 2 main types: Type A, the mild lesion, oedema alone; and Type B, the severe lesions consisting of erosion and/or haemorrhage with or without oedema. Histological assessment revealed a close correlation between mucosal damage and dose of adrenaline. Thus oedema occurred alone at the low dose of adrenaline, but in combination with erosion and haemorrhage at higher doses.

Gastric acid secretion, gastric mucosal blood flow and the gastric mucosal barrier are perhaps the 3 major pathogenic determinants of stress-related gastric mucosal erosive injury. Sethbhakdi, Pfeiffer and Roth (1970) described a model producing acute gastric lesions in the rabbit by i.p. adrenaline, 0·4 mg/kg, after ligation of the pylorus. The ulcers were acid-dependent. Antacids greatly reduced the severity of ulceration. After truncal vagotomy ulcers could not be produced. Davenport and Barr (1973) demonstrated

that ischaemia produced by s.c. injection of vasopressin or i.v. infusion of noradrenaline did not break the gastric barrier in the dog. However, Skillman *et al.* (1970) reported that acute gastric mucosal injury was produced in the rabbit by a short period of haemorrhagic shock. Bile easily damages the gastric barrier and has been shown to produce acute gastric mucosal ulceration (Ritchie and Shearburn, 1976). The damage is manifested by an increase back diffusion of hydrogen ions as well as an intraluminal influx of sodium and potassium ions.

H_2-receptor antagonists significantly reduce stress-related mucosal damage in a number of animal models (Strauss, Stein and Wise, 1978; Levine, Sirinek and Pruitt, 1979). The mode of action is to decrease the intraluminal acid concentration during stress and to facilitate blood flow in the gastric mucosa (Levine *et al.*, 1979). However, the receptor blocker did not decrease hydrogen ion back-diffusion in dogs, either in the resting or stressed state (Gurll, Zinner and Callahan, 1977).

Recently there has been growing caution about the use of cimetidine therapy in patients with acute gastric disorders. Priebe, Skillman and Bushnell (1980) described a controlled clinical trial which demonstrated that cimetidine was inferior to antacid in preventing acute gastrointestinal bleeding in critically ill patients. It is postulated that the secretory state of the gastric mucosa is an additional pathogenic determinant of gastric erosive injury. The bicarbonate in the secretion can act as a buffer agent and protects the gastric mucosal wall. Cimetidine impairs the secretory state of the mucosa and decreases the intracellular buffering capacity against acid erosion. Thus cimetidine is not as effective as antacid, which does not interfere with the secretion mechanism of the gastric mucosa in preventing acute gastrointestinal bleeding.

In the present study, by microscopic examination and assessment of gastric mucosal damage, cimetidine was found to give no significant protection against the stress lesions induced by a total dose of 1·2 mg/kg adrenaline in the rabbits. The dose of adrenaline was chosen partly because it had a submaximal effect in producing lesions and would thus be more suitable for detection of the therapeutic effect of cimetidine, and partly because larger doses are poorly tolerated (Beraha *et al.*, 1980). The cimetidine doses tested were 1, 3 and 6 times that given to patients with peptic ulcer. Further studies are in progress to monitor the intraluminal acid concentration and the gastric blood flow during stress and after the administration of therapeutic agents.

The present study confirms the previous finding that severe mucosal lesions are associated with depleted mucosal histamine. The significance is not fully understood at present and further exploration of this phenomenon is clearly indicated. Histamine, acting as a gastric secretagogue and as a vasoactive agent, has been proposed to have a pathophysiological role in the breakdown of the gastric mucosa barrier (Drapanas *et al.*, 1971).

In conclusion, histological examination revealed that the pattern of production of gastric lesions and the histology of the lesions in the present rabbit model are similar to those of lesions found in stress states in man. Experimental lesions were related to dose of adrenaline, and were not prevented by cimetidine. Mucosal histamine may have a role in the aetiology of the mucosal lesion. In view of the known similarity in microvascular anatomy between rabbit and man, further study of the aetiological mechanism and the efficacy of therapeutic agents may be of direct clinical relevance.

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