

Production of Gastric Hemorrhage in Rats by Multiple Stresses. (28555)

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Interest in the production of experimental gastric ulcers has increased as the use of rapid and uncomplicated stress methods has become more widespread. Restraint, starvation, environmental changes and drugs have all been shown to produce gastric ulcers in rats in 24 hours(1-3). Studies on combinations of these factors indicated stressors synergized in their effects, since exposure of rats to restraint and starvation produced a higher incidence of gastric lesions than either stress alone(4), while restraint and cold stress were synergistic as stressors in production of hypothermia in rats(5). This report concerns a study of the effect of a combination of stressors (cold and restraint in starved rats), in an attempt to produce gastric lesions rapidly, and a study of the mechanism by which the stomach was damaged.

Methods. Male Holtzman rats, 120-150 g, were starved for 24 hours and then placed in restraint without prior anesthesia. The animals were restrained either by stapling them into a piece of window screen or by putting them into a piece of perforated conduit tubing that was plugged with corks at each end. Immediately after restraint, the animal was placed in a cold room (5-6°C) and, at the end of the test time, removed from restraint and killed with ether or pentobarbital. The stomach was removed, inflated with air, then cut along the greater curvature, and examined for the appearance of hemorrhage. Any hemorrhagic area over 1 mm was considered as positive; neither the number nor the severity of the hemorrhage was considered. In the drug studies, all agents were injected intraperitoneally (I.P.), unless otherwise noted, 30 minutes before restraint. Gastric secretion studies were done in the pylorus-ligated rats as described by Shay *et al.*(6) and in the chronic fistula rat as described by Brodie *et al.*(7). Volume of gastric content was measured in a graduated test tube and free acidity and total acidity were measured by titration of gastric juice with 0.01 N NaOH to pH 3.5 and

8.5, respectively, on a Beckman Zeromatic pH meter. In the study of the relationship of gastric blood supply and gastric lesions, all gastric arteries and veins were ligated under ether anesthesia, with care taken not to disturb the vagus nerve. Colonic temperatures were obtained with an Ellab electric thermometer.

Results. *Time-response curve for cold + restraint-induced gastric hemorrhage.* The production of gastric hemorrhage at varying time periods by cold alone, restraint alone and the combination is given in Table I. The combination of cold and restraint in the starved rat produced a synergistic effect at intervals of 30 and 60 minutes. Average incidence of hemorrhage in one hour (cold + restraint) in 40 control groups of 10 rats per group was 93%.

The lesions produced by cold + restraint were purely gastric hemorrhage, and the gross appearance of the stomach after one hour of cold + restraint is illustrated in Fig. 1. The massive hemorrhage was easily visible through the serosa when the stomach was removed from the animal, but careful wiping of the surface with a gauze sponge removed the hemorrhage and no mucosal damage was evident, either grossly or microscopically.

An attempt was made to produce mucosal damage by repeatedly restraining the animals in the cold twice a day for 60-minute periods for 4 days (total of 8 trials). On autopsy, after the eighth restraint period there was a lesion incidence of 100% but mucosal damage was still not evident. A study of recovery from the lesions (10 rats/group) indicated that, at 4 hours after recovery from

TABLE I. Gastric Hemorrhage in Rats. Time-response curve.

Duration of stress (min)	No. rats with hemorrhage		
	Cold	Restraint	Cold + restraint
15	2/20	5/20	8/20
30	2/20	5/20	14/20
60	4/20	6/20	18/20

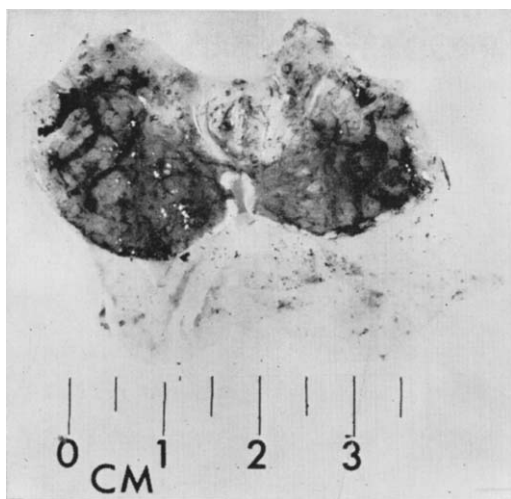


FIG. 1. Gastric hemorrhage in rat stomach produced by 1 hour cold + restraint stress.

the stress, 90% of the animals had gastric hemorrhage, at 8 hours the incidence of hemorrhage was 70%, while at 24 hours after cold + restraint, only 10% of the rats had gastric lesions.

Pharmacological analysis of cold + restraint-induced gastric hemorrhage. The effects of selected drugs on lesion incidence of stress-induced hemorrhage are shown in Ta-

ble II; anticholinergics, ganglionic blocking agents, certain CNS depressants and epinephrine blocked the stress-induced hemorrhage, while adrenergic blocking agents, anti-serotonin and antihistaminic drugs had little effect.

Effect of cold + restraint on gastric secretion in the rat. Cold + restraint stress significantly reduced the volume of gastric secretion in pylorus-ligated rats, but did not reduce gastric acidity (Table III). An attempt was made to duplicate the lesions obtained after 60 minutes cold + restraint by ligation of gastric blood vessels. Ligating the gastric blood vessels for one hour without any other stressors produced 100% (10/10) incidence of gastric hemorrhage which grossly appeared to be identical to the lesions produced by cold + restraint. However, when gastric secretion was collected in pylorus-ligated animals with gastric blood vessels ligated, there was a marked decrease in both volume and gastric acidity while cold + restraint only reduced gastric volume. Cold + restraint markedly reduced both volume and gastric acidity in chronic fistula rats (Table III), and the depression lasted for 2 hours

TABLE II. Effect of Drugs on Cold + Restraint-Induced Hemorrhage in Rats.

Drug	Dose range (mg/kg I.P.)	ED ₅₀ * (mg/kg I.P.)
<i>Anticholinergics</i>		
Scopolamine methyl bromide	.05- .20	.08
Atropine methyl nitrate	.05- .20	.13
" sulfate	.20- 1.00	.46
<i>Ganglionic blocking agents</i>		
Mecamylamine hydrochloride	5.0 - 20.0	8.2
<i>Adrenergic neuron blocking agent</i>		
Bretylium tosylate	8.0 - 30.0	Not active
<i>Adrenergic blocking agents</i>		
SY-21	10.0 - 40.0	" "
<i>Central nervous system depressants</i>		
Chlorpromazine hydrochloride	4.0 - 16.0	5.2
Trifluoperazine hydrochloride	30.0 - 60.0	42.5
Meprobamate	100.0 - 500.0	350.0
Morphine sulfate	5.0 - 20.0	Not active
Pentobarbital sodium	20.0 - 45.0	" "
<i>Antihistamine</i>		
Pyrilamine maleate	30.0 - 60.0	" "
<i>Antiserotonin</i>		
Lysergic acid diethylamide	.1 - 10.0	" "
<i>Adrenergic agents</i>		
Epinephrine hydrochloride (subcutaneously)	.25- 1.0	.48

* ED₅₀ calculated from visually fitted linear function of the log probit plot of data; 10 rats were used at each dose level tested. Doses of drugs are given as salt weights.

TABLE III. Effect of Stress on Gastric Secretion in the Rat.

Test	No. rats	Volume (ml)	Free acid (mEq/l)	Total acid (mEq/l)
<i>Pylorus ligation—1 hr</i>				
Control	14	2.5	77.5	102.7
Cold + restraint	15	.6*	88.0	119.5*
Control	10	2.6	85.7	113.2
Ligation of gastric blood vessels	9	.9*	9.3*	63.8*
<i>Chronic gastric fistula rat</i>				
1-hr collection	No. rats	Volume (ml)	Free acid output† (μEq)	
Control	9	1.5	56.5	
Cold + restraint	9	.6†	18.0†	

* Significantly different from control at .05 level as determined by Mann-Whitney U test(8).

† Group differences significantly different at .05 level as determined by Wilcoxon matched pairs signed ranks test(8).

‡ Free and total acid concentrations are not given; 5/9 rats had gastric samples too small to analyze.

when the animals were removed from the stress situation.

Effect of cold + restraint on rat body temperature. When rats were removed from 60 minutes of cold and restraint stress, they appeared sedated, and many of the animals showed loss of the righting reflex, which was suggestive of a hypothermic state. The effect of both cold and restraint and of the combination of cold + restraint on colonic temperature in the rat is shown in Fig. 2. Restraint or cold alone for one hour produced a slight drop in temperature, while cold + restraint produced a precipitous fall in body temperature. It required 3 hours for the cold + restraint rats to return to basal temperature levels.

Discussion. The combination of cold and restraint stress produced marked hypothermia and gastric hemorrhage in the rats in one hour. In contrast to the adaptation to

the hypothermic response on repeated exposure to the stress combination(5), there was no adaptation to the production of gastric bleeding; however, it was not possible to produce mucosal damage by the repeated stressing.

Superficially, the lesions produced by cold + restraint resemble those of restraint alone in gross appearance and the effect of drugs on the lesion incidence in both tests was similar(4). However, the studies on gastric secretion indicate that increased gastric acidity is a factor in restraint lesions(7), while non-acid, possibly vascular, factors appear to be involved in cold + restraint gastric hemorrhage, since in the chronic fistula rat, both volume and gastric acidity were reduced during the stress period and derangement of the gastric blood supply in the pylorus-ligated rat produced massive gastric hemorrhage in one hour without increase in gastric acid.

Since drugs, such as anticholinergics, ganglionic blockers and certain central nervous system depressants, reduce lesion incidence, it is possible that there is a centrally mediated cholinergic mechanism involved in production of the hemorrhage. The data on gastric secretion suggest that the vascular factors are more important than acid factors in production of gastric hemorrhage, since volume and acidity of gastric juice are reduced by the stress. However, it remains to be determined if this stress procedure produces gastric hemorrhage by an alteration in gastric blood flow and/or changes in vascular

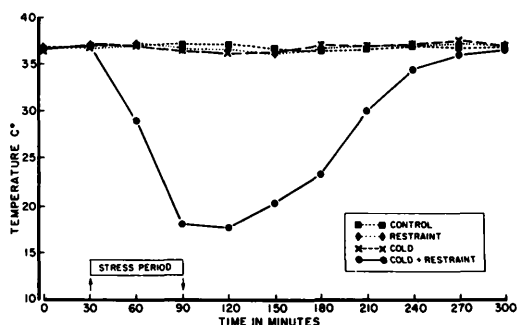


FIG. 2. Effect of stress on rat colonic temperature. Cold + restraint for 60 min. produced marked hypothermia in the rat (5 rats/group).

tone of the gastric blood vessels at the time that gastric secretion is depressed.

Summary. Gastric hemorrhage was produced in 93% of rats subjected to cold + restraint stress for 60 minutes. Mucosal damage was not evident and could not be induced by repeated stress periods. Anticholinergics, ganglionic blocking agents, certain central nervous system depressants and epinephrine reduced the incidence of hemorrhage. Gastric volume was reduced by cold + restraint in the pylorus-ligated and chronic gastric fistula rat, while gastric acidity was only reduced during stress in the fistula rats. The cold + restraint produced a marked hypothermic response and body temperature was depressed for 3 hours following the stress.

1. Selye, H., *The Physiology and Pathology of Exposure to Stress*, Acta, Inc., Montreal, 1950.
2. Brodie, D. A., Hanson, H. M., *Gastroenterology*, 1960, v38, 353.
3. Segal, H. L., *Am. J. Med.*, 1960, v29, 780.
4. Hanson, H. M., Brodie, D. A., *J. Appl. Physiol.*, 1960, v15, 291.
5. Bartlett, R. G., Jr., Bohr, V. C., Helmendach, R. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1954, v86, 395.
6. Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., Siplet, H., *Gastroenterology*, 1945, v5, 43.
7. Brodie, D. A., Marshall, R. W., Moreno, O. M., *Am. J. Physiol.*, 1962, v202, 812.
8. Siegel, S., *Nonparametric Statistics for the Behavioral Sciences*, McGraw Hill Book Co., Inc., New York, 1956.

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Antibody Response to Brucella Antigen in Patients with Rheumatoid Arthritis.* (28556)

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Among the theories attempting to explain the presence of rheumatoid factor (RF) in the serum of patients with peripheral rheumatoid arthritis is one in which RF is considered to be a macroglobulin (19S) antibody to the patient's 7S gamma globulin(1). Relevant to this concept are reports that patients with rheumatic disease are hyperreactive in their humoral antibody response(2,3). Recently Meiselas *et al.*, noted, in rheumatoid subjects, a significant hyperreactivity to Brucella antigen which evokes a macroglobulin antibody response(4).

Because such observations have implications for the significance of rheumatoid factor, a study was made: (a) to determine whether the antibody response to Brucella antigen in patients with rheumatoid arthritis

would prove to be higher than in those with osteoarthritis, a condition in which there is chronic articular disease while rheumatoid factor and other serological abnormalities are characteristically absent, and (b) to ascertain whether a relationship exists in these patients between the titer of the Brucella antibody and the titer of rheumatoid factor.

Method. The subjects were 44 ambulatory patients from the Arthritis Clinic of Univ. of California Medical Center. After physical examination and appropriate testing, they were classified according to the diagnostic criteria of the American Rheumatism Assn. Thirty-three of the patients had rheumatoid arthritis; 11 had osteoarthritis. After a sample of serum was collected, each patient was inoculated subcutaneously with 0.5 ml of Brucella vaccine (Parke-Davis) containing *Brucella abortus* and *melitensis*, 2,000 million killed bacteria per ml. Two weeks later

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