A Continuous Monitoring of Mucosal Integrity and Secretory Activity in Rat Stomach: A Preparation Using a Lucite Chamber

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Abstract—We assembled a new system using a lucite chamber and rat stomach for simultaneous measurement of transmucosal potential difference (PD) and luminal pH as indicators of the mucosal integrity and the secretory activity, respectively. The biological preparation involved only the glandular mucosa and responded to a variety of mucosal damaging agents by different degrees of PD reduction, pH increases and histological damages. When the mucosa was exposed for 10 min to 1 M NaCl, the reduced PD was restored with time, reaching the baseline values within 2 hr with histological restitution. Titration of gastric effluent showed that after the exposure, acid secretion ceased and a considerable amount of HCO₃⁻ was evident in the lumen, followed by re-secretion of acid. These secretory changes corresponded with those of luminal pH; this remained elevated for 1 hr after the exposure and returned to the basal values 2 hr later. The histological restitution as well as the PD recovery after damage were significantly interfered with by indomethacin (5 mg/kg, s.c.) or vasopressin (10 unit/kg/hr, i.v.), respectively, at the dose which inhibited the increased pH responses caused by 1 M NaCl or reduced the mucosal blood flow. These results suggest that this system may be useful for studying physiological changes of gastric mucosa after acute injury and for screening drugs that may have an effect on the repair process.

Self-defensive mechanisms of gastric mucosa have been suggested to be important for maintaining the mucosal integrity under adverse conditions (1–3). These involve functional alterations such as inhibition of acid secretion, increase of mucus/HCO₃⁻ secretion and mucosal blood flow (1, 3–5), but also the dynamic aspect of the surface epithelial cell [restitution] as well (1, 6, 7). The in vitro preparation has been the experimental condition of choice for evaluating these mucosal responses after acute injury. However, since the experimental milieu bathing the mucosal tissue in vitro differs from the physiological conditions on basis of blood supply and nerve innervation, the data obtained in such systems cannot be directly extrapolated to in vivo conditions. We have, therefore, approached these problems using the rat whole stomach preparation, but the interpretation of the results was sometimes difficult because the system involves the forestomach (non-glandular portion) as well as the glandular mucosa. Thus, it is noteworthy to develop a system by which both functional and morphological changes are simultaneously monitored in a specific site of the mucosa under physiological conditions.

In the present study, we assembled a new experimental system using a lucite chamber and the rat stomach, and demonstrated the usefulness of the system for studying physiological changes of gastric mucosa in response to acute injury.

Materials and Methods
Male Sprague Dawley rats (300–350 g) were used. The animals, which were kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access
to tap water for 24 hr prior to the experiments.

**Apparatus and surgical procedures:** The design of the lucite chamber and the apparatus of the gastric perfusion system are shown in Figs. 1 and 2. The chamber consists of two parts: one part is a lucite base and the other part is a plastic rim which has three holes on the side wall. Two holes are cannulated for perfusing the mucosa with saline (154 mM NaCl) at a rate of 1 ml/min, and the other is inserted with an agar bridge for determination of transmucosal PD. The animals were anesthetized with intraperitoneally administered urethane (1.25 g/kg, Nakarai), and the trachea was cannulated to ensure a patent airway. The stomach was exposed through a midline incision and delivered onto the abdominal surface by gentle traction on the spleen, and both the esophagus and pylorus were ligated. The lucite base was lowered over the animal, and the stomach was drawn through the center hole with the forceps applied only to the forestomach. The stomach was then opened along the greater curvature from the middle

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**Fig. 1.** A lucite chamber (upper) and a rat gastric mucosa mounted in a chamber (lower).

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**Fig. 2.** Schematic illustration of the lucite chamber system and the order of collections of tubes for determination of transmucosal PD and luminal pH in a rat stomach.
part of the forestomach to the area where the.epiploic artery is terminated, and the edges
were pinned out by gently stretching the
glandular mucosa. The plastic rim was then
applied and tightened down on the mucosa.
Under these conditions, the exposed area
was exclusively the glandular mucosa, mostly
consisting of the corpus region (the antrum
portion was minimally exposed over the
surface). The chamber was set at the level of
the abdominal wall so that the external wall
of the stomach remained inside the abdominal
cavity. The body temperature was maintained
at a rectal temperature of around 37°C by
exposing the animal to the heating lamp. The
saline solution was kept in a stock bottle and
gassed with 100% O₂ to minimize the con-
tamination of CO₂ and placed in a water bath
heated at 37°C. The perfusion rate was
controlled by two peristaltic pumps (Mitsumi
Sci, Inc.), each located at the middle of an
entry and exit tube (2 mm in diameter) con-
nected to the chamber. The exit tube was
connected to a pH glass electrode of the
flow type (Horiba Model 6901-25T) to
determine the pH of the gastric effluent. The
circuit for PD recording was completed with
an indifferential electrode (another agar
bridge) positioned in the abdominal cavity.
These electrodes led to a pH meter-millivolt
meter (Yokokawa Denki, Type 2575) through
two separate beakers filled with saturated KCl
solution in which a balanced calomel elec-
trode was placed. Changes in PD were con-
tinuously monitored using a Hitachi two-
channel recorder (Model 056) simultaneously
with those of pH. On the other hand, deter-
mination of the actual amount of acid or
alkali in the lumen was performed by intro-
ducing an automatic titrator (Hiranuma
Comtite-7) in the above perfusion system,
and the titration was made at pH 7.4 using a
pH-stat method and by adding 50 mM NaOH
or 10 mM HCl to the reservoir.

Experimental protocols: Approximately 1 hr
after both PD and pH had stabilized, the
perfusion system was interrupted, and the
mucosal solution was withdrawn. The mucosa
was then exposed to 2 ml of various mucosal
damaging agents such as 50% ethanol (10
min), 1 M NaCl (10 min), 20 mM taurocholic
acid (30 min, Sigma Chemicals) and 20 mM
aspirin (30 min, Sigma) or to 2 ml of various
concentrations of NaCl (10 min). Taurocholic
acid and aspirin were applied to the mucosa
with 50 mM HCl. After the exposure, the
mucosa was rinsed well with saline, another
2 ml of saline was instilled, and the perfusion
system resumed. Monitoring of pH was also
interrupted for 10 or 30 min, while the mucosa
was exposed to the above agents, whereas
PD was continuously measured throughout a
test period (2 hr). In some cases, the effects
of indomethacin (Sigma) and vasopressin
(Sigma) were examined on the changes in
PD and pH seen after exposure to 1 M NaCl.
Indomethacin (5 mg/kg) was given sub-
cutaneously 30 min before exposure to 1 M
NaCl, and vasopressin (10 unit/kg/hr) was
infused intravenously during a test period. In
separate experiments, the mucosa was
excised immediately after exposure to various
concentrations of NaCl and also in the case
of 1 M NaCl at various time intervals after the
exposure. Tissue samples were immersed in
10% formalin, processed for routine light
microscopy, sectioned at 5 μm, and stained
with hematoxylin and eosin.

Determination of gastric mucosal blood
flow: To examine the effects of indomethacin
and vasopressin on gastric mucosal blood
flow, we used the aminopyrine clearance
technique according to the method described
by Jacobson et al. (8) and modified by
Kitagawa et al. (9). A polyethylene tube was
inserted into the stomach through the pylorus
from a slit in the duodenum for instillation
and withdrawal of gastric solution. Constant
plasma aminopyrine level was maintained by
continuous infusion of aminopyrine (Sigma,
6.6 mg/kg/hr) at a rate of 1.2 ml/hr, starting
30 min after a single dose of 30 mg/kg given
intravenously by a bolus injection. The
stomach was filled with 2 ml of gastric
solution (glycine buffered solution, pH 3.5).
The gastric solution was changed every 15
min, and the collected juice was analyzed for
volume and titrated with 0.1 N NaOH to pH
7.0 for titratable acidity. Acid output was
calculated by subtracting the acidity of the
initial gastric juice (glycine buffered solution)
from the titratable value of each final sample
and expressed as μEq/15 min. Samples of
arterial blood was collected from the tail.
artery at 60 min after the onset of aminopyrine infusion and at the end of the experiments. The content of aminopyrine in the plasma and gastric juice was measured according to the method of Brodie and Axelrod (10). Gastric mucosal blood flow was determined every 15 min and expressed as ml of blood/15 min. Approximately 1 hr after basal acid output had stabilized, the animals were given indomethacin (5 mg/kg) subcutaneously or infused with vasopressin (10 unit/kg/hr) intravenously, and both acid output and mucosal blood flow were measured for 1.5 hr thereafter.

Statistics: Data are presented as the mean±S.E. of values determined every 10 or 15 min from 4 to 6 rats per group. Statistical analysis was performed using a two-tailed Dunnett's multiple comparison test (11), and values of P<0.05 were regarded as significant.

| Table 1. Effects of various mucosal damaging agents on transmucosal potential difference and luminal pH in the chambered rat gastric mucosa |
|-----------------------------------------------|
| Treatment | No. of rats | Potential difference (−mV) Before | After | Luminal pH Before | After |
| Saline (154 mM NaCl) | 6 | 35.5±3.5 | 33.0±1.5 | 3.7±0.2 | 3.7±0.2 |
| 50% Ethanol | 6 | 33.0±1.3 | 9.5±2.0* | 3.8±0.2 | 6.7±0.1* |
| 1 M NaCl | 6 | 33.3±1.8 | 9.0±1.3* | 3.9±0.1 | 6.5±0.1* |
| 20 mM Taurocholic acid | 6 | 36.0±3.5 | 12.3±3.8* | 3.6±0.1 | 5.4±0.3* |
| 20 mM Aspirin | 6 | 35.0±2.5 | 12.5±1.8* | 3.9±0.2 | 4.3±0.3 |

Values are presented as the mean±S.E. from 6 rats per group. The stomach was exposed to 2 ml of each agent for 10 min (50% ethanol, 1 M NaCl) or 30 min (saline, 20 mM taurocholic acid, 20 mM aspirin), and it was perfused with saline before and after the exposure. The values in Before and After mean those observed immediately before and 10 min after exposure to each agent. *Statistically significant difference from the values in Before, at P<0.05.

| Table 2. Effects of various concentrations of NaCl on transmucosal potential difference and luminal pH in the chambered rat gastric mucosa |
|-----------------------------------------------|
| Concentration of NaCl | No. of rats | Potential difference (−mV) Before | After | Luminal pH Before | After |
| 0.15 M | 4 | 34.2±1.6 | 34.8±1.6 | 3.4±0.1 | 3.4±0.2 |
| 0.25 M | 5 | 35.0±1.2 | 29.1±2.1* | 3.5±0.1 | 3.5±0.2 |
| 0.50 M | 5 | 34.6±1.8 | 23.8±1.6* | 3.5±0.1 | 3.8±0.2 |
| 0.75 M | 5 | 36.8±2.0 | 20.2±1.2* | 3.5±0.1 | 5.4±0.2* |
| 1.0 M | 6 | 35.6±1.2 | 9.1±1.8* | 3.4±0.1 | 6.3±0.1* |

Values are presented as the mean±S.E. from 4 to 6 rats per group. NaCl solution (2 ml) was applied topically to the chambered mucosa for 10 min, and the mucosa was perfused with saline (0.15 M NaCl) before and after the exposure. Values in Before and After mean those observed immediately before and 10 min after exposure to NaCl. *Statistically significant difference from the values in Before, at P<0.05.
magnitude being 26.3±2.4 mV in the mucosa exposed to 1 M NaCl (Table 2 and Fig. 3). The PD remained unaltered in response to 0.15 M NaCl, but significantly was reduced after exposure to NaCl at 0.25 M or greater. The pH was markedly elevated after exposure to NaCl at 0.75 M and 1.0 M. Exposure of the mucosa to 0.25 M and 0.5 M NaCl did not cause significant rise of the pH, despite the PD being significantly decreased. Histological study showed that 10 min exposure to NaCl induced various degrees of damage in the surface cells at the concentrations of 0.25 M or greater, and an extensive damage was observed at 0.75 M and 1.0 M (not shown).

Effect of 1 M NaCl on gastric PD, pH and
secretory responses: Exposure of the chambered mucosa for 10 min to 1 M NaCl reduced the PD from -34.6±1.8 mV to -8.7±2.1 mV, but the reduced PD was gradually normalized with time, reaching the values of -30.1±1.6 mV at a 2 hr post exposure (Fig. 4). The pH was markedly increased from 3.5±0.1 to 6.4±0.1 within 20 min and remained elevated for 1 hr, followed by a decrease to 4.0±0.2 at 2 hr post exposure. In the mucosa exposed to 1 M NaCl, acid secretion (3.4±0.6 μEq/10 min) ceased within 30 min, and a significant alkalinization (0.2–0.3 μEq/10 min of HCO$_3^-$) was evident in the lumen for 1 hr, followed by re-secretion of acid in the later period. The surface epithelial layer was extensively damaged when examined immediately after exposure to 1 M NaCl, and the injured portion was covered with a thick layer of mucus containing cell debris. However, the mucosa injured by 1 M NaCl was partially recovered with surface epithelial cells at 1 hr post exposure, and epithelial continuity was observed at 2 hr after the mucosa had been damaged by NaCl (not shown).

Effects of indomethacin and vasopressin on mucosal blood flow, acid output, and changes in PD and pH responses: The levels of gastric acid output and mucosal blood flow were 2.1±0.4 μEq/15 min and 2.8±0.6 ml/15 min, respectively, under anesthetized conditions. Subcutaneously administered indomethacin (5 mg/kg) had no effect on the mucosal blood flow, while intravenous infusion of vasopressin (10 unit/kg/hr) sig-

Fig. 5. Effects of indomethacin (5 mg/kg, s.c.) and vasopressin (10 unit/kg/hr, i.v.) on gastric acid secretion and mucosal blood flow in anesthetized rats. Data are presented as the percentage (%) for the corresponding control values in each group and represent the mean±S.E. of values determined every 15 min from 6 rats. *Statistically significant difference from normal rats, at P<0.05.
significantly decreased the mucosal blood flow to 28.2±9.2% of control values (Fig. 5). Acid output was also markedly reduced by vasopressin, but remained unaltered in response to indomethacin.

**PD and pH:** Pretreatment of the animals with indomethacin (5 mg/kg) did not affect PD responses of the chambered mucosa caused by 1 M NaCl, but significantly inhibited the increase of pH and the recovery process of PD seen after exposure to 1 M NaCl (Figs. 6 and 7). Intravenous infusion of vasopressin (10 unit/kg/hr) also delayed the recovery of PD in the mucosa exposed to 1 M NaCl, although this agent rather enhanced the increase of pH seen after the exposure. Both agents interfered with the histological restitution after damage, and the epithelial continuity was not observed even at 2 hr post exposure (not shown).

**Discussion**

The biological preparation described herein responds to a variety of mucosal damaging agents and allowed a study of adaptive changes of gastric mucosa after acute injury.

![Figure 6](image-url)

**Fig. 6.** Effects of indomethacin (5 mg/kg, s.c.) and vasopressin (10 unit/kg/hr, i.v.) on the PD and pH responses of the chambered mucosa after exposure for 10 min to 1 M NaCl. Data in pH are presented as the mean±S.E. of values read every 10 min from 6 rats, and those in PD recovery are expressed as the percentage (%) for the degrees of PD reduction observed immediately after the exposure and are presented as the mean±S.E. of values determined every 10 min from 6 rats. *Statistically significant difference from the controls, at P<0.05.
Fig. 7. Representative figures showing the effects of indomethacin (5 mg/kg, s.c.) and vasopressin (10 unit/kg/hr, i.v.) on changes in PD and pH of the chambered mucosa after exposure for 10 min to 1 M NaCl.

Since this preparation includes normal blood supply and nerve innervations, the data obtained may be more useful for understanding of the physiological events in the mucosa than those observed in the in vitro experiments. Although the system is essentially the same as that reported previously using the rat whole stomach preparation (2-4), the present method is more sensitive to changes in PD and pH, probably because the system consists of only the glandular mucosa but does not include the non-glandular portion of the stomach. Moreover, since the mucosa is exteriorized and can be observed directly from outside, the method would follow macroscopical alterations in the mucosa very closely with time, in association with the functional events that occurred after acute injury.

The utilization of pH deflection for measuring gastric secretory activity is essentially the same method as was reported by Ghosh and Shield (12), who demonstrated the validity of luminal pH to determine acid secretory responses to secretagogues. Continuous recording of the pH has, however, obvious limitations; it cannot distinguish acid secretion from alkaline secretion and/or acid back-diffusion. These problems are solved by introducing an automatic titrator and by measuring the actual amounts of acid and alkali appearing in the lumen at pH 7.4. On the other hand, gastric PD has been considered as an indicator of the mucosal integrity and used as a sensitive method for assessing the mucosal injurious activity of drugs (13, 14). In agreement with the findings by others (1, 6, 15), the degrees of PD reduction were closely associated with the severity of damage in the gastric mucosa. Moreover, the present system monitors the recovery process of PD after removal of the irritant from the mucosa and correlates these changes with the histological observations on the mucosa. It is known that the generation of PD in the damaged mucosa composed of exfoliating the surface epithelium is dominated by alterations of the tissue electrical resistance accompanying the morphological restitution (1, 6, 16), and a close relationship has been found between these two factors. Thus, monitoring of PD may be used as an
indicator of the re-establishment of the mucosal integrity as well as the index of the degrees of mucosal injury.

The major advantage of this system is the simultaneous observation of PD and gastric secretory responses in the same tissue, so that a correlation can be obtained between these two events. The luminal pH was markedly increased after exposure to damaging agents, and it reached the maximal values within 30 min, followed by a return to the baseline values about 2 hr later when the reduced PD was almost completely normalized. These changes in PD and pH were much clearly observed in the present system when compared to the rat whole stomach preparation. The involvement of non-glandular mucosa in the latter system may somehow interfere with the net changes that occur in the glandular mucosa in response to injury. Different pH responses to aspirin and other agents were consistent with previous results obtained in the whole stomach preparation (2, 3), suggesting an involvement of endogenous prostaglandins (PGs) in the increased pH response of the chambered mucosa against injury. In fact, many investigators (2, 5, 17, 18) reported that PG production and/or release was markedly increased in the gastric mucosa after exposure to hypertonic NaCl and bile salts. Administration of an exogenous PG increased the luminal pH, probably due to both inhibition of acid secretion and stimulation of alkaline secretion (2, 3). Since we previously showed in the whole stomach preparation that indomethacin had no effect on the gastric secretory responses caused by 1 M NaCl in the cimetidine-treated rats (2), it is likely that the increased pH responses may be ascribed to inhibition of acid secretion caused by enhanced release of PGs in the gastric mucosa in response to injury. The present finding that the increased pH reverted to the baseline about 2 hr post exposure may suggest that the enhanced release of PGs takes place within less than 2 hr after mucosal injury.

Attenuation of the increased pH response by indomethacin significantly delayed the PD recovery and the morphological restitution after damage. This is consistent with the findings of Svanes et al. (19), who showed using in vitro preparations of frog fundic mucosa that low luminal pH inhibits and high nutrient HCO₃⁻ promotes the process of mucosal repair after damage with 1 M NaCl. Morris and Wallace (15) showed that if H⁺ remains in the lumen after injury, the basal lamina is destroyed, and thus the substratum necessary for restoration of the epithelial continuity is removed. Thus, the increased pH response (alkalinization) may be a factor for maintaining the microenvironment in which the mucosal repair can proceed. Certainly, the increased pH response alone cannot account for the rapid tissue recovery, since a decrease of mucosal blood flow caused by vasopressin significantly interfered with the process of mucosal restitution, without altering the pH response of the mucosa. We previously showed that application of 1 M NaCl to the rat stomach significantly increased the mucosal blood flow and that this hyperemic response disappeared in the presence of indomethacin (4). Thus, it may be assumed that the increased blood flow response also involves endogenous PGs in its regulation as the increased pH response does and that the lack of this phenomenon partly accounts for the deleterious influence of indomethacin on the restitution process after damage. These functional alterations observed after mucosal injury may be potentially important in the recovery process after acute mucosal damage and prevent the later extension of such an injury to severe lesions.

Finally, our apparatus has the advantage of not only giving a continuous simultaneous recording and display of PD and pH but also providing a direct visualization of the mucosal changes to injurious agents. This method may, therefore, be useful for understanding of gastric mucosal defensive mechanisms against acute injury and for evaluation of drugs which affect gastric mucosal integrity and secretory activity.

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