Comparative Effects of Aliphatic Alcohols on the Gastric Mucosa (37044)

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One of the proposed theories of the etiology of gastric ulcer is a decrease in the resistance of gastric mucosa so that it is susceptible to attack by acid gastric juice. The properties of the mucosa which normally make it resistant to digestion are not fully known. However, several indices of mucosal permeability such as ion fluxes and transmucosal potential difference are altered by various chemical agents. Alterations of these indices have been implicated in the production of gastric erosions (1, 2).

Davenport (1, 3) found that weak organic acids had to be in their nonionized form to alter the permeability of the gastric mucosa and many other compounds which affect permeability are lipid soluble. Thus lipid solubility seems to be required of an agent to alter ion fluxes and electrochemical potential difference. No chemical structural specificity for agents to alter the mucosa has been found but systematic studies have not been made to investigate structural requirements.

The purpose of these experiments was to compare the effects of methanol, ethanol, *n*-propanol, and *t*-butanol on the gastric mucosa and to relate any differences in their effects to some physical or chemical differences among the alcohols.

Methods. A thorough description of the animal model, apparatus, and methods used in this study has been published (2). Briefly, four dogs with vagally denervated (Heidenhain) pouches were studied over a 6 mo period. Each animal was fasted for 18 hr prior to a test. Then atropine, 15 μg/kg, was injected intramuscularly to ensure basal secretion and the pouch rinsed thoroughly. Secretions were collected for consecutive 15 min periods and an experiment was begun when no secretions were present.

Each experiment consisted of three to six 30 min observation periods. At the beginning of each 30 min period, 40 ml of a solution of 100 m*M* HCl + 54 m*M* NaCl were placed in the pouch, the pouch contents were mixed, and a 5 ml sample was withdrawn and saved. At the end of the period, the contents of the pouch were drained and measured to the nearest 0.1 ml. The pouch was then rinsed with a solution of 100 m*M* HCl + 54 m*M* NaCl. During the first 30 min period the solution consisted of 100 m*M* HCl + 54 m*M* NaCl in distilled water. During period 2, the alcohol under study dissolved in the acid–saline solution was instilled. During periods 3 through 6, the solutions were the same as in period 1.

The initial and final samples were analyzed for titratable acidity, sodium, potassium and chloride. The concentration of acid was measured by titrating a 0.2 ml sample of the gastric contents with 0.2 *N* NaOH to pH 7.0 using a glass electrode and an automatic buret (Autoburet, Radiometer, Copenhagen). Sodium and potassium concentrations were measured by automated flame photometry (flame photometer, Instrumentation Laboratory Inc., Boston). Chloride concentration was measured by the chloridometer automatic titrator (Bucher-Cotlove, Fort Lee, NJ).

Transmucosal potential difference (PD) was measured as previously described (2). Readings were taken to the nearest 0.5 mV at 2 min intervals during the experiment. An average of the 2 min readings was taken as the PD during each period.

Oil–water partition coefficients were determined by using ^{14}C labeled alcohols (New England Nuclear). Ten milliliter of *n*-heptane were placed in a 50 ml volumetric flask. To this, 250 μl of alcohol (1 μCi/ml) and 9.75

ml of the acid–saline solution were added. The flask was placed in a water bath at 37° and agitated for 4 hr. The radioactivity in 1 ml of each layer (aqueous and organic) was determined in a Nuclear Chicago Unilux II scintillation counter. Corrections were made for quenching and the partition coefficient was calculated by the following formula:

$$\text{Partition coefficient} = \frac{\text{corrected cpm in 1 ml of organic phase}}{\text{corrected cpm in 1 ml of aqueous phase}}.$$

Gastric absorption of each alcohol was determined also by using ^{14}C labeled alcohols. One microcurie of radioactive alcohol was added to 1.5 moles of unlabeled alcohol in 40 ml of the acid–saline solution. This solution was used during period 2 of an experiment. The rest of the experimental procedure was identical to that of nonradioactive alcohols. Radioactivity was determined in aliquots taken at the beginning and end of period 2 and in the rinse solution. Corrections were made for quenching and the concentrations of labeled alcohol was determined in each solution. Absorption was calculated from the formula:

$$\% \text{ absorbed} = \frac{(\text{alcohol})_{in} \times \text{vol}_{in} - (\text{alcohol})_{out} \times \text{vol}_{out}}{(\text{alcohol})_{in} \times \text{vol}_{in}}.$$

Concentration-effect curves for the alcohols were made by plotting the difference in PD (Δ PD) or ion flux (Δ ion flux) between periods one and two against the molar concentration of the alcohol. Regression analyses were performed by the method of least squares (4).

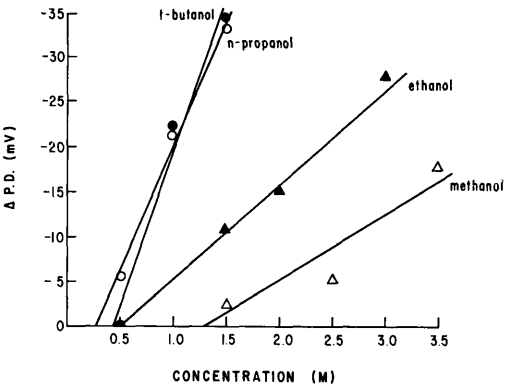

FIG. 1. Change in transmucosal potential difference (△ PD) induced by various molar (*M*) concentrations of the alcohols. Each point is the mean of at least 3 experiments. Regression coefficients of all lines were significant ($p < 0.01$).

Results. Control values. The transmucosal potential difference (PD) and net fluxes of H^+, Na^+, and Cl^- during the control periods (period 1) for each animal are shown in Table I. There was little variation in the PD from day to day in the same animal. Net fluxes of K^+ were small and tended to be constant. The net fluxes of H^+ and Na^+ varied over a wider range and net fluxes of Cl^- had the greatest variability. The values were similar for the four animals except that the net values of Na^+ and H^+ were somewhat greater for dog No. 2.

Effects of the alcohols. All of the alcohols caused a fall in PD (Fig. 1). The PD began to decrease 0.5 to 2 min after instillation of the alcohol–acid–saline solution. Peak effects occurred in 10 to 15 min. The PD generally returned toward control levels during the remainder of the 30 min period. During subsequent 30 min periods when only acid–saline solution was in the pouch, the PD returned

TABLE I. Transmucosal Potential Differences and Net Fluxes of Ions During Period 1 (Control Period).

Dog no. 1	PD (mV)	H^+ Flux (μEq)	Na^+ Flux (μEq)	Cl^- Flux (μEq)
1.	−62 ± 1.4[a]	−180[b] ± 39	137 ± 15	−35 ± 57
2.	−64 ± 1.0	−259 ± 33	307 ± 34	−33 ± 90
3.	−66 ± 1.2	−137 ± 43	124 ± 14	41 ± 37
4.	−67 ± 1.0	−138 ± 38	158 ± 15	40 ± 42

[a] Av ± 1 SEM, n = at least 12.

[b] Negative value indicates net flux from the lumen of the pouch, positive value indicates net flux into the lumen of the pouch.

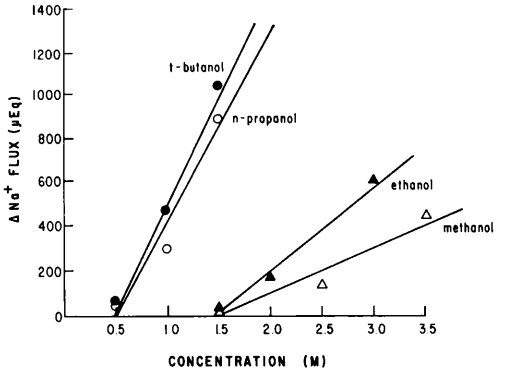

FIG. 2. Change in net flux of Na^+ ($\triangle$ Na^+) into the lumen induced by various molar (M) concentrations of the alcohols. Each point is the mean of at least 3 experiments. Regression coefficients of all lines were significant ($p < 0.01$).

to control levels. The time required for complete recovery depended upon the degree of change induced by the alcohol; the greater the decrease in PD, the longer for recovery. The decrease in PD was greater as the concentration of each alcohol was increased. In addition, except for *t*-butanol, the magnitude of the change was proportional to the carbon-chain length of the alcohol. The longer chain alcohols were effective at a lower molar concentration. Also, for a given increase in concentration, the longer chain alcohols caused a greater change in PD than did the shorter chain alcohols.

The net flux of Na^+ ion into the lumen of the pouch was increased over control by each of the alcohols (Fig. 2). As with changes in PD, the net flux of Na^+ increased as the concentration of each alcohol was increased and, on a molar basis, the longer carbon-chain alcohols were more effective.

The effect on net H^+ flux varied depending upon the alcohol used and its concentration (Fig. 3). Methanol and *t*-butanol caused either no change or an increase in the net flux of H^+ from the lumen of the pouch. The higher the concentration of either alcohol, the greater the net flux and *t*-butanol was more effective at lower concentrations than was methanol. Ethanol and *n*-propanol had effects which depended upon their concentration. Low concentrations of either of these alcohols caused a net flux of H^+ into the lumen of the pouch (secretion of H^+). Higher concentrations of *n*-propanol caused a net loss of H^+ from the pouch (Fig. 3). In two experiments, 4.0 M ethanol likewise produced a net loss of H^+. At the concentrations tested, ethanol was more effective at causing secretion of H^+ while *n*-propanol was more effective at causing a net loss of H^+ from the pouch.

The alcohols produced no consistent effects on the net flux of Cl^- ion (Fig. 4). However, in all experiments, the net flux of cations was closely paralleled by the net flux of Cl^-. For example, with 0.5 M *n*-propanol there was a net flux of 183 μEq of Na^+ and 144 μEq of H^+ into the pouch. This was balanced by a net flux of 363 μEq of Cl^- into the pouch. When 1.5 M *n*-propanol was in the pouch, there was net flux of 1028 μEq of Na^+ into the pouch and a net flux of 637 μEq of H^+ out of the pouch. The difference between the net fluxes of the two cations was balanced by the net flux of 331 μEq of Cl^- ion into the pouch. Potassium fluxes remained fairly constant (5–10 μEq) and were always into the pouch.

Partition coefficients. The *n*-heptane-aqueous coefficients for methanol, ethanol, *n*-propanol, and *t*-butanol were 0.032, 0.048, 0.071, and 0.137, respectively. A plot of the concentration of each alcohol which caused a change in the PD of 18 mV against the partition

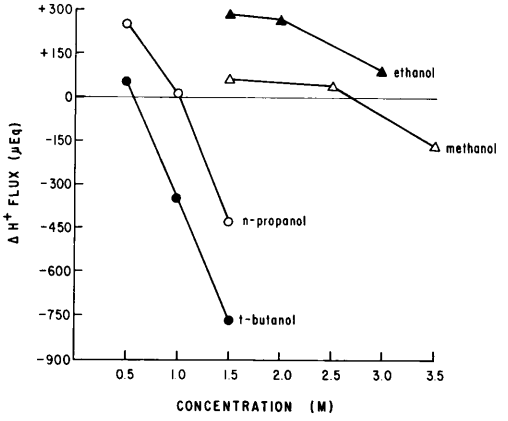

FIG. 3. Change in net flux of H^+ ($\triangle$ H^+) induced by various molar (M) concentrations of the alcohols. Positive values indicate flux into the pouch; negative values, flux out of the pouch. Each point is the mean of at least 3 experiments.

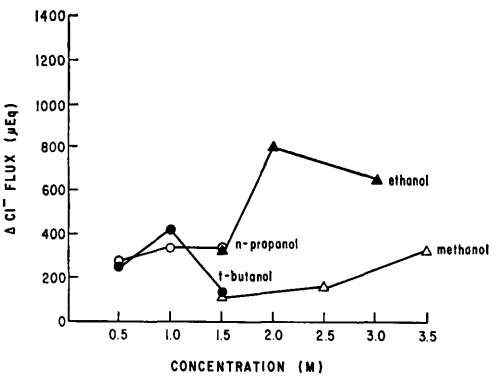

FIG. 4. Changes in net flux of Cl^- (Δ Cl^-) induced by various molar (M) concentrations of the alcohols. Each point is the mean of at least 3 experiments.

coefficient is shown in Fig. 5. Except for *t*-butanol, there is good correlation between the ability of the alcohol to effect the PD and its partition coefficient; the more lipid soluble the alcohol, the lower the concentration needed to affect the PD.

Gastric absorption. The average percentage absorptions for each alcohol were: methanol 42.5%, ethanol 47.9%, and *n*-propanol 40.8%, ($n = 3$ for each alcohol), and *t*-butanol 35% ($n = 2$).

Discussion. It is well established that lipid-soluble compounds can readily penetrate biological membranes: Schanker *et al.* (5) tested the gastric absorption of a series of chemicals and related their degree of absorption to their lipid solubility. They found that the more lipid-soluble the compound the greater its absorption. All of the alcohols used in this study were absorbed from the pouch. We did not find a correlation between lipid solubility and the amount absorbed, but this was not thoroughly investigated. We did find an excellent correlation between the lipid solubility of the straight chain alcohols and their ability to affect the PD of the gastric mucosa (methanol < ethanol < *n*-propanol). However, *t*-butanol was about twice as lipid soluble as *n*-propanol, but, on a molar basis, was no more effective than *n*-propanol. Thus, the ability of an agent to affect the mucosa does depend upon its lipid solubility, but other properties of the agent may play a role.

Elwin (6) found that of the alcohols with carbon chain lengths of C-1 to C-4, only ethanol and *n*-propanol stimulated the release of gastrin from the gastric antrum. Other studies have shown that even though a large number of compounds can release gastrin from antral mucosa, chemicals which have a carbon-chain length of two or three are the most effective (7). We found no such structural requirements for agents to affect the parameters which we measured. We did find that the presence of ethanol and *n*-propanol in the fundic pouches induced a secretion of H^+ into the pouch. The mechanism of stimulation of acid secretion by these alcohols is not known, but may have been due to a local or systemic effect of the alcohol (8, 9).

Just how the agents are affecting the gastric mucosa is not known. Davenport (10) proposed that the chemicals damage a "mucosal barrier" to allow an increase in the net flux of H^+ from the lumen into the mucosa. The backflux of H^+ then leads to a series of events to produce damage. However, Shanbour, Miller and Chowdhury (11) have recently shown that one of the first effects of alcohol is to decrease the transport of Cl^- into the gastric lumen. Thus, the PD and ion fluxes would be altered due to an effect on active transport of ions. Our measurements of ion fluxes were made only after the alcohols were in contact with the mucosa for 30 min. We did not study the temporal course of the changes in fluxes induced by the alcohols.

Many agents which are suspect in either causing or exacerbating mucosal erosions have been tested for their effects on the net fluxes

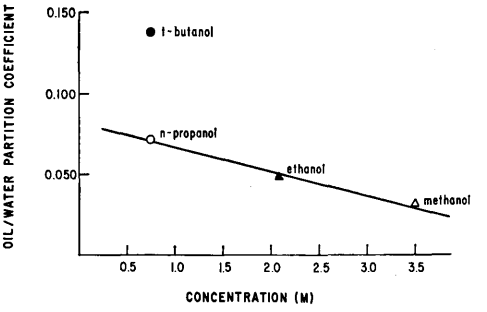

FIG. 5. Correlation between the oil/water partition coefficients of the alcohols and the concentration of each alcohol required to change the PD by 18 mV.

of H^+ and other ions. Generally, agents which cause a net flux of H^+ from the lumen also cause a fall in PD and an increase in the net flux of Na^+ into the lumen. However, as our results indicate, this relationship is not always true. For example, 2.0 *M* ethanol induced a net secretion of H^+ ions but it caused a fall in the PD of 15 mV and an increase in the net flux of Na^+ into the pouch. Thus, two parameters indicated mucosal damage while the net flux of H^+ indicated no change. There possibly was an increase in the flux of H^+ from the pouch, but this was masked by the secretory effects. We found the most sensitive and probably the most reliable monitors of mucosal integrity to be the transmucosal potential difference and the net flux of Na^+.

Summary. We studied the effects of a series of aliphatic alcohols on the gastric mucosa of Heidenhain pouches in four unanesthetized dogs. Transmucosal potential difference (PD), and the net fluxes of H^+, Na^+, and Cl^-, were determined in the presence of various concentrations of methanol, ethanol, *n*-propanol, and *t*-butanol. Each alcohol caused a decrease in the PD and an increase in the net flux of Na^+ ion into the lumen of the pouch. On a molar basis, *t*-butanol = *n*-propanol > ethanol > methanol in their effects on PD and net Na^+ flux. Except for *t*-butanol there was a linear relationship between the ability of the alcohol to alter PD and the oil–water partition coefficient of the alcohol. Low concentrations of ethanol and *n*-propanol caused a net flux of H^+ ion into the pouch (secretion). Higher concentrations of these alcohols and all concentrations of methanol and *t*-butanol caused a net loss of H^+ ion from the pouch. The net flux of Cl^- ion tended to parallel and equal the net flux of cations.

We conclude that (a) there is no optimal carbon-chain length between C-1 and C-4 for the "damaging" effects of alcohol on the mucosa, (b) the degree of "damage" is best correlated with the lipid solubility of the damaging agent, and (c) there is good correlation between the PD changes and the change in net Na^+ flux but not the change in net H^+ flux.

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