PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 415-417 (1977)

The Effect of Stress on the Gastric Mucosal Barrier in Rats[1] (39683)

J. LAWRENCE WERTHER AND ISADORE HOROWITZ

Division of Gastroenterology of the Department of Medicine, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029

The gastric mucosa presents a barrier to the back-diffusion of hydrogen ions (H^+) from the gastric lumen (1, 2). Such substances as bile (3), weak acids (4), and acetazolamide (2) may alter and increase the permeability of this barrier resulting in increased back-diffusion of H^+ ions and subsequent tissue damage. Critically ill man has increased H^+ back-diffusion across the gastric mucosa (5). Experimental shock in dogs produces gastric erosions, but an increase in permeability to H^+ has not been demonstrated (6). Rats subjected to restraint-stress develop ulceration of the stomach (7, 8), and their gastric secretory rates are reported as markedly decreased (9); however, increased acid back-diffusion has not been demonstrated (10). In order to reevaluate the role of H^+ back-diffusion in restrained rats, we modified previously described techniques for small animals by adapting methods used in this laboratory in dogs and in intact man (3, 11). The findings presented herein indicate that increased rates of H^+ back-diffusion occur across the gastric mucosa of stressed rats.

Methods. Eighteen male Sprague–Dawley rats weighing 250–300 g were used. Nine control animals were fasted for 48 hr with full access to water in raised mesh-bottom cages to obtain maximal gastric emptying. Nine rats to be restrained were fasted for 24 hr. They were restrained in wire cages for 24 hr and two supplements of 2.5 ml of 0.9% sodium chloride were given subcutaneously to each rat. Both control and restrained rats were anesthetized with intraperitoneal pentobarbital (4 mg/100 g body weight) and maintained under anesthesia by additional small quantities of pentobarbital intraperitoneal as needed. Nonvagotomized pylorus-ligated stomachs were prepared with cannulae in the esophagus (12). The stomach was washed gently with 2 ml of isotonic solution containing HCl 55 meq/liter and NaCl 100 meq/liter. The test solution was identical to the wash solution but contained phenol red (10 mg/liter) as indicator.

Acid instillation was performed similarly to our previous canine instillation experiments (3) with slight modifications. For greater accuracy all solutions were weighed. A 10-ml syringe containing 8 ml of test solution was attached to the cannula in the esophagus. Four ml of fluid were instilled into the stomach and then removed and mixed with the remaining 4 ml to determine residual volume. This was repeated twice. Four milliliters were used as the instillation test solution; the remaining 4 ml was weighed and used as an initial aliquot. At the end of 1 hr, the gastric contents were removed and weighed. If the animal's condition was satisfactory, another instillation was performed. No more than three instillations per rat were done because of the risk of ulcerations in such preparations after 4–6 hr (13). Preliminary experiments had revealed no ulcerations developed in 3 hr-pylorus-ligated rats. Hydrogen concentration was determined by titration to pH 7.0 using a Radiometer electrometer titrator. Sodium and potassium concentration were measured by flame photometer using lithium internal standards. Phenol red was determined by spectrophotometer (14).

Ionic fluxes, residual volumes, hydrogen secreted and hydrogen neutralized were determined using assumptions previously described by us and others utilizing the "two component hypothesis" (5, 10, 11). Statistical significance of means was determined by Student's *t* test.

Ulcerations were graded on a scale of 1 to 4 by gross visual inspection: 1, one ulceration; 2, two to three ulcerations; 3, three to eight ulcerations; 4, more than eight ulcerations.

Results. H^+ and sodium fluxes are pre-

[1] Supported by NCI grant No. 1 R01 CA15541-01.

ISSN 0037-9727

sented in Table I. Restrained rats had a highly significant ($P < 0.001$) increase in the rate of hydrogen ion back-diffusion across the gastric mucosa. Restrained animals did not secrete significantly less acid than control animals. The amount of H^+ neutralized was similar in both groups. Sodium fluxes were similar in control and restrained rats.

Twenty-four hours of restraint produced varying amounts of gastric erosions in all nine restrained animals; no lesions were observed in control animals.

There was a high correlation ($r = +0.88$) between the severity of erosions and the magnitude of H^+ ion back-diffusion in the restrained rats (Table II).

Discussion. Our results indicate that restraint stress in rats increases the rate of back-diffusion of H^+ across the gastric mucosa. This increase correlated well with the severity of the induced mucosal ulceration. This increased permeability of the gastric mucosa may explain some of the published results on hypoacidity of restrained rats (9).

The acid-instillation technique for measuring ionic fluxes across the gastric mucosa in small animals utilized in this study has several advantages over previous techniques (12) in that it allows one to measure residual volume and both parietal cell and nonparietal secretion. In one previously utilized method (12), initial ion values are assumed to be those of the stock solution. Our initial ion values are determined by an aliquot obtained after washing the gastric mucosa with test solution. The drop in H^+ and phenol red concentration from the stock solution to the initial aliquot at times was as high as 20%. Furthermore, instillate volumes of 8 ml in 300-g rats were used by others (12); we found that such a volume produced tension in the gastric wall with intragastric pressure of 24 cm/H_2O. Our volume of 4 ml more nearly approached normal distention of the stomach as measured by other (11).

The method of Overholt (16) requires incisions through the lumen, with repeated insertions of tubing through this opening. They (16) did not include correction for nonparietal secretion nor an estimate of acid secreted by the stomach during the experiment. Their values for H^+ back-diffusion in control sham vagotomized rats are greater than that observed by others when corrections for secretion are made (10, 12). This could also be related to their use of a greater concentration of H^+ (100 meq/liter) in the test solution.

Gerety and Guth (10) were able to overcome the above drawbacks but introduced another. Their instillation solution contained HCl at 100 meq/liter. However, concentrations of 80 meq/liter of HCl result in induced damage to the gastric mucosa in rats (12) and gastric hemorrhage in 70% of vagotomized rats (15). The use of this concentration of HCl might explain the large H^+ backdiffusion in these workers' control group (−66.0 μeq/hr), which is even greater than the back-diffusion (−47.3) in our restrained rats.

In our experiments, sodium fluxes in stressed animals were not significantly different from controls. This may have resulted from the relatively high concentration of NaCl (100 meq/liter) in our test solution. Moody (17) also found little correlation between H^+ flux and Na^+ flux in experimental hypovolemic shock in dogs.

Recent work has emphasized impaired microcirculation of the stomach as a prime factor in stress-induced ulceration (18). Restraint induced stress in rats results in areas of ischemia of the glandular mucosa. Normal intact mucosa continued to secrete undiluted acid which was postulated to autodigest the susceptable ischemic area (18).

TABLE I. Ionic Fluxes during Acid Instillations in Control Rats and Restrained Rats.

	H+ Back-diffusion (μeq/hr)	Hydrogen secreted (μeq/hr)	Hydrogen neutralized (μeq/hr)	Sodium flux (μeq/hr)
Control $n = 22$	9.2 ± 11.0	33.8 ± 5.7	11.7 ± 1.5	68.1 ± 10.5
Restrained $n = 25$	−47.3 ± 7.2[a,c]	20.0 ± 6.7[b]	16.2 ± 2.0[b]	97.0 ± 12.5[b]

[a] $P < 0.001$.
[b] $P < 0.2$
[c] Minus sign signifies flux of ion from lumen to mucosa.

TABLE II. MAGNITUDE OF H+ IN RESTRAINED RATS WITH DIFFERENT DEGREES OF ULCERATION.

Severity of ulceration	Number	H+ Back-diffusion
Erythema	5	$-10.65 \pm 9.73^{a,b}$
1	3	-42.79 ± 7.96^{c}
2	6	-40.36 ± 7.87^{d}
3	6	-70.74 ± 17.19
4	5	-66.10 ± 17.13

[a] Erythema vs 1 or 2. $P < 0.005$.
[b] Erythema vs 3 or 4. $P < 0.001$.
[c] One vs 3 or 4. $P < 0.01$.
[d] Two vs 3 or 4. $P < 0.01$.

Hase (18, 19) showed that stressed rats with ulcerations had greater acid secretion than stressed rats without ulceration. Back-diffusion was not determined in these studies. We show no difference in rates of acid secretion. The importance of intraluminal acid in the pathogenesis of restraint ulcers is further demonstrated by the observation that vagotomy and anticholinergics decrease the severity of stress induced ulcers (9, 20). Our studies indicate that back-diffusion of secreted acid may be involved in the restraint induced mucosal ulcerations.

Summary. To obtain evidence about the controversial role of the gastric mucosal barrier to back-diffusion of hydrogen ions in the pathogenesis of gastric ulcerations during restraint-stress, rats were subjected to restraint stress for 24 hr and acid instillations performed in their pylorus ligated stomachs with a technique adapted from previously described methods for acid instillation in canine dog pouches. Since the rat's pylorus ligated stomach does not tolerate solutions with high hydrogen ion (H^+) concentrations, test solutions containing H^+ of 55 meq/liter were used. Hydrogen ion secretion did not decrease in these stressed rats, but there was a marked increase in H^+ back-diffusion across the gastric mucosa which was well correlated with the gross visual damage. Abnormal gastric mucosal permeability to hydrogen ion may play a pathogenic role in restraint-stress induced ulceration.

1. Code, C. F., Higgins, J. A., Moll, J. C., and Orvis, A. L., J. Physiol. **166,** 110 (1963).
2. Werther, J. L., Hollander, F., and Altamirano, M., Amer. J. Physiol. **209,** 127 (1965).
3. Dyck, W. P., Werther, J. L., Rudick, J., and Janowitz, H. D., Gastroenterology **56,** 488 (1969).
4. Davenport, M. W., Gastroenterology **46,** 245 (1964).
5. Skillman, J. J., Gould, S. A., Chung, M. B., and Silen, W., Ann. Surg. **172,** 564 (1970).
6. Moody, F. G., and Aldrette, J. S., Surgery **70,** 154 (1971).
7. Selge, H., Stehle, R. L., and Collip, J. B., Canad. M. A. J. **34,** 339 (1936).
8. Brodie, D. A., and Valitski, L. S., Proc. Soc. Exp. Biol. **113,** 998 (1963).
9. Menguy, R., Amer. J. Dig. Dis. **5,** 911 (1960).
10. Gerety, D. S., and Guth, P. H., Amer. J. Dig. Dis. **17,** 1012 (1972).
11. Chapman, M. A., Werther, J. L., and Janowitz, H. D., Gastroenterology **55,** 344 (1968).
12. Lindner, A. E., Amer. J. Physiol. **207(3),** 606 (1964).
13. Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Greenstein, M., and Siplet, H., Gastroenterology **5,** 43 (1945).
14. Hunt, J. N., and Knox, T. M., J. Physiol. (London) **163,** 34 (1962).
15. Brodie, D. A., and Chase, B. J., Gastroenterology **56,** 206 (1969).
16. Overholt, B. F., Brodie, D. A., and Chase, B. J., Gastroenterology **56,** 651 (1969).
17. Moody, F. G., and Adrete, J. S., Surgery **70,** 154 (1971).
18. Hase, T., and Moss, B. J., Gastroenterology **65,** 224 (1973).
19. Hase, T., Anderson, P. R., and Mehlman, B., Dig. Dis. **20(5),** 443 (1975).
20. Hanson, H. M., and Brodie, D. A., J. Appl. Physiol. **15,** 291 (1960).

Received October 17, 1976. P.S.E.B.M. 1977, Vol. 154.