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RELATIONSHIP BETWEEN LESION FORMATION AND PERMEABILITY OF RAT GASTRIC MUCOSA TO H^+ AND OTHER CATIONS

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1 The relationship between lesion formation and ionic permeability has been investigated in rat gastric mucosa *in vivo*. Changes in these parameters were measured in the mucosa treated topically with prostaglandins E_2 and A_2 and/or aspirin. Particular attention was paid to the net flux of H^+ ions across the gastric mucosa.

2 The effect of aspirin concentrations of 5 mM, 20 mM and '40 mM' (the latter, a suspension in a saturated solution) was investigated. Aspirin concentrations of 20 mM and '40 mM' produced a marked increase in lesion formation and increased the net mucosal to serosal flux of H^+ ions. Aspirin 5 mM produced a significant increase in lesion formation but did not cause a significant change in net H^+ ion flux. This result suggests that aspirin can have a direct irritant effect on the gastric mucosa and that the back diffusion of H^+ ions is not a pre-requisite for the development of overt mucosal ulceration.

3 The effect of topically applied prostaglandin E_2 (PGE_2) on aspirin-induced gastric mucosal damage was investigated. Concentrations of PGE_2 of 10^{-5} M and 10^{-4} M ameliorated aspirin-induced damage, but these changes were not necessarily accompanied by a significant reduction in net H^+ ion flux. Again, this result is not consistent with a direct relationship between lesion formation and mucosal permeability to H^+ ions.

4 Since PGA_2 did not ameliorate aspirin-induced mucosal damage, the protective effect of PGE_2 could not be attributed to its conversion to PGA_2 in the acidic environment of the gastric lumen.

5 Changes in gastric mucosal potential difference (p.d.) and net fluxes of Na^+ and K^+ ions may occur without a concomitant change in the permeability of the gastric mucosa to acid back-diffusion. Thus, the assumption cannot be made that a change in the permeability of the gastric mucosa to one particular ion reflects a general increase in ionic permeability.

Introduction

The formation of gastric mucosal lesions following topical acetylsalicylic acid (aspirin) is dependent on the presence of luminal acid (Cooke, 1973; Fromm, 1978). However, the precise role of acid in the development of these lesions is unclear. It is well established that an acidic environment is required to maintain aspirin (pK_A 3.5) in its non-ionized form, thus increasing the lipid solubility of the molecule and the rate of absorption across the mucosal membrane (Cooke, 1973; Fromm, 1978). Following absorption, it is possible that aspirin causes gastric mucosal damage through a direct irritant effect (Rainsford, 1977), perhaps involving such mechanisms as the inhibition of active ion transport (Fromm, 1978) and the inhibition of oxidative metabolism (Rainsford & Whitehouse, 1980).

Davenport (1967) has proposed that aspirin *per se* does not cause gastric ulceration, but that the compound initially disrupts the gastric mucosal 'barrier' which allows the back-diffusion of H^+ ions down a steep concentration gradient; an effect which causes mucosal damage and haemorrhage.

Further evidence, albeit circumstantial, that acid back-diffusion may be important in the development of gastric ulceration is derived from experiments using the stable analogue of prostaglandin E_2, 16,16-dimethyl PGE_2. The latter compound reduces both gastric lesion formation and the back-diffusion of H^+ ions induced by aspirin in rats (Bommelaer & Guth, 1979) and dogs (Miller & Tepperman, 1979).

In the present experiments the effects of prostaglandins E_2 and A_2 and/or aspirin on lesion formation and several indices of gastric mucosal permeability have been studied in anaesthetized rats. Particular attention has been paid to the relationships between lesion formation and mucosal permeability to H^+

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ions since this might provide some insight into the importance of acid back-diffusion in the development of aspirin-induced mucosal damage.

Methods

Female Wistar rats (170–210 g) were deprived of food overnight, but allowed water *ad libitum*. The rats were anaesthetized with urethane (1.25 g/kg s.c.), the trachea intubated and a jugular vein cannulated. The abdomen was opened by a midline incision and the stomach exposed. An incision was made in the duodenum about 1 cm from the pyloric sphincter, and the gastric lumen was rinsed thoroughly with isotonic mannitol solution. A polythene tube was tied into the stomach via the duodenal incision, exteriorized through a stab-wound in the right flank and connected to a 3-way tap. This tube was used to instil solutions into and drain solutions from the stomach.

Gastric mucosal potential difference (p.d.) was measured using polythene catheters filled with 1% w/v agarose in 4 M KCl. One catheter was passed through the mouth and down the oesophagus and positioned in the stomach with a tie at the gastro-oesophageal junction. A reference electrode was placed in a subcutaneous saline-filled bleb in a hind-foot. The agarose-KCl bridges were connected to a high input impedance d.c. amplifier via calomel electrodes placed in separate beakers of saturated KCl solution. The amplifier was connected to a Devices chart recorder calibrated on a 0–100 mV scale.

Each experiment was divided into five 30 min periods. During each of the first two periods 4 ml of a control solution was placed in the stomach. This solution contained HCl 100 mM, NaCl 10 mM, mannitol 80 mM and [^{14}C]-polyethylene glycol-4000([^{14}C]-PEG, 5 g/litre and 1 μCi/litre) as a volume marker. The osmolarity of the solution was 300–302 mosm/kg as measured by depression of freezing point (Advanced 3W II Osmometer). Preliminary experiments showed that 97.9 ± 1.5% ($n = 6$) of the [^{14}C]-PEG could be recovered from the stomach, this result indicating that the compound was suitable for use as a volume marker in these experiments.

During the third period of the experiment a 'test' solution (4 ml) was placed in the stomach. In most experiments this solution was similar to the 'control' solution except that mannitol was replaced with acetylsalicylic acid, such that osmolarity remained constant. The concentrations of aspirin used were 5 mM, 20 mM and '40 mM'. This latter solution contained the quantity of aspirin theoretically required to achieve a 40 mM concentration, but it was not possible to dissolve all the aspirin and consequently a suspension of aspirin in a saturated solution was obtained. In some experiments a buffered aspirin solution was used. This was prepared by dissolving sufficient aspirin for a 20 mM solution with an equimolar amount of NaOH. The osmolarity of the solution was made up to 300 mosm/kg by the addition of mannitol, and the pH was 4.5; at this pH, 91% of the drug is ionized (Cooke, 1973).

During periods 4 and 5 the 'control' solution was again placed in the stomach. At 10 min intervals during each period, adequate mixing of the gastric contents was ensured by gently withdrawing the solution from the stomach into a syringe and injecting the sample back into the stomach.

Between each period the stomach was rinsed out thoroughly with three 4 ml aliquots of isotonic mannitol solution. Preliminary experiments showed that the third wash of mannitol contained a mean of 1.2% of the radioactivity initially instilled into the stomach. This result indicated that any contamination of solutions between periods was minimal.

Prostaglandins were applied topically to the gastric mucosa. The rats were predosed with these compounds during the second period of the experiment, and also treated during the period of aspirin damage (period 3). The prostaglandins were initially dissolved in ethanol at a concentration of 10 mg/ml. Further dilutions were then made in 0.01% (v/v) Tween 80 (Sigma). For the addition of PGE_2 or PGA_2 to the acidic solutions not more than 0.5 ml of the Tween 80 preparation was added to a 15 ml sample.

The concentration of H^+ ions in the gastric aspirate was measured by titration against 0.1 N NaOH using a Radiometer-TTT60 autotitrator. This measurement was not made during period 3 of the experiment since the titration value would reflect both unabsorbed aspirin and HCl. The concentrations of Na^+ and K^+ ions were measured with a Radiometer FLM3 flame photometer. The activity of [^{14}C]-PEG in the solutions was determined by liquid scintillation spectrometry and the total volume of the final solution in the stomach was calculated using the formula:–

$$V_o = \frac{V_i \times PEG_i}{PEG_o}$$

where V_o = volume of gastric aspirate, V_i = volume of instilled solution (4 ml), PEG_o = [^{14}C]-PEG activity of gastric aspirate, PEG_i = [^{14}C]-PEG activity of instilled solution.

The total amounts of H^+, Na^+ and K^+ in the solutions were calculated from the product of volume and concentration. The net ion flux was then calculated as the difference between the initial and final amounts in μEq/30 min.

At the end of each experiment the stomach was removed and opened along the greater curvature.

The extent of lesion formation in the glandular region of the stomach was estimated using a grid system, and expressed as a percentage of the total surface area of the glandular mucosa.

Materials

Acetylsalicylic acid (aspirin) and polyethylene glycol-4000 (BDH Ltd), [^{14}C]-polyethylene glycol-4000 (Radiochemical Centre, Amersham), mannitol (Sigma), prostaglandins E_2 and A_2 (PGE_2 and PGA_2, Cambrian) were used. All solutions were pre-warmed for 5 min at 37°C before instillation into the stomach.

Analysis of results

In analysing the data, allowance has been made for inter-preparation variability and any time-dependent changes that might occur during the course of a 2.5 h experiment. For this purpose the effect of aspirin on the measured parameters, recorded during periods 4 and 5, has been expressed as the change (Δ) from the corresponding control periods; the measurements for period 3 are not shown since they were affected by changes in the composition of the instillate. Thus, the total flux of each ion during the hour following aspirin treatment (periods 4 and 5 combined), was compared with the total flux during the hour prior to aspirin (periods 1 and 2 combined), and the appropriate Δ values calculated. For p.d., each Δ value was calculated as the difference between the control value at the end of period 2 and the test value at the end of period 5.

Results have been expressed as mean ± s.e.mean. The difference between two samples was examined statistically using the Mann Whitney U test as described by Siegel (1956). A two-tailed test was used and P values of less than 0.05 were considered to be significant.

Results

Control experiments

As shown in Table 1, when control conditions were maintained throughout all five 30 min experimental periods, transepithelial p.d., net mucosal to serosal (m–s) flux of H^+ and net serosal to mucosal (s–m) flux of Na^+ remained relatively constant. However, the net serosal to mucosal (s–m) flux of K^+ fell during the course of the experiment, the change being particularly marked between periods 1 and 2. In addition, a small degree of lesion formation occurred even in the absence of topical aspirin.

Comparison of buffered and unbuffered aspirin

Since the main purpose of the present study was to investigate the possible role of acid in the damage induced by aspirin in the gastric mucosa, it was important to establish the absolute necessity of acid in lesion formation in the present experimental situation. Therefore experiments were carried out to compare the effects of 20 mM aspirin, both in buffered and unbuffered solutions, on gastric mucosal permeability and lesion formation.

The results are shown in Figure 1. Unbuffered 20 mM aspirin produced significant changes in p.d. and ion flux compared with the corresponding control values and caused marked lesion formation. However, when buffered, the same concentration of aspirin failed to produce any significant changes in these parameters.

The effect of increasing concentrations of unbuffered aspirin

Having established the dependence of the damaging effects of topical aspirin on the presence of acid, the relationship between gastric mucosal permeability,

Table 1 Control experiments: lesion formation, transepithelial potential difference (p.d.) and net ion fluxes in rat gastric mucosa during five consecutive 30 min periods

30 min periods	**p.d.* (mV)	***net m–s flux H^+* (μEq/30 min)	***net s–m flux Na^+* (μEq/30 min)	***s–m flux K^+* (μEq/30 min)	*% lesion formation*
1	52.8 ± 1.7	42.8 ± 4.5	20.5 ± 1.9	2.9 ± 0.5	
2	53.2 ± 1.2	56.3 ± 6.9	17.8 ± 1.8	2.0 ± 0.1	
3	52.7 ± 0.9	50.4 ± 9.0	18.8 ± 1.6	1.8 ± 0.1	
4	52.5 ± 1.0	50.6 ± 8.9	21.5 ± 1.0	1.6 ± 0.2	
5	50.0 ± 2.4	50.1 ± 4.9	20.0 ± 1.0	1.4 ± 0.2	7.7 ± 0.8

*The polarity of the p.d. was such that the mucosal surface was negative with respect to the reference electrode.
**m–s indicates that the net ion flux was in the direction of mucosa to serosa. s–m indicates that the net ion flux was in the direction of serosa to mucosa.
Each value is the mean of six observations.

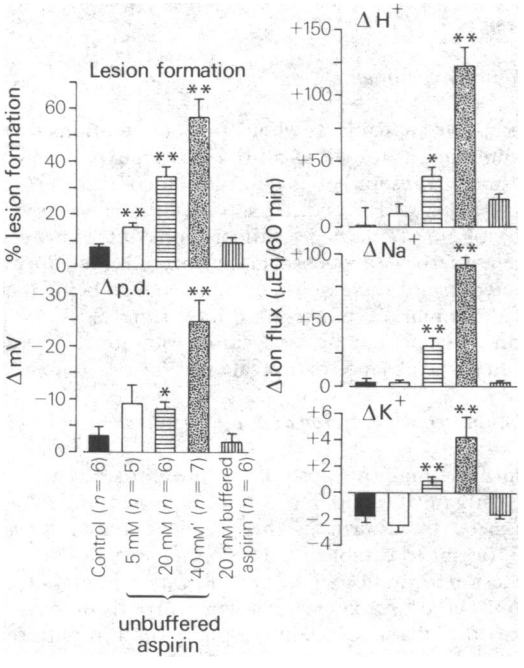

Figure 1 The effect of aspirin on lesion formation, transepithelial potential difference (p.d.) and net ion fluxes in rat gastric mucosa. The control Δ values are calculated from the original data recorded in Table 1. The sign given to the Δ values (ordinate axes) refers to the direction in which the changes occurred: + indicates a mean increase; − indicates a mean decrease. Statistical comparisons are made with the appropriate control data; $*P<0.05$, $**P<0.01$. *n* as indicated.

especially to H^+ ions, and lesion formation was investigated using three concentrations of aspirin viz., 5 mM, 20 mM and '40 mM'. The results are recorded in Figure 1.

The most significant observation in this series of experiments was that the topical application of 5 mM aspirin to the gastric mucosa caused a significant increase in gastric mucosal lesion formation without a concomitant increase in the rate of back-diffusion of H^+ ions. Indeed, measurement of net s–m flux of Na^+ and K^+ ions also failed to reveal any change in the ionic permeability of the gastric mucosa, and there was no significant change in transepithelial p.d.

The higher concentrations of unbuffered aspirin of 20 mM and '40 mM' produced significant increases in lesion formation, p.d. and the rate of flux of H^+, Na^+ and K^+ ions, indicating a definite increase in mucosal permeability.

Effect of prostaglandin E_2 on the gastric mucosa

As a prerequisite for studying the effect of PGE_2 on the aspirin-induced changes, it was necessary to determine the effect of PGE_2 alone. Lesion formation, p.d. and ion flux values are recorded in Table 2 and compared with the corresponding control values given in Table 1. At the high concentration of 10^{-4} M, PGE_2 had no significant effect on mucosal p.d., lesion formation or net flux of H^+ or K^+ ions. A small but significant increase in net s–m flux of Na^+ ions did occur during period 5 of the experiment. However, since the change in Na^+ ion flux was not consistent (it did not occur during periods 3 and 4) and no change in mucosal permeability was revealed by the other parameters measured, it was considered that this would not interfere with subsequent interpretation of the effect of PGE_2 on the aspirin-induced changes.

The effect of topical prostaglandin E_2 on aspirin-treated gastric mucosa

As topical '40 mM' aspirin caused the most marked and distinct changes in all of the parameters measured, this dose of aspirin was used in experiments on PGE_2. The results are shown in Figure 2. The series of '40 mM' aspirin controls was repeated, and there

Table 2 The effect of topical prostaglandin E_2 (PGE_2 10^{-4} M) on lesion formation, transepithelial potential difference (p.d.) and net ion fluxes in rat gastric mucosa

30 min periods	*p.d.* (mV)	*net m–s flux H^+* (μEq/30 min)	*net s–m flux Na^+* (μEq/30 min)	*net s–m flux K^+* (μEq/30 min)	*% lesion formation*
1	55.4 ± 1.1	32.5 ± 6.7	22.6 ± 1.9	2.9 ± 0.2	
2	54.0 ± 2.8	44.2 ± 3.2	21.0 ± 1.5	1.8 ± 0.1	
3	52.4 ± 3.0	44.9 ± 2.9	26.0 ± 2.7	1.7 ± 0.2	
4	52.0 ± 1.8	48.1 ± 6.0	24.2 ± 2.4	1.5 ± 0.2	
5	50.6 ± 2.8	43.0 ± 4.2	26.3 ± 1.7*	1.4 ± 0.2	6.0 ± 1.4

PGE_2 (10^{-4} M) was applied topically to the gastric mucosa during periods 2 and 3.
Each value is the mean of 5 observations.
Statistical comparison of p.d., ion fluxes and lesion formation was made with the corresponding control values in Table 1. $*P<0.05$.

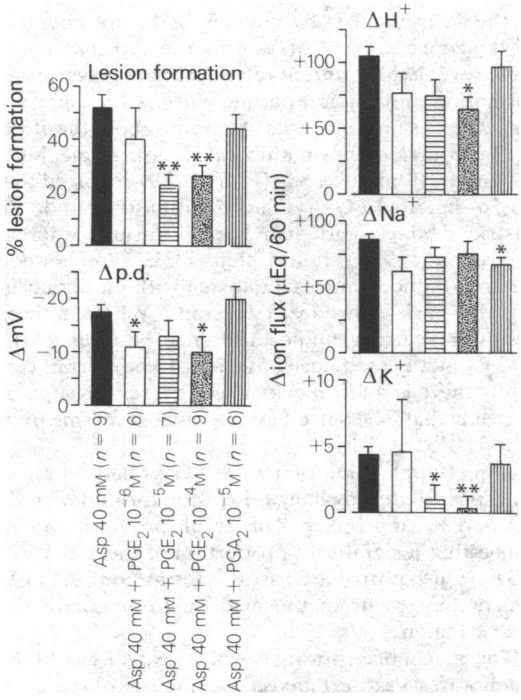

Figure 2 The effect of prostaglandin E_2 (PGE_2) or PGA_2 on aspirin (Asp)-induced changes in lesion formation, transepithelial p.d. and net ion fluxes in rat gastric mucosa. The sign given to the Δ values (ordinate axes) is explained in Figure 1. Statistical comparisons are made with the data obtained using aspirin alone: $*P<0.05$, $**P<0.01$. *n* as indicated.

were no significant differences in lesion formation, Δ p.d. or Δ ion flux values for the two sets of data (cf. Figure 1); a result indicating the consistent nature of the response to topical '40 mM' aspirin.

PGE_2 produced a complex effect on the changes induced by aspirin. The prostaglandin did inhibit aspirin-induced lesion formation, but not in a dose-related manner. At a concentration of 10^{-5} M, PGE_2 reduced lesion formation from $52.1 \pm 5.1\%$ to $23.0 \pm 3.6\%$, and this was not further diminished by a tenfold increase in the concentration of PGE_2. Concentrations of PGE_2 of 10^{-6} M and 10^{-4} M significantly reduced the fall in transepithelial p.d., although only 10^{-4} M PGE_2 ameliorated aspirin-induced lesion formation. Conversely, 10^{-5} M PGE_2 inhibited lesion formation without significantly changing the p.d. The results also indicate that Na^+ ion flux is a poor index of mucosal damage since concentrations of PGE_2 of both 10^{-4} M and 10^{-5} M reduced lesion formation without producing a significant effect on net Na^+ ion flux. It is of interest to note that a significant inhibition of lesion formation (10^{-5} and 10^{-4} M PGE_2) was accompanied by a significant reduction in net K^+ ion flux into the gastric lumen.

In addition, the results show that PGE_2, at concentrations of 10^{-6} M and 10^{-5} M, produced respectively small and large (significant) inhibitions of lesion formation without any significant changes in the aspirin-induced m–s flux of H^+ ions. At a concentration of 10^{-4} M, PGE_2 significantly reduced both lesion formation and the net m–s H^+ ion flux.

The effect of topical prostaglandin A_2 on aspirin-treated gastric mucosa

Since PGE_2 is converted to PGA_2 in the presence of acid (Bindra & Bindra, 1977), the possibility existed that PGE_2 was converted to PGA_2 in the gastric lumen and that the inhibition of lesion formation was due to the activity of the latter compound. However, the results given in Figure 2 show that this is unlikely because 10^{-5} M PGA_2 failed to inhibit significantly aspirin-induced lesion formation.

Discussion

In the present experiments the relationship between gastric lesion formation and ionic permeability of the gastric mucosa, particularly to H^+ ions, has been studied. Besides measuring net m–s flux of H^+ ions, mucosal permeability was assessed by measuring transepithelial p.d. and net s–m flux of Na^+ and K^+ ions (Ivey, 1971). However, it should be pointed out that these latter parameters, particularly p.d. and net K^+ ion flux, should be interpreted with some caution (Thjodleifsson & Wormsley, 1977; Fromm, 1979). For example, the transepithelial p.d. may be affected not only by mucosal permeability (resistance), but also by active ion transport (Tarnawski & Ivey, 1980). In addition, an increase in net s–m K^+ ion flux may be the result of both a change in mucosal permeability and the exfoliation of cells into the gastric lumen (Davenport, 1965). Nevertheless, it was considered that measurement of p.d. and net fluxes of H^+, Na^+ and K^+ ions would provide more information about mucosal permeability than measurement of H^+ ion flux alone.

In this study a low incidence of gastric lesion formation was observed in the absence of topical aspirin. This effect might be caused by the insertion of polythene catheters into the stomach, or by the prolonged exposure to exogenous HCl. However, the effect of aspirin or PGE_2 was always compared with the low level of lesion formation observed in the control experiments.

The data with buffered and unbuffered 20 mM aspirin confirm previous observations (several authors cited by Cooke, 1973) that the presence of acid in the gastric lumen is an absolute requirement for the ulcerogenic effect of topical aspirin. This result is

predictable since the pK_A value for aspirin is 3.5, and at higher pH values the lipid solubility and rate of absorption of the compound are diminished (Cooke, 1973). The role of acid back-diffusion in the aetiology of aspirin-induced gastric mucosal damage is a more contentious subject. Topical application of 5 mM aspirin to the gastric mucosal surface produced a significant increase in the level of lesion formation without changing the rate of acid back-diffusion. This result suggests that under these conditions aspirin damages the gastric mucosa through a direct irritant effect, and not via an increase in the rate of flux of H^+ ions into the mucosal tissue. Raising the concentrations of aspirin to 20 mM and '40 mM' increased both the degree of lesion formation and the rate of acid back-diffusion in a dose-related manner. A possible interpretation of these observations is that relatively low concentrations of topically applied aspirin damage the gastric mucosa through a direct irritant action, but that this is not severe enough to allow a detectable increase in the net m–s flux of H^+ ions across the mucosa. In the presence of higher concentrations of aspirin the damage to the gastric mucosa was sufficient to allow a measurable flux of H^+ ions down their concentration gradient. It is possible that this flux of acid into the mucosal tissue then exacerbates the direct damaging effect of aspirin.

The topical application of PGE_2 alone to the gastric mucosa had little effect on the parameters measured, although it did cause a small, inconsistent increase in net s–m Na^+ ion flux. It is reported that topical 16,16-dimethyl PGE_2 stimulates the secretion of HCO_3^- *in vivo* (Kauffman, Reeve & Grossman, 1980), and it is probable that this secretion is coupled to Na^+ co-ion (Allen & Garner, 1980). However it is unlikely that the increase of flux of Na^+ ions into the gastric lumen, observed in the present work, is due to stimulation of $NaHCO_3$ secretion by PGE_2 since no apparent increase in net m–s flux of H^+ ions occurred. Bolton & Cohen (1979) found that topical 16,16-dimethyl PGE_2 stimulated the net s–m fluxes of both Na^+ and Cl^- ions. A similar effect of PGE_2 in the present study would explain the small secretion of Na^+ ions, although such a mechanism could not be confirmed since Cl^- ion flux was not measured.

Concentrations of PGE_2 of 10^{-5} M and 10^{-4} M ameliorated the gastric mucosal damage induced by '40 mM' aspirin. Although this effect of 10^{-4} M PGE_2 was accompanied by a significant reduction in the aspirin-induced net m–s H^+ ion flux, the effect of the lower concentration of PGE_2 was not. Thus in the present work the inhibition of lesion formation was not necessarily accompanied by a significant reduction in the rate of acid back-diffusion. The maximal inhibition of aspirin-induced lesion formation by PGE_2 was only 56%, and it is possible that treatment of the gastric mucosa with PGE_2 in the presence of a lower concentration of aspirin (i.e. 20 mM) might have revealed a different relationship between lesion formation and H^+ ion permeability. At the present time there is no concensus of opinion about the effect of E prostaglandins on mucosal H^+ ion permeability in the rat. Bommelaer & Guth (1979) reported that 16,16-dimethyl PGE_2 reduced both aspirin-induced gastric damage and acid back-diffusion, whereas Puurunen (1980) found that PGE_2 ameliorated ethanol-induced mucosal damage without diminishing the back-diffusion of H^+ ions. Whether these results reveal a genuine difference between PGE_2 and its methylated derivative is not known, but certainly there is no firm evidence at present to support the view that PGE_2 itself affects mucosal permeability to H^+ ions.

Direct application of 10^{-5} M PGA_2 had no effect on aspirin-induced changes in lesion formation, p.d., H^+ and K^+ ion fluxes. This result provides no evidence that a significant proportion of the activity of PGE_2 can be attributed to the formation of PGA_2 which may occur in the acid environment of the gastric lumen.

The mechanism through which exogenous PGE_2 ameliorates gastric mucosal damage is obscure although it may involve the replacement of endogenous PGE_2, the amount of which is diminished by aspirin treatment (Konturek, Piastucki, Brzozowski, Radecki, Dembinska-Kiec, Zmuda & Gryglewski, 1981). One possibility is that PGE_2 increases gastric mucosal blood flow and thus prevents excessive accumulation of acid within the mucosal tissue (Whittle, 1980). Indeed, topical administration of 16,16-dimethyl PGE_2 to resting canine gastric mucosa does cause vasodilatation (Cheung, 1980). Another possibility is that PGE_2 averts mucosal damage through the stimulation of mucus and/or bicarbonate secretion (Allen & Garner, 1980). Topical application of PGE_2 to the rat gastric mucosa does stimulate mucus production (Bolton, Palmer & Cohen, 1978), but as discussed above, there was no evidence in the present study that topical PGE_2 caused luminal alkalinization.

Several mechanisms have been implicated in the gastric damage induced by aspirin (Rainsford, 1977; Rainsford & Whitehouse, 1980; Whittle, 1980) including an increased permeability of the mucosa to H^+ ions (Davenport, 1967). However, considering all of the data presented in the present investigation, it is possible that lesion formation in rat gastric mucosa is not primarily determined by the permeability of the epithelium to H^+ ions. This conclusion is based on the observations that under certain circumstances aspirin may damage the mucosa without an increase in acid back-diffusion, and similarly PGE_2 may inhibit aspirin-induced damage without affect-

ing mucosal permeability to the H^+ ions. Nevertheless, as discussed by Bugat, Thompson, Aures & Grossman (1976), it is still possible that under conditions where a gross change in H^+ ion permeability was not observed, a small localized back-diffusion of H^+ occurred which escaped detection.

The value of measuring changes in p.d. and fluxes of Na^+ and K^+ ions as indices of mucosal permeability must also be considered. The data obtained using aspirin alone do suggest that significant changes in net m-s H^+ ion flux are accompanied by concomitant changes in the other parameters. However, the experiments using both aspirin and PGE_2 (10^{-6} M and 10^{-5} M) showed that changes in p.d. and net flux of K^+ ions may occur without a concomitant change in the rate of acid back-diffusion. Thus, the assumption cannot be made that a change in the permeability of the mucosa to one particular ion indicates a general increase in ionic permeability.

References

ALLEN, A. & GARNER, A. (1980). Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. *Gut*, **21**, 249–262.

BINDRA, J.S. & BINDRA, R. (1977). *Prostaglandin Synthesis*. p. 342. New York: Academic Press.

BOLTON, J.P. & COHEN, M.M. (1979). Effect of 16,16-dimethyl prostaglandin E_2 on the gastric mucosal barrier. *Gut*, **20**, 513–517.

BOLTON, J.P., PALMER, D. & COHEN, M.M. (1978). Stimulation of mucus and nonparietal cell secretion by the E_2 prostaglandins. *Am. J. dig. Dis.*, **23**, 359–364.

BOMMELAER, G. & GUTH, P.H. (1979). Protection by histamine receptor antagonists and prostaglandin against gastric mucosal barrier disruption in the rat. *Gastroenterology*, **77**, 303–308.

BUGAT, R., THOMPSON, M.R., AURES, D. & GROSSMAN, M.I. (1976). Gastric mucosal lesions produced by intravenous infusion of aspirin in cats. *Gastroenterology*, **71**, 754–759.

CHEUNG, L.Y. (1980). Topical effects of 16,16-dimethyl prostaglandin E_2 on gastric blood flow in dogs. *Am. J. Physiol.*, **238**, G514–G519.

COOKE, A.R. (1973). The role of acid in the pathogenesis of aspirin-induced gastrointestinal erosions and haemorrhage. *Am. J. dig. Dis.*, **18**, 225–237.

DAVENPORT, H.W. (1965). Potassium fluxes across the resting and stimulated gastric mucosa; injury by salicyclic acid and acetic acids. *Gastroenterology*, **49**, 238–245.

DAVENPORT, H.W. (1967). Salicylate damage to the gastric mucosal barrier. *New Eng. J. Med.*, **276**, 1307–1312.

FROMM, D. (1978). Salicylate and gastric mucosal damage. *Pediatrics*, **62**, 938–942.

FROMM, D. (1979). Gastric mucosal 'barrier'. *Gastroenterology*, **77**, 396–398.

IVEY, K.J. (1971). Gastric mucosal barrier. *Gastroenterology*, **61**, 247–257.

KAUFFMAN, G.L., REEVE, J.J. & GROSSMAN, M.I. (1980). Gastric bicarbonate secretion: effect of topical and intravenous 16,16-dimethyl prostaglandin E_2. *Am. J. Physiol.*, **239**, G44–G48.

KONTUREK, S.J., PIASTUCKI, I., BRZOZOWSKI, T., RADECKI, T., DEMBINSKA-KIEC, A., ZMUDA, A. & GRYGLEWSKI, R. (1981). Role of prostaglandins in the formation of aspirin-induced gastric ulcers. *Gastroenterology*, **80**, 4–9.

MILLER, T.A. & TEPPERMAN, B.L. (1979). Effect of prostaglandin E_2 on aspirin-induced gastric mucosal injury. *J. Surg. Res.*, **26**, 10–17.

PUURUNEN, J. (1980). Effect of prostaglandin E_2, cimetidine and atropine on ethanol-induced gastric mucosal damage in the rat. *Scand. J. Gastroent.*, **15**, 485–488.

RAINSFORD, K.D. (1977). Gastrointestinal and other side-effects from the use of aspirin and related drugs; biochemical studies on the mechanisms of gastrotoxicity. In *Aspirin and Related Drugs: their Actions and Uses. Agents & Actions*, suppl. 1, pp. 59–70. ed. Rainsford, K.D., Brune, K. & Whitehouse, M.W. Basel: Birkhauser.

RAINSFORD, K.D. & WHITEHOUSE, M.W. (1980). Biochemical gastroprotection from acute ulceration induced by aspirin and related drugs. *Biochem. Pharmac.*, **29**, 1281–1289.

SIEGEL, S. (1956). Nonparametric Statistics for the Behavioural Sciences. pp. 116–127. Tokyo: McGraw-Hill Kogakusha Limited.

TARNAWSKI, A. & IVEY, K.J. (1980). Effect of pentagastrin on potential difference in rat stomach. *Proc. Soc. exp. Biol. Med.*, **163**, 162–166.

THJODLEIFSSON, B. & WORMSLEY, K.G. (1977). Back-diffusion – fact or fiction. *Digestion*, **15**, 53–72.

WHITTLE, B.J.R. (1980). Role of prostaglandins in the defence of the gastric mucosa. *Brain. Res. Bull.*, **5** (suppl. 1) 7–14.

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