PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **147**, 280–283 (1974)

Increased Phenolsulfonphthalein Absorption Following Disruption of the Gastric Mucosal Barrier by Urea (38326)

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(Introduced by E. D. Jacobson)

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Increased absorption of hydrogen ions by the gastric mucosa has been implicated in the etiology of gastritis and benign gastric ulcer (1, 2). However, the mechanisms responsible for enhanced "back diffusion" of hydrogen ion are obscure. Under resting conditions, the oxyntic gland area of the normal gastric mucosa of man and the dog limits the diffusion of hydrogen ion out of the gastric lumen into the mucosa and the diffusion of sodium and potassium in the opposite direction. This barrier can be altered by the topical application of synthetic detergents, bile and urea (3), thereby rendering the mucosa incapable of maintaining a high intraluminal concentration of H^+ and permitting increased influx of Na^+, K^+ and water. These agents have in common the ability to alter the organization of lipoprotein and to break hydrogen bonds (4). It has been postulated, therefore, that disruption of the mucosal barrier is the result of destruction of the tight junctions between surface epithelial cells leading to increased mucosal permeability (5). Validation of this hypothesis requires as a first step the demonstration of absorption by the gastric mucosa of a lipid insoluble substance to which it is not normally permeable because of the size of the probing molecule. Accordingly, in the present study, mucosal absorption of phenolsulfonphthalein (PSP; M. W. 354) was determined in dogs before and after the topical application of 4 *M* urea. In addition, associated changes in the net fluxes of H_2O, H^+, K^+ and Cl^- and the net and bidirectional fluxes of Na^+ were assayed in order to document the fact that disruption of the mucosal barrier had actually occurred.

Materials and Methods. A. Animal preparation. Seventeen adult mongrel dogs, weighing between 16 and 23 kg, were used in this study. In each dog, at a preliminary operation, a vagally denervated pouch of full thickness gastric wall was constructed from the greater curvature of the corpus, pedicled on the short gastric vessels and drained via a six-inch Roux-en-Y limb of jejunum. Previous work indicated that this maneuver is necessary to prevent the development of patchy areas of gastric atrophy in the pouch. The studies described below were undertaken three to four weeks later because pouches drained in this way are histologically normal at this time (6).

Following a 24-hr fast each animal was lightly anesthetized with sodium pentobarbital. The abdomen was opened and the pouch was cannulated with minimal manipulation through a small distal enterotomy. An umbilical tape was tied around the cannula at the level of the gastrojejunostomy to insure a water-tight seal. Prior to the instillation of the test solutions, each pouch was lavaged with several 30 ml aliquots of 80 m*M* saline solution at 37°. Residual air and wash solution were evacuated from the pouch, and the abdomen was closed leaving the cannula exteriorized.

B. PSP absorption. In ten animals a precise volume of test solution containing 80 m*M* HCl, 80 m*M* NaCl, 3–5 μCi of ^{24}Na and 1.25 μM of PSP (sample A) was instilled into the pouch.

Thirty minutes later the solution was removed (sample B) and the pouch was washed with saline solution (sample C). The volume of each sample was determined. In five dogs, 30 ml of 4 *M* urea was then instilled into each pouch for 30 min. Following removal of the urea solution, the pouch was washed with saline solution and a second 30 min PSP flux using the same test

solution was performed. The remaining five animals served as controls in that no urea was instilled between PSP fluxes.

Each sample was analyzed for (1) relative radioactivity using a δ well counter and (2) PSP concentration; after buffering to pH 10 with 100*g*/dl bicarbonate solution, the absorbency of each sample was determined using a spectrophotometer (wave length 550 nm). The concentration of PSP in each sample was calculated from a previously constructed linear graph which related absorbency to serial dilutions of the test solution.

The following equations were used to calculate the net absorption of PSP:

$$\text{PSP Instilled} = ([\text{PSP}]_A) \times \text{Volume A},$$

$$\text{PSP Recovered} = \frac{\text{Volume C } ([\text{PSP}]_C) \times \text{CPM}_C/\text{mlC}}{\text{CPM}_B/\text{mlB}} + \text{Volume B } ([\text{PSP}]_B),$$

$$\text{PSP Absorbed} = \text{PSP instilled} - \text{PSP recovered}.$$

C. Flux studies. In seven dogs a precise volume of 37° test solution containing 80 m*M* HCl, 80 m*M* NaCl and 3–5 μCi of ^{24}Na (sample A) was introduced into the pouch. A 10 ml aliquot (sample A) was withdrawn and the study period was begun. Thirty min later the pouch was emptied as completely as possible (sample C), and the pouch was washed with saline solution (sample D). The volumes of samples C and D were determined.

Thirty milliliters of 4 *M* urea were then instilled into each pouch. Thirty min later the urea was removed, and a second flux was performed in the same manner as before. Each sample was analyzed for (1) total acid by titration of an aliquot to pH 7 with 0.01 *N* NaOH, using phenol red as an indicator, (2) concentration of sodium and potassium using a flame photometer, and (3) relative radioactivity using a δ well counter.

The following equations, based on the work of Visscher *et al.* (7), were used to determine the net flux of water, hydrogen ion and potassium, and the net and bidirectional flux rates of sodium.

A. Net water flux (Q_{H_2O})

$$\text{Initial pouch volume (IPV)} = \text{volume A} \times \frac{\text{CPM}_A/\text{mlA}}{\text{CPM}_B/\text{mlB}} - \text{volume B},$$

$$\text{Final pouch volume (FPV)} = \text{volume C} + \frac{\text{CPM}_D/\text{mlD} \times \text{volume D},}{\text{CPM}_C/\text{mlC}}$$

$$Q_{H_2O} = \text{FPV} - \text{IPV}.$$

B. Net ionic flux (Q_i) for H^+ and K^+.

$$Qi = \text{final [i]} \times \text{FPV} - \text{initial [i]} \times \text{IPV}.$$

C. Bidirectional fluxes of sodium.

$$\text{Sodium outflux}^1 = \frac{\text{CPM}_B\text{mlB} \times \text{IPV} - \text{CPM}_C/\text{mlC} \times \text{FPV}}{[^{24}\text{Na}] \text{ Avg.}}$$

$$\text{Sodium influx}^2 = \text{Net sodium flux} + \text{sodium outflux}.$$

The usual assumptions concerning the use of radioisotopes to measure unidirectional ionic flux rates were made. In addition, it was assumed that all residual radioactivity was recovered from the pouch during the final wash. This latter assumption is valid because the relative radioactivity of subsequent washings was indistinguishable from background relative radioactivity.

D. Determination of mucosal area. At the conclusion of each experiment the animal was sacrificed by injecting saturated potassium chloride solution intravenously. The pouch was harvested, and the mucosa was separated from the muscle layers by sharp dissection. The mucosa was flattened on drawing paper, and the mucosal area was determined using a planimeter.

The mean (± SE) was determined for each parameter, and significance was evaluated by using a Student's *t* test.

Results. PSP absorption by canine gastric corporeal mucosa before and after urea instillation is summarized in Table I. Prior to urea instillation an average of 0.13 ± 0.04 μ*M* of PSP/30 min/100 cm^2 mucosal area disappeared from the pouches. Following urea, however, an average of 0.63 ± 0.06 μ*M* 30 min/100 cm^2 mucosal area was absorbed. Analysis of this data comparing PSP absorption from each pouch (rather than per 100 cm^2 mucosal area) before and after urea showed the same magnitude of change indicating that shrinkage of the gastric mucosa after urea was not responsible for the increased absorption. No significant alteration in PSP ab-

[1] Flux from lumen to blood.

[2] Flux from blood to lumen.

TABLE I. PSP Absorption Across Canine Gastric Mucosa Before and After Topical Urea.

Study group	No. dogs	PSP absorption (mean ± SE μM/30 min/ 100 cm² mucosal area)
A. Controls	10	0.13 ± 0.04
B. After 30 min of anesthesia	5	0.17 ± 0.09
C. After 30 min of urea	5	0.63 ± 0.09*

* $P < 0.01$ with respect to either A or B.

sorption was noted as a result of 30 min of anesthesia alone.

Associated changes in ionic and water flux rates are indicated in Table II. Prior to urea instillation, the pouches gained an average of 1.6 ± 0.4 ml of water, 241 μEq Na^+, 15 μEq of K^+ and 184 μEq of Cl^-/30 min/100 cm² mucosal area. Net H^+ loss from the pouch was −156 μEq/30 min/100 cm² mucosal area. Following urea, net luminal water, Na^+ and K^+ gain and net H^+ loss increased markedly. Net Cl^- flux was also diminished significantly.

Discussion. Disruption of the gastric mucosal barrier by urea was first described by Davenport who postulated that the observed ionic flux changes might be a consequence of increased mucosal permeability (3). In a passive system, ions diffuse through semipermeable membranes in accordance with concentration and electrical gradients (8). The gastric mucosal membrane potential, which is normally negative in the lumen with respect to blood, approaches zero following urea instillation (5). The change in membrane potential might account, at least in part, for the observed increase in diffusion of H^+ out of the pouch, but it cannot explain the enhanced flux of Na^+ and K^+ into the pouch since this movement is against the change in electrical gradient. Another possible explanation for the altered movements of H^+, Na^+ and Cl^- after urea would be impaired active ion transport (9). Decreased active transport of H^+ and Cl^- from serosa to mucosa and Na^+ from mucosa to serosa would yield results indistinguishable from those of this study. A more appropriate interpretation of the observed flux changes is that urea instillation results in increased mucosal permeability, permitting increased cationic diffusion in accordance with concentration gradients alone (for Na^+ and K^+, from blood to lumen and for H^+, from lumen to blood). The present study was undertaken to assess the latter interpretation by determining whether or not urea altered the mucosal absorption of PSP.

Mathematical analysis of experimental data suggests that lipid insoluble substances diffuse through biological membranes via water-filled intercellular pores, which limit diffusion by virtue of their radius, charge and length (10). Using lipid insoluble probing molecules of graded radii, Durbin *et al.* (8) estimated that 93% of the pores in frog gastric mucosa have a radius 2.5 A° while 7% have a radius of 60 A°. Villegas (11), in similar experiments, obtained data best described mathematically by a single pore population 3–4.5 A° in radius. PSP is lipid insoluble and cannot transverse cell membranes. Its diffusion path is the same as that of water and small ions. If the membrane-pore concept is valid, the relative impermeability of normal gastric mucosa to PSP must relate principally to its molecular size. A CPK model (12) of PSP constructed by one of us (J. C. B.) has an approximate unhydrated radius of 6 A°, larger than the unhydrated radius of any of the other ionic species used in the present study or the estimated radii of all but a fraction of the pores of the gastric mucosa. The demonstration, therefore, of more than a fourfold increase in PSP absorption following urea instillation indicates that mucosal permeability is increased and suggests that increased pore size may be responsible. In addition, it provides evidence that the observed alterations in ionic fluxes are, in fact, the result of altered mucosal permeability as has been postulated.

TABLE II. Ionic and Water Fluxes Across Canine Gastric Mucosa during Anesthesia and Following Topical Urea. (Flux rate ± SEM/30 min/100 cm² (+) = pouch gain; (−) = pouch loss)

	Control	After urea
Vol (ml)	+1.6 ± 0.4	+3.3 ± 0.5*
Net H^+ (μEq)	−156 ± 29	−402 ± 20*
Net Na^+ (μEq)	+241 ± 34	+572 ± 58*
Na Out (μEq)	77 ± 15	141 ± 15*
Na In (μEq)	292 ± 11	756 ± 46*
Net K^+ (μEq)	+15 ± 1	+30 ± 3*
Net Cl^- (μEq)	+184 ± 15	+133 ± 12*

* $P = < 0.01$ with respect to control.

Variability in pore size may represent sequential stages of cellular maturity. It has been proposed that although the tight junctions between cells become increasingly incompetent with aging, the ratio of incompetent to competent junctions remains low in normal mucosa and the "mucosal barrier" to ionic diffusion is maintained. Damage to the mucosa, on the other hand, may alter this ratio, permitting enhanced ionic diffusion to occur (13). Of interest in this regard is the observation that changes in ionic fluxes similar to those seen with urea can be produced when frog mucosa is suspended in Ca^{++} free solution containing EDTA (14). Sedar and Forte (15) have demonstrated by electron-microscopy that this treatment disrupts the tight junctions of intracellular pores, increasing their radii several fold.

The present study indicates that increased mucosal absorption of hydrogen ion following urea instillation is the result of increased mucosal permeability. Although similar alterations have been implicated as precursors to erosive gastritis and benign gastric ulcer, the precise pathophysiologic mechanism of increased "back diffusion" of hydrogen ion is unknown. Davenport (16) has postulated that hydrogen ion diffusing into the mucosa causes release of tissue histamine, increased capillary permeability, venous stasis, and ultimately hemorrhage. Evidence to support this hypothesis is incomplete, however, and further study is required. Bloom and associates (17) have called attention to many of the pitfalls in the use of dilution indicators (such as PSP) and emphasized the importance of validating the recovery rate of the indicator under the specific experimental conditions. On the basis of the observations reported herein, it is strongly recommended that PSP not be used as a volume marker in experimental or clinical studies in which the integrity of the gastric mucosal barrier is assessed.

Summary. The absorption of phenolsulfonphthalein from canine Heidenhain pouches before and after the topical application of 4 *M* urea was determined. In addition, associated changes in the net fluxes of H_2O, H^+, K^+ and Cl^- and the net and bidirectional fluxes of Na^+ were assayed to document disruption of the mucosal barrier. PSP absorption before urea instillation averaged 0.13 ± 0.4 μM/30 min/100 cm^2 mucosal urea. Following urea, an average of 0.63 ± 0.06 μM/30 min/100 cm^2 mucosal area was absorbed. Associated ionic and water flux rates demonstrated significantly increased "back diffusion" of H^+ and influx of Na^+, K^+ and water into the lumen. The observation of over a fourfold increase in PSP absorption after urea instillation indicates that mucosal permeability is increased and suggests that increased effective pore area is responsible. It is concluded that PSP should not be used as a volume marker in studies in which the integrity of the gastric mucosal barrier is altered.

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Received March 20, 1974. P.S.E.B.M., 1974, Vol. 147.