Gastric adaptation to injury by repeated doses of aspirin strengthens mucosal defence against subsequent exposure to various strong irritants in rats

T Brzozowski, P C Konturek, S J Konturek, H Ernst, J Stachura, E G Hahn

Abstract
Gastric adaptation to injury during repeated doses of acetyl salicylic acid (ASA) is a well documented finding but it is not known whether this adaptation affects the tolerance of the mucosa to other strong irritants. Gastric adaptation was induced by repeated daily doses of acidified ASA (100 mg/kg in 1.5 ml of 0.2 N HCl) given intragastrically (series A) rats. Control rats with an intact stomach were given daily intragastric vehicle only (1.5 ml of 0.2 N HCl) (series B). After full adaptation to ASA (5 days), rats were challenged again with acidified ASA or, for comparison, with strong irritants such as 100% ethanol, 200 mM acidified taurocholate, or 25% NaCl for 1 hour or with water immersion and restraint for 3-5 hours. The first dose of ASA produced numerous gastric lesions and deep histological necrosis accompanied by a fall in the gastric blood flow, negligible expression of epidermal growth factor (EGF) and transforming growth factor α (TGFα) or their receptors, and no evidence of mucosal proliferation. As adaptation to ASA developed, however, the areas of gastric lesions were reduced by more than 80% and there was a noticeable decrease in deep necrosis, a partial restoration of gastric blood flow, an approximately four-fold increase in EGF expression (but not in TGFα) and its receptors, and an appreciable increase in mucosal cell proliferation compared with vehicle treated rats. Increases in the mucosal expression of EGF receptors and the luminal content of EGF were also found in ASA adapted animals. In ASA adapted rats subsequently challenged with 100% ethanol, 200 mM TC, 25% NaCl, or stress, the area of the gastric lesions and deep histological necrosis were appreciably reduced compared with values in vehicle treated rats. This increased mucosal tolerance to strong irritants was also accompanied by the return of the gastric blood flow towards control levels and further significant increases in the mucosal expression of EGF receptors and mucosal cell proliferation. Gastric adaptation to ASA enhances the mucosal resistance to injury by strong irritants probably as a result of the restoration of the gastric blood flow and increased cell proliferation that may result from increased mucosal expression of EGF and its receptors.

Keywords: gastric adaptation, gastric blood flow, cytoprotection, epidermal growth factor.

Long term gastric adaptation develops in response to repeated exposure of the gastric mucosa to various irritants. This adaptation was originally shown in gastric mucosa exposed to non-steroidal anti-inflammatory drugs (NSAID) including acetyl salicylic acid (ASA) and indomethacin, necrotising substances, but neither the mechanism of this adaptation nor its influence on gastric mucosal defence against the damage by necrotising substances has been explored.

ASA and related agents given for the first time in a single dose may cause widespread damage to the surface epithelium and deep haemorrhagic lesions. The pathogenesis of NSAID gastropathy is poorly understood but it has been attributed to the topical irritation of the mucosa associated with the inhibition of mucosal prostaglandin generation and vascular injury as a result of neutrophil activation and thrombi formation.

We reported recently that with repeated daily doses of ASA, mucosal adaptation develops within three to four days and persists for about six days; there is increased mucosal tolerance to further insults by ASA without significant injury. Which factors or mechanisms are responsible for this long term adaptation and how the development of the adaptation could contribute to the integrity of the gastric mucosa and its defence against the injury by strong irritants remains unexplained.

This study was designed:
- To examine the role of gastric mucosal blood flow, mucosal expression of epidermal growth factor (EGF) and transforming growth factor α (TGFα) and their receptors, and mucosal cell proliferation in the mechanism of gastric adaptation to ASA;
- To compare the injurious effects of strong irritants on the ASA adapted stomach and the unadapted, vehicle treated stomach; and
- To assess the mechanisms responsible for the increased mucosal defence of the ASA adapted gastric mucosa against various strong irritants.

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Methods

PRODUCTION OF GASTRIC LESIONS AND INDUCTION OF GASTRIC ADAPTATION

Acute gastric lesions were induced by an intragastric application of acidified ASA in male Wistar rats weighing 180-220 g. The animals were fasted overnight in individual cages but had free access to water. There were two series of rats, A and B, each comprising 80 rats. Series A animals received intragastric ASA (100 mg/kg dissolved in 1·5 ml of 0·2 N HCl daily) and series B animals were given intragastric vehicle only (1·5 ml of 0·2 N HCl daily).

Gastric adaptation in series A rats was achieved by repeated intragastric administration of acidified ASA, daily for five consecutive days as described in detail previously.18 Briefly, one group of rats received acidified ASA at a dose of 100 mg/kg at 10·00 am and was killed one hour later (day 0). Another group also received acidified ASA (100 mg/kg) at 10·00 am but one hour later was refed until 5·00 pm, fasted overnight, given acidified ASA the next day at 10·00 am, and killed one hour later (day 1). Other groups underwent the same schedule of refeeding and refasting for 2, 3, or 4 consecutive days (day 2, 3, and 4, respectively) after the first exposure to ASA and were then killed. Next day, after five repeated daily doses of acidified ASA, when the adaptation to ASA was fully developed, and after an 18 hour fast rats of series A were divided into five groups (each consisting 8-10 animals) and challenged intragastrically with 1·5 ml of one of the following: acidified ASA, 100% ethanol, 200 mM taurocholate, or 25% NaCl and killed after one hour or underwent water immersion and restraint stress and were killed after 3-5 hours as described before.12 For comparison, unadapted, vehicle treated rats with an intact stomach (series B) treated intragastrically for four days with 1·5 ml of saline were divided into five groups of 8-10 rats each and were challenged with the same ulcerogens as the rats in series A.

MEASUREMENT OF THE GASTRIC BLOOD FLOW

One hour after each of the topical ulcerogens had been applied intragastrically or after 3-5 hours of stress, the rats were lightly anaesthetised with ether and the gastric blood flow was measured in the oxyntic gland area of the stomach by means of local H2 gas clearance technique using an electrolytic regional blood flow meter (Biomedical Science, Model RBF-2, Japan). The double needle electrodes were inserted through the serosa into the mucosa, one electrode was used for local generation of H2 gas and the other for the measurement of tissues H2. With this technique, the H2 generated by the electrode is carried away by the flowing blood and the polarographic current detector gives the decreasing tissue H2 as the clearance curve, which is then used to calculate absolute values of the blood flow (ml/100 g-min) in the tissue as described previously.19 The mucosal blood flow was measured in the oxyntic gland area of the stomach that did not show macroscopically visible mucosal lesions, and the mean value of three consecutive recordings was calculated and expressed as a percentage of the control values recorded in the oxyntic mucosa of the stomach in vehicle treated rats. The stomachs were then removed, opened along the greater curvature, and pinned open for macroscopic determination of the area and the number of gastric lesions by computerised planimetry (Morphomat 10, Carl Zeiss, Berlin, Germany) by two investigators unaware of the treatment given.

DETERMINATION OF MUCOSAL GENERATION OF PROSTAGLANDIN E2

In series A and B rats, mucosal biopsy samples of the oxyntic gland area (about 100 mg) were taken immediately after the animals had been killed to determine the mucosal generation of prostaglandin E2 by specific radioimmunoassay (RIA) as described previously.19 The mucosal sample was placed in preweighed Eppendorf vials and 1 ml of Tris buffer (50 mM, pH 3-5) was added to each vial. The samples were finely minced (for about 15 seconds) with scissors, washed, and centrifuged for 10 seconds, and the pellet was resuspended in 1 ml of Tris. Each sample was then incubated on a vortex mixer for 1 minute and centrifuged for 15 seconds. The pellet was weighed and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) and kept at −20°C until RIA. Prostaglandin E2 was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The capability of the mucosa to generate prostaglandin E2 was expressed in ng/g wet tissue weight.

DETERMINATION OF MUCOSAL EXPRESSION OF EGF AND TGFβ AND THE MUCOSAL CONTENT OF EGF

For the determination of the luminal concentration of EGF, the cardia and the pylorus were ligated (after the blood flow had been measured) and 1 ml of cold saline was instilled into the stomach to wash out the gastric contents. Each sample was centrifuged: the supernatant was collected, neutralised with 0·1 N Na(OH) to pH 7·0, and frozen at −20°C until EGF RIA. In series A and B, the mucosal samples (about 100 mg) were excised from the oxyntic gland area of the stomach for determination of the mucosal EGF content by RIA.18 The tissue samples were immediately weighed, homogenised in ice cold 0·32 M Tris-HCl buffer, and centrifuged: the supernatant was collected and frozen at −20°C until EGF radioimmunoassay. The EGF antiserum (gift of Dr H Gregory, ICI, Alderley Park, UK), raised in rabbits against human EGF was used at a final dilution of 1:210 000 and this antiserum recognised equally rat and human EGF. Iodinated ([3-125I]iodotyrosyl) peptide and rat EGF were calibration standards (Amersham, UK). The detection limit of the assay was
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0.01 nmol/l. The intrassay and interassay precision were about 12% and 10%, respectively.

MUCOSAL HISTOLOGY AND EXPRESSION OF EGF AND TGFα AND THEIR RECEPTOR

In some experiments, standardised strips incorporating the total width of stomachs were fixed in 10% buffered formalin and paraffin sections were stained with haematoxylin and eosin (H & E) for histological evaluation. A Nikon microscope equipped with microplan II digital image system was used for the quantitative histological examination (morphometry) of the sections. Coded specimens of mucosa stained with H & E were evaluated quantitatively under 500× magnification in blinded conditions. The disrupted surface of the mucosal strips denuded of epithelium, the deep necrotic lesions penetrating the mucosa, and the strip length with regeneration of the surface epithelium cells were measured and expressed as a percentage of the total. Coded specimens of mucosa stained with H & E were evaluated quantitatively under 500× magnification in blinded conditions. In addition, serial sections of paraffin embedded blocks were dewaxed, rehydrated, slides pretreated with citrate buffer (pH 6) in a microwave oven (3×5 min), and immunostained with specific monoclonal antibodies against EGF (1:40, GF0.1, Oncogene), TGFα (1:20, GF10

<table>
<thead>
<tr>
<th>Lesion area (nm²)</th>
<th>PGE₂ (ng/g) (%)</th>
<th>GBF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (control)</td>
<td>220 (13)</td>
<td>100</td>
</tr>
<tr>
<td>Aciddified ASA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>59.6 (8-4)</td>
<td>23 (4)</td>
</tr>
<tr>
<td>Day 1</td>
<td>17 (3)</td>
<td>65 (4)</td>
</tr>
<tr>
<td>Day 2</td>
<td>12.7 (4-3)*</td>
<td>12 (2)</td>
</tr>
<tr>
<td>Day 3</td>
<td>8.5 (3-7)*</td>
<td>13 (3)</td>
</tr>
<tr>
<td>Day 4</td>
<td>5.2 (1-8)*</td>
<td>10 (1)</td>
</tr>
</tbody>
</table>

*Indicates a significant decrease in the area of gastric lesions below the value recorded with ASA given at day 0. +Indicates a significant increase above the value of GBF obtained with ASA given at day 0.

Figure 1: Typical macroscopic appearance of the stomach of rats after the first challenge (upper level) with aciddified ASA (on the left), 100% ethanol (in the middle) or water immersion and restraint stress (on the right) in the unadapted animals. The lower level shows the stomach after the challenge with the aciddified ASA (on the left), 100% ethanol (in the middle), or stress (on the right) in rats adapted to repeated administration of aciddified ASA. Note that the lesions in ASA adapted stomach were small and scattered mostly in the oxyntic gland area.

Table 1: Mean area of gastric lesions, prostaglandin E₂ (PGE₂) generation, and gastric blood flow (GBF) (expressed as percentage of the control value in the mucosa treated with vehicle) after the first (day 0) administration of aciddified aspirin (ASA) (100 mg/kg) and ASA given at the same dose for four days. Results are mean (SEM) for 8–10 rats

Results

GASTRIC DAMAGE INDUCED BY ASA AND DEVELOPMENT OF THE ADAPTATION TO ASA

Aciddified ASA, given orally for the first time (once), produced an increase in gastric erosions at a dose of 100 mg/kg that reached a mean area of about 60 mm² (Table I). The area of gastric lesions was already significantly decreased the next day after the rechallenge with aciddified ASA. After the next 4 days of ASA rechallenge, a marked decrease in the area of macroscopic gastric lesions was observed, indicating that full adaptation to aciddified ASA had been achieved (Fig 1).

Gastric blood flow, which in the intact, vehicle treated stomach averaged 49 (7) ml/min per 100 g, was significantly decreased by about 60% after the first exposure to ASA (Table I). Then, after rechallenge with ASA (day 1), it tended to return to control values over the next four consecutive exposures to ASA (Table I). Mucosal generation of prostaglandin E₂ in the vehicle treated mucosa averaged about 220 (13) ng/g of wet tissue: the single exposure to aciddified ASA reduced the mucosal generation of prostaglandin E₂ by about 90% (Table I). The suppression of prostaglandin E₂ generation persisted during all successive days of ASA treatment.

In the rats with an intact, unadapted stomach, intra gastric aciddified ASA (100 mg/kg in 0.2 N HCl), 100% ethanol, 200 mM TC, 25% NaCl, or exposure to 3-5 hours of stress resulted in the formation of gastric mucosal

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lesions with mean areas of 58 (6), 82 (8), 40 (5), 32 (4), or 10 (1) mm² respectively (Fig 2).
This was accompanied by a severe reduction in the gastric blood flow compared with the value obtained in gastric mucosa treated only with vehicle. In rats with ASA adaptation subsequently challenged on day 5 with acidified ASA (100 mg/kg), 100% ethanol, 200 mM TC, 25% NaCl, or stress, the areas of gastric lesions were reduced by 95%, 88%, 92%, 93%, or 90% respectively compared with respective values obtained in vehicle treated, unadapted control rats exposed to the same irritants. This reduction in the formation of acute gastric lesions in ASA adapted rats exposed to the irritants was accompanied by a significant rise in the gastric blood flow compared with that observed after each irritant in unadapted animals.

**HISTOLOGICAL FINDINGS**
Single exposure to acidified ASA resulted in hyperaemia of the gastric wall with acutely inflamed and oedematous submucosa. In the gastric mucosa of rats exposed to ASA, the deep necrotic lesions occupied about 25% of the mucosal strip length (Table II). About 45% of the mucosa was, however, denuded of the surface epithelium in these rats and no regeneration of gastric mucosa was observed. In contrast, the gastric mucosa of rats adapted to ASA treatment showed remarkable regenerative changes, with elongation of the neck and foveolar areas lined with cuboidal regenerative epithelium. In the submucosa, the inflammatory infiltrate was mostly composed of polymorphonuclear and mononuclear cells. The macroscopic injury was considerably reduced – only about 5% of the mucosa was denuded of surface epithelium and 4% of the mucosal length was involved in deep necrotic lesions.

In unadapted rats with an intact stomach, single insults of acidified ASA, 100% ethanol, 200 mM TC, 25% NaCl, or stress meant that deep mucosal necrosis occupied about 27%, 34%, 28%, 19%, or 14% of mucosal strip length, respectively, and the lack of regenerative changes was noticed in these animals. In contrast, rats adapted to ASA and challenged with 100% ethanol, 200 mM TC, 25% NaCl, acidified ASA, or stress, showed a significant reduction in the deep necrosis associated with the increase in mucosal regeneration compared with changes observed in unadapted rats exposed to each of the irritants (Table II).

**MUCOSAL EXPRESSION OF EGF AND TGFα AND THEIR RECEPTORS AND LUMINAL AND MUCOSAL CONTENTS OF EGF**
As shown in Figure 3, the expression of EGF, which was negligible in the intact, vehicle treated mucosa (staining intensity about 0-18 (0-05)), was increased in the ASA adapted stomach – approximately fourfold mainly at the neck area of gastric glands and threefold at the gland base (Figs 4, 5, and 6). The expression of TGFα in the vehicle treated, unadapted mucosa was relatively higher than that of EGF, with staining intensity averaging about 1-5

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**TABLE II** Quantitative histology of gastric mucosa after first exposure (day 0) to acidified ASA (100 mg/kg) and after four subsequent exposures to acidified ASA during 8 day’s treatment with that agent and then with or without challenge with strong irritants. Mean (SEM) of 8-10 rats per group. Results are expressed as a percentage of the mucosal strip length.

<table>
<thead>
<tr>
<th></th>
<th>Denuded surface</th>
<th>Deep necrosis</th>
<th>Mucosal regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASA:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>45 (6-5)</td>
<td>25 (4-3)</td>
<td>0</td>
</tr>
<tr>
<td>Day 4</td>
<td>5-8 (1-2)*</td>
<td>2-8 (0-4)*</td>
<td>47-3 (8-3)*</td>
</tr>
<tr>
<td>Irritant in unadapted stomach:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>58-2 (7-4)</td>
<td>34-4 (6-5)</td>
<td>0</td>
</tr>
<tr>
<td>200 mM taurocholate</td>
<td>33-5 (6-5)</td>
<td>28-8 (5-6)</td>
<td>0</td>
</tr>
<tr>
<td>25% NaCl</td>
<td>24-8 (3-8)</td>
<td>19-5 (2-2)</td>
<td>0</td>
</tr>
<tr>
<td>ASA</td>
<td>46-6 (5-5)</td>
<td>27-4 (4-1)</td>
<td>0</td>
</tr>
<tr>
<td>WRS</td>
<td>39-1 (4-2)</td>
<td>14-2 (2-2)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Irritant in ASA adapted stomach:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>5-4 (1-2)*</td>
<td>3-9 (0-8)*</td>
<td>38-6 (4-2)*</td>
</tr>
<tr>
<td>200 mM taurocholate</td>
<td>4-2 (0-9)*</td>
<td>2-5 (0-6)*</td>
<td>43-7 (6-9)*</td>
</tr>
<tr>
<td>25% NaCl</td>
<td>2-8 (0-7)*</td>
<td>1-2 (0-3)*</td>
<td>52-2 (9-3)*</td>
</tr>
<tr>
<td>ASA</td>
<td>5-0 (1-5)</td>
<td>2-9 (0-7)*</td>
<td>42-5 (7-5)*</td>
</tr>
<tr>
<td>WRS</td>
<td>2-7 (0-8)*</td>
<td>1-8 (0-5)*</td>
<td>37-9 (3-8)*</td>
</tr>
</tbody>
</table>

*Significant change compared with value obtained after first exposure to ASA. WRS=water restraint stress.

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**Figure 3:** Expression of epidermal growth factor (EGF) (in arbitrary units from 0=no EGF staining to 3=maximal staining) in the gastric mucosa adapted to ASA and in the mucosa of intact, vehicle treated stomach at the neck of the gastric glands. Mean (SEM) of 5 rats. *Indicates a significant increase above the control value in unadapted rats.
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Figure 4: Gastric mucosa adapted to ASA. Prominent apical immunoreactivity for epidermal growth factor in the base and the neck regions of gastric glands (original magnification ×63).

Figure 5: Prominent staining for epidermal growth factor in the lumen and apical part of the neck region of the gland in the stomach of rats adapted to ASA (original magnification ×250).

Figure 6: Vehicle treated rats. Weak staining for epidermal growth factor observed only in the neck region of the gastric glands (original magnification ×63).

In the vehicle treated gastric mucosa, EGF receptor (EGFr) was expressed in some neck cells, mainly at their luminal plasma membranes, and in some single parietal cells (Fig 7). After first exposure to acidified ASA, the expression of EGFr was diminished or absent in the area of deep necrotic lesions, while in non-necrotic mucosa the expression of EGFr was not significantly affected compared with that in the intact gastric mucosa (Table III). After repeat ASA insults, a significant increase in EGFr expression was observed in non-necrotic mucosa (Fig 8). In some surface epithelium cells, especially in regenerating mucosa with elongated foveolae and neck region, the expression was seen in both foveolar and neck cells. In these areas of regenerative mucosa, increases in the expression of EGFr in the neck region and the deeper areas of oxyntic mucosa were also observed. The expression of EGFr was diminished or absent in the areas of deep necrotic lesions in unadapted rats challenged with 100% ethanol, 200 mM TC, or 25% NaCl. In contrast, the gastric mucosa of rats adapted to ASA and then challenged with 100% ethanol, 200 mM TC, or 25% NaCl showed enhancement in expression of EGFr similar to that in rats adapted to ASA and challenged with ASA and other irritants, especially in areas with strong regeneration of the mucosal surface area.

Table III shows the immunoreactivity of EGF in the gastric mucosa and in gastric juice in rats adapted to repeated ASA insults with or without the challenge with various strong irritants. The single exposure to ASA failed to affect significantly the mucosal and luminal content of EGF compared with that measured in animals with an intact stomach. The luminal and mucosal EGF contents gradually increased with consecutive ASA insults compared to values in rats exposed to a single dose of ASA. The mucosal content of EGF
remained unchanged in unadapted rats challenged with 100% ethanol, 200 mM TC, or 25% NaCl but it reached significantly higher values in rats exposed to repetitive ASA insults. In rats adapted to ASA and challenged with acidified ASA, 100% ethanol, 200 mM TC, 25% NaCl, or stress, there was a significant increase in the luminal and mucosal contents of EGF compared with values in respective unadapted rats exposed to each of the irritants.

### Discussion

The present study confirms our previous observations\(^{17,18}\) that the rat stomach has the ability to resist further damage despite continuous exposure to acidified ASA. This adaptation is accompanied by restoration of the gastric blood flow, increased expression of EGF and its receptors, and an increase in the luminal EGF contents. Furthermore, the gastric mucosa adapted to ASA is remarkably resistant to the injury caused by necrotising substances such as absolute ethanol, 200 mM TC, 25% NaCl, or by stress. This reduction in the formation of acute gastric lesions in the ASA adapted stomach in response to challenge with noxious agents is accompanied by gastric hyperaemia, a noticeable increase in the mucosal expression of EGF and its receptor, and an impressive increase in mucosal cell regeneration.

Adaptation describes the phenomenon in which visible gastric mucosal injury lessens or resolves completely despite continued insults of injurious agents such as ASA. We have reported that the gastric mucosa adapts to repeated ASA\(^{17,18}\) or stress\(^{12}\) insults and that this is accompanied by gastric hyperaemia, probably mediated by sensory nerves but not by endogenous prostaglandin, at least in the case of adaptation to ASA.\(^{17,18}\) In this study, the first exposure to ASA reduced gastric blood flow considerably but upon adaptation to injury by repeated exposure to ASA, the gastric blood flow tended to return to a value similar to that recorded in the intact mucosa. Mucosal generation of prostaglandin E\(_2\) was inhibited with the first dose of ASA and remained suppressed throughout adaptation to ASA and subsequent insults by various irritants, confirming that endogenous prostaglandins are not required for the development of this adaptation, the accompanying gastric hyperaemia, or increased mucosal defence against various topical irritants.

The role of prostaglandins in the short term adaptation of the gastric mucosa to damage by various ulcerogens was originally examined by Robert et al.\(^{22,23}\) They first showed that oral administration of mild irritants such as 10–20% ethanol, 5% NaCl, or 5 mM TC prevented the massive mucosal damage caused by necrotising agents such as 100% ethanol, 25% NaCl, 80 mM TC, 0·6 N HCl, or 0·2 N NaCl. This effect resembled that induced by exogenous application of prostaglandins\(^{22-24}\) and has been called adaptive cytoprotection. Moreover, mild irritants were found to increase the mucosal capability to generate...
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occurred protective effect of mild
reinforcing or necrotising agents.
It is of interest that adaptive
cytoprotection induced by one type of mild
irritant such as 20% ethanol resulted in an
increased mucosal tolerance to the injury
cauised by a high concentration of not only the
same type of irritant – that is, 100% ethanol
(homocytoprotection) but also of other strong
irritants such as 25% NaCl, strong acid or
strong base (cross protection). The cross
adaptation of mild irritants was further con-
firmed by showing that mild stress induced in
rats by short restraint of animals in the cage,
attenuated the formation of gastric lesions
caused by severe stress as well as by the
exposure of the mucosa to 40% ethanol. The
seven days pretreatment with mild stress or a
low concentration of ethanol prevented the
formation of mucosal lesions in rats subjected
to strong restraint stress or absolute ethanol.
In another study, Uramoto et al have shown that
the gastric mucosa of restraint loaded rats
exerted a greater resistance to the damaging
action of absolute ethanol. In their study, the
protective effect of mild stress was significantly
mitigated by vagotomy or indomethacin sug-
gesting that vagal influences or endogenous
prostaglandin, or both, could be involved in the
mechanism of this cross adaptation.
It is of interest that cross adaptation to mild
irritants has also been demonstrated in vitro,
using cultured surface epithelium treated with
strong ulcerogens. This protective action of mild
irritants was abolished by indomethacin
reinforcing the crucial role of endogenous
prostaglandins in the mediation of this
adaptive response in vitro.

The major finding of our present study is the
demonstration for the first time that mucosa
adapted to repetitive insults of ASA is more
resistant to the damage induced by other
necrotising agents such as 100% ethanol,
200 mM TC, or 25% NaCl. Adaptation to ASA
occurred despite complete suppression of
prostaglandin generation in the gastric
mucosa, and yet the adapted gastric mucosa
was less susceptible to the damage caused by
topical ulcerogens or stress. This is in keeping
with the study of Shorrock et al, carried out in
man, which showed that the adaptation to
indomethacin does not depend upon the local
formation of prostaglandins but is accom-
panied by an increase in the gastric blood flow.

It is interesting that the mucosa adapted to
ASA, which by itself is an acid dependent
ulcerogen, showed enhanced resistance to
damage by both an acid dependent irritants (for
example, stress, TC, or ASA) and acid inde-
pendent irritants (for example, 100% ethanol
or hypertonic NaCl).
Enhanced blood flow and increased cell
proliferation were recently proposed as the
major mechanisms responsible for the
adaptation to ASA but the role of growth
factors, in particular an overexpression of
EGF and its receptors, in this adaptation was
limited to stress induced lesions. Our detailed
histological assessment showed that the first
exposure to ASA leads to widespread damage
of the surface epithelium and deep necrotic
lesions extending into the gastric glands. This
first exposure to ASA caused extensive desqua-
mation of mucosal surface and deep necrosis
but both these changes were reduced during
the development of adaptation to the
ulcerogen and, in the same time, a marked
mucosal regeneration was observed. This
regeneration was remarkably impressive after
repeated ASA insults as shown by the elonga-
tion of gland neck and foveolar areas lined with
cuboidal regenerative epithelium. The mech-
anism of this process is not clear but the
finding that regenerative changes occurred
after single exposure to ASA suggests that the
repetitive dose indicates that initial mucosal damage
is necessary for the rapid restitution and is later
followed by the cell proliferation most evident in the
regenerative zone. This is in keeping with original observation of Schmidt et al that
adaptive protection requires direct contact
and initial surface cell damage and can not be
demonstrated in intact, undamaged gastric
mucosa. Interestingly, gastric adaptation initi-
ated by the first exposure of the gastric mucosa
to an irritant is not limited to ulcerogenic
action of salicylate type of NSAID such as aspirin because, as reported recently, rats
dosed repeatedly with a non-salicylate NSAID
agent, such as diclofenac, also showed progres-
sive reduction in macroscopic and deep
necrotic damage. Further evidence that adap-
tation to ASA is non-specific was provided by
Wallace et al by showing that with chronic
ASA administration, the gastric mucosa
becomes significantly more resistant to damage
induced by the new challenge not only with
ASA but also with other NSAID such as
indomethacin or naproxen.

Similarly to ASA in our study, the initial
exposure to 100% ethanol, 25% NaCl, or
200 mM TC caused widespread damage to the
surface epithelium and deep necrotic lesions
accompanied by a fall in the gastric blood flow
and the absence of the expression of receptors
for EGF. However, rats adapted to ASA and
then challenged with 100% ethanol, 200 mM
TC, or 25% NaCl showed a marked reduction
in ulcerogenesis, and this was accompanied by
a significant rise in gastric blood flow and by
expression of EGF and its receptors to an
extent similar to that observed in rats adapted
to ASA. This suggests that the enhanced
production of EGF and augmentation of
specific receptor sites for this peptide may con-
tribute to the adaptation and to the enhanced
resistance of the gastric mucosa to injury
caused by necrotising agents. The regeneration
of gastric mucosa during gastric adaptation to
ASA is probably mediated predominantly by
EGF because in addition to the rise in the
mucosal expression of EGF and its receptor,
the luminal contents of immunoreactive EGF were significantly increased during adaptation and remained raised in ASA adapted rats challenged with strong irritants. Salivary EGF could play a part in the process of mucosal repair and adaptation because, as previously observed, the formation of submucosal EGF was significantly increased after the exposure of gastric mucosa to acid dependent or acid independent ulcerogens. Indeed, in our present study the luminal content of EGF was markedly increased after adaptation to ASA and after challenge with strong irritants, indicating that EGF of salivary origin may interact with specific receptors over expressed in the mucosa and this may be essential for the regeneration observed during adaptation to these ulcerogens. This notion is supported by our recent observation that removal of salivary glands abolished gastric adaptation to stress and reduced that of ASA and this was accompanied by a significant decrease in the luminal contents of EGF. Since damaged gastric mucosa is capable of secreting EGF from the novel cell lineages, it is likely that gastric luminal EGF in the ASA adapted stomach originates from local gastric production rather than from the excessive release by salivary glands. That the changes in EGF and EGFl could be, at least partly, epiphenomena related to excessive mucosal regeneration and may not be directly responsible for the resistance of the epithelium to further damage has not been excluded.

Our previous study using specific receptor binding assay showed that normal gastric mucosa expresses receptors for TGFα and EGF, and that both peptides were equally effective in protection against the damage induced by strong irritants. That an augmented local expression of TGFα which shares a common high affinity receptor with EGF and is greatly increased after acute mucosal damage with hyperosmolar solution, could also participate in the gastric adaptation to ASA and enhanced resistance of the ASA adapted mucosa against the damage by strong irritant has not been excluded. Our present results show, however, that although normal mucosa exhibits relatively higher expression of TGFα than of EGF, the ASA adapted mucosa exhibits an enhanced expression only of EGF but shows no further increase in TGFα expression. It is reasonable, therefore, to assume that EGF rather than TGFα plays a crucial role in the gastric adaptation to ASA and in the subsequent increase in the mucosal tolerance to necrotising substances.
35 Wallace JL, McKnight GW. Gastric adaptation to aspirin (ASA) is non-specific and prostaglandin-independent. Gastroenterology 1993; 104: A222.


