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BIOCHEMICAL GASTROPROTECTION FROM ACUTE ULCERATION INDUCED BY ASPIRIN AND RELATED DRUGS

K. D. RAINSFORD*‡ and M. W. WHITEHOUSE†

*Biochemistry Department, University of Tasmania Medical School, GPO Box 252C, Hobart, Tasmania, Australia, 7001, and †Department of Experimental Pathology, John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia, 2601

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Abstract—Adjuncts that serve as: (a) buffers to neutralize drug acidity, and/or (b) solubilizers of acidic drugs, or (c) certain nutrients (e.g. glucose, acetate), considerably reduced the gastric mucosal injury induced by orally administered aspirin (and other non-steroidal anti-inflammatory drugs) in stressed and starved rats. Gastroprotection against aspirin and related drugs was obtained by supplying the mucosa with glucose with intermediates or precursors of the tricarboxylic acid cycle (that may be absorbed directly from the gastric lumen). Glucose alone was not sufficiently gastroprotective. Gastroprotection with tricarboxylic acid cycle precursors given with glucose appears to be due to the effects of these nutrients in restoring ATP synthesis in the gastric mucosa. D-glutamate and D-aspartate were deaminated by homogenates prepared from saline-washed rat fundic mucosa (yielding α-oxo acids for the tricarboxylic acid cycle). These amino acids could be substituted for the L-forms in combination with glucose to yield gastroprotection from damage by aspirin. Studies in domestic pigs (a species with a pseudo-human stomach) established that acute and chronic oral administration of the aspirin + acetate + glucose combination (1:3:3 molar proportions) was less irritating to the gastric mucosa than aspirin alone. These results were confirmed in acute studies in monkeys. Sodium and potassium salts were superior to calcium and ammonium salts as the buffer component in these improved (i.e. less gastrotoxic) aspirin formulations tested in rats. Bicarbonate was not effective in preventing aspirin gastrotoxicity in stressed-sensitized rats, but is effective in non-stressed rats.

Gastric mucosal ulceration and/or gastric haemorrhage are frequent and serious side-effects in man encountered following the ingestion of aspirin and other non-steroid anti-inflammatory (=NSAI) drugs [1–3]. Currently, strategies for reducing these gastric side-effects have mostly involved using special pharmaceutical preparations (e.g. soluble, sustained-release or 'enteric-coated' tablet forms) or substituting precursor forms = pre-drugs, e.g. the paracetamol ester of aspirin (benorylate), or mesecla-zone, a tricyclic precursor of 5-chlorosalicyclic acid [4].

These formulations or derivatives may be less ulcerogenic but they can hardly be regarded as eliminating the gastrotoxicity [4–6]. With some, e.g. benorylate, the derivative may not be as therapeutically effective as the parent drug (aspirin) [4, 7, 8]. Resorting to pre-drugs or other derivatives usually involves considerable extra cost in the synthesis and safety evaluation of these new derivatives.

Another approach currently of interest is the development of procedures to fortify some of the natural defence mechanisms in the stomach. Manipulation of certain metabolic systems in the gastric mucosa might overcome some of the deleterious metabolic effects of NSAI drugs (e.g. on cellular respiration, mucus biosynthesis) which seem to underlie the pathogenesis of gastric mucosal damage [2, 9–11].

‡ To whom correspondence should be addressed at: Pharmacology Dept., Biozentrum der Universität Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.

Previous studies have established that prior *ad lib.* access to glucose, or the concurrent oral administration of mixtures or individual amino acids, reduces the gastric irritancy or ulcerogenic activity of aspirin in starved rats [12–15]. These findings suggest that the metabolic status of the gastric mucosa could be an important factor in the genesis of mucosal damage.

We have studied the effects of prior, or concurrent, oral/parenteral administration of glucose and other nutrients on (a) overall gastric mucosal damage, and (b) changes in essential mucosal metabolites (glucose, lactate and ATP) induced by aspirin and other NSAI drugs. The object of this study was to identify some metabolic effects of these drugs on the stomach *in vivo* which could predispose the mucosa to ulceration. Since the concurrent oral administration of an amino acid mixture has been found to reduce the therapeutic efficacy of aspirin [15], we decided to monitor the anti-inflammatory activity of the experimental drug formulations together with gastrotoxicity in the one animal, using an assay developed for this purpose [15].

METHODS

Gastric ulcerogenic–anti-oedemic activity. Male and female Hooded (Tas Uni) and SPF Wistar (ANU) rats (160–180 g body weight) were starved in wire mesh cages (i.e. no bedding) for 24 hr and allowed water *ad lib.* before the experiments. Water was withdrawn during the experiments. Animals were dosed orally with 2 ml of NSAI drug and/or

nutrient–buffer mixtures prepared by homogenizing all the components together in distilled water immediately before administration [15, 16]. Details of the drug–nutrient–buffer mixtures used will be found in the tables and Results. Solutions for intraperitoneal injection (Table 4) were all adjusted by volume to achieve isotonic concentrations wherever possible.

Following drug–nutrient–buffer administration, the animals were stressed by exposure to cold (−15°, 45 min) at standard animal density per cage [15]. In studies where the anti-inflammatory activity was monitored (together with ulcerogenicity), animals were given a sub-plantar injection of 1 mg sodium carrageenan in 0·1 ml saline into one rear paw and an injection of 0·1 ml saline in the other paw (control) immediately after the cold stress. Ninety minutes later the carrageenan-induced paw oedema (inflammation) was measured with a micrometer screw gauge as the difference in thickness [=Δ pt. (mm)] between the saline- and carrageenan-injected paws. Immediately after this the animals were killed by stunning and cervical fracture, the stomachs excised and rinsed lightly with saline. The number and severity of gastric lesions was recorded and used to calculate the lesion index [16]. In studies where the anti-oedemic measurements were not made, the gastric ulcerogenic activity was measured 2 hr after drug administration.

Biochemical assays. Blood and tissue levels of glucose and lactate were assayed by the GOD-Period and lactate dehydrogenase methods [17, 18]. For ATP measurements, sections of the fundic mucosa were freeze-clamped between stainless steel tongs precooled in liquid nitrogen. Small sections of frozen mucosal tissue were rapidly weighed and homogenized in boiling 0.1 M morpholino-propanesulfonic acid (MOPS) buffer, pH 7.4. After centrifuging the chilled homogenate, the ATP content of the supernatant was assayed [19] with a Luminescence Biometer (Du Pont Instruments, Wilmington, DE) using firefly luciferase (Sigma Chem. Co., St Louis, MO).

Plasma corticosterone was measured spectrofluorometrically [20]. Blood was collected in heparinized tubes from groups of 3–4 rats 2 hr following treatment with cold stress (−15°, 45 min) and with either aspirin (200 mg/kg in 1 ml H_2O p.o.), aspirin and nutrient (see Table 5) or 1 ml H_2O alone (control group). The plasma was extracted with *iso*-octane and chloroform, and the fluorescence measured with a spectrofluorimeter (Aminco-Bowman, Silver Spring, MD) following treatment of the chloroform extract with concentrated sulphuric acid [20].

D-amino acid oxidase activity of saline-rinsed rat gastric mucosa was determined following preparation of 1:5 w/v mucosal homogenates in 0.05 M Tris–HCl, pH 8.4. The enzyme activity was measured polarographically in the presence of 6 μM FAD and 60 mM D-aspartate [21].

Absorption of the acetate anion from Na acetate and glucose mixtures was determined by measuring the ^{14}C content in blood and in the gastric and (where applicable) intestinal contents of pyloric-ligated or non-operated rats dosed with Na (1-^{14}C)-acetate (Radiochemical Centre, Amersham, U.K.) and/or glucose or aspirin. Pyloric/ligation of rats was performed immediately after the instillation of 3 ml of 0.25 M glucose plus 0.25 M sodium acetate and 5 μCi Na(1-^{14}C)-acetate with or without 150 mg/kg aspirin. Samples of blood and gastric contents were withdrawn at approximately 10–15 min intervals. Blood (0.1 ml) was digested in a solubilizing medium (NCS, Packard Instrument Co, IL) and the radioactivity determined in a liquid scintillation counter after adding 10 ml toluene–Triton X-100 scintillant [22].

Acute and chronic studies in pigs. Female Landrace cross pigs (11–15 kg) were given single (acute) or repeated (in chronic studies) doses orally for 10 days, of either: (i) 30 mg/kg/day aspirin together with glucose and sodium acetate (in molar proportions of 1:3:3, respectively, in 30 ml water), or (ii) aspirin alone (30 mg/kg/day), or (iii) water alone (30 ml). The techniques as described previously were used [6]. The animals were fasted for 24 hr and allowed water *ad lib.*, then killed (by the captive bolt technique) 1 hr following the final dosing. The stomach and upper gastrointestinal tract were excised, washed free of debris and routinely photographed. Sections of gastric and intestinal mucosa were taken for histological examination after formalin-fixation. Paraffin embedded sections were stained with haematoxylin and eosin or periodic acid Schiffs reagent + Alcian blue.

RESULTS AND DISCUSSION

Effects of some nutrients/buffers as gastroprotective agents. Oral or parenteral administration of glucose with either L-aspartic acid, L-glutamic acid, sodium or potassium acetate or with various intermediates of the tricarboxylic acid (TCA) cycle (in molar proportions of 1:3:3 with aspirin = 1) was particularly effective in reducing the gastric mucosal damage induced by oral aspirin (150 mg/kg p.o.) in (cold) stress-sensitized rats (Tables 1–2). The protective effects of these combinations of glucose with individual tricarboxylic or amino acids, when given orally, was generally more effective than when these nutrients were given separately (Tables 1, 2, and data not shown on other combinations).

Part of the gastroprotective actions of the organic anions is due to their buffering of the gastric/drug acidity (Tables 1–2). For instance, co-administration of either neutral phosphate buffer (pH 6.8) or any of the following sodium salts: formate, benzoate, malate, maleate, malonate—together with glucose—also effectively reduced the gastric mucosal damage induced with aspirin (150 mg/kg p.o.) (Table 1).

However, addition of sodium bicarbonate, either alone or in combination with glucose, did not prevent aspirin-induced mucosal damage unless the glucose was given prior to the dose of aspirin + equimolar bicarbonate mixture. For instance, aspirin (150 mg/kg p.o.) + equimolar $NaHCO_3$ with glucose (at −45 min i.p.) produced 12.3 ± 8.0 lesions (N = 4) and lesion index (LI) = 24.3, c.f. aspirin alone = 26.3 ± 7.5 (N = 5) lesions (LI = 39.6) and aspirin + Na HCO_3 + glucose (p.o. in 1:3:3 molar proportions) = 32.7 ± 1.3 (N = 5) lesions (LI = 46.0) (all values mean ± S.E.M., with N animals per group). Clearly, the main effect of glucose pre-treat-

Table 1. The effects of oral co-administration of glucose and/or organic and inorganic acid buffers on aspirin-induced gastric damage and carrageenan oedema in stress-sensitized rats*

Expt. No.	Treatment	Lesion numbers (mean ± S.E.)	Lesion index	Paw oedema mean Δpt (mm)
1	H_2O (control)	0	0	2.53
	Aspirin alone	15.0 ± 2.0	26.5	1.05
	Aspirin with glucose	23.0 ± 3.0	31.0	ND
	Aspirin with glucose and:			
	sodium acetate	2.0 ± 1.0†	9.4	1.25
	potassium acetate	2.0 ± 2.0†	5.6	1.50
	calcium acetate	0†	0	1.30
	ammonium acetate	0†	0	1.20
2	H_2O (control)	0	0	2.23
	Aspirin alone	13.3 ± 3.4	25.3	1.07
	Aspirin with glucose and:			
	sodium lactate	7.3 ± 7-0	14.7	1.12
	sodium pyruvate	10.7 ± 0.9	22.0	1.40
	sodium formate	3.7 ± 3.6†	7.0	1.37
	sodium butyrate	2.0 ± 1.5†	9.4	1.32
	sodium malonate	0.3 ± 0.3†	3.9	1.25
	sodium dihydrogen phosphate	8.7 ± 3.3	21.0	1.32
	disodium hydrogen phosphate	0.3 ± 0.2†	7.6	1.40
	sodium benzoate	2.0 ± 1.5†	9.4	1.32
3	Aspirin alone	42.3 ± 5.6	57.9	ND
	Aspirin with trisodium citrate	12.8 ± 3.1†	24.6	ND
	Aspirin with sodium malate	4.5 ± 0.5†	15.8	ND
	Aspirin with sodium maleate	21.3 ± 5.1	33.8	ND
	Aspirin with glucose and:			
	trisodium citrate	12.5 ± 4.1	24.3	ND
	disodium citrate	16.7 ± 4.3	28.0	ND
	sodium malate	2.8 ± 0.9†	13.8	ND
	sodium maleate	24.2 ± 8.3	36.0	ND

* Male Wistar rats (starved for 24 hr) were dosed orally (in groups of three to five each) with aspirin (150 mg/kg in 1 ml H_2O) and/or metabolites/buffers (in molar proportions of 1:3 aspirin to each of the metabolites), or H_2O (for controls). The rats were cold-stressed (−15°, 45 min) and subsequently injected in one rear paw with 0.1 ml 1% w/v carrageenan in saline and 0.1 ml saline (control) in the other rear paw. Ninety min later the difference in paw thickness between saline and carrageenan injected paws was measured (Δpt). The animals were then killed, gastric contents washed out with saline and the number and severity of lesions used to calculate the lesion index as described.

† Statistically significant reduction in number of lesions ($P < 0.05$, Student's t-test) compared with aspirin alone.

ND = not determined.

ment was to reduce the severity of lesions and hence the lesion index. The sodium salt of aspirin (prepared by careful addition of NaOH to pH 5.6) was less irritant than aspirin itself, whereas added bicarbonate (pH = 5.5) was equally irritant (data not shown). This suggests that excess bicarbonate has some physiological effects which may counteract the beneficial effects of solubilizing the drug. Sodium bicarbonate partially reduces mucosal damage when given orally with an equimolar quantity of aspirin to starved but non-stressed rats [16] and also in man [23, 24]. Addition of excess sodium bicarbonate to aspirin (as in some commercial preparations) does reduce aspirin-induced damage in stressed rats [15]. Chronic administration of relatively high doses (100 mg/kg) aspirin with equimolar bicarbonate causes as many gastric lesions to pigs as observed with the same dose of aspirin itself [6]. Thus the gastroprotective effects of adding bicarbonate to aspirin mixtures may depend on the relative quantities of aspirin or bicarbonate given, as well as on the effects of exposure to stress states. It appears that bicarbonate may only be effective when given in equimolar quantities with low doses of aspirin or when present in excess with high doses of aspirin. Under conditions of exposure to stress states the effectiveness of bicarbonate as a gastroprotectant appears to decline.

It was found that the type of cation employed in the buffer mixture affects the degree of gastroprotection so attained. Ammonium and calcium ions are evidently less effective than sodium or potassium ions in this respect. For instance, aspirin (150 mg/kg p.o.) co-administered with ammonium acetate (1:3 molar proportions, respectively) produced 26.0 ± 2.0 (mean ± S.E., N = 5) lesions (LI = 38.0), cf. aspirin alone = 34.7 ± 6.3 (N = 5) lesions (LI = 48.4) and aspirin + sodium acetate (1:3 molar proportions) which produced 9.0 ± 5.0 (N = 5) lesions (LI = 20.0). Moreover, co-dosing animals with

Table 2. Effects of co- or prior oral administration of metabolites and/or buffers on aspirin-induced gastric mucosal damage and carrageenan oedema in stress-sensitized rats*

Expt. No.	Treatment	Number of lesions (mean ± S.E.)	Lesion index	Paw oedema mean Δpt (mm)
1	H_2O (control)	0	0	1.80
	Aspirin alone	20.3 ± 2.3	32.6	0.77
	—with glutamine (−10 min)	9.0 ± 1.2†	20.0	1.43
	—with lysine (−10 min)	10.3 ± 5.2	21.3	1.85
	—with arginine (−10 min)	10.3 ± 0.9	22.0	1.28
	Aspirin alone	10.3 ± 7.4	21.6	0.82
	—with glutamine (−60 min)	16.0 ± 4.6	29.7	0.90
	—with aspartic acid (−60 min)	7.3 ± 1.5†	19.7	1.15
	—with glucose (−60 min)	19.7 ± 2.2	32.3	0.75
	—with glucose + aspartic acid (−60 min)	2.7 ± 1.3†§	10.4	0.80
2	H_2O (control)	0	0	2.05
	Aspirin alone	18.7 ± 2.2	31.7	0.87
	—with glucose + glutamic acid (−10 min)	1.0 ± 0.6†	8.4	0.92
	—with glucose + asparagine (−10 min)	13.3 ± 2.3	25.0	1.18
3	H_2O (control)	0	0	2.37
	Aspirin alone	31.0 ± 0.5	45.0	1.33
	—with monosodium glutamate	1.7 ± 1.2†	3.4	1.03
	—with monosodium glutamate + glucose	2.3 ± 1.2†	7.7	1.37
	—with glycine + glucose	12.6 ± 4.8†	24.3	1.07
	—with alanine	31.3 ± 12.4	45.0	ND
4	Aspirin (150 mg/kg)	39.3 ± 9.2	50.8	ND
	3,5-Dibromo aspirin (282 mg/kg)	8.3 ± 5.6†	16.3	ND
	—with sodium acetate + glucose	0.3 ± 0.2‡	3.0	ND
	—with disodium citrate + glucose	0‡	0	ND
5	H_2O (control)	0	0	2.40
	Aspirin	22.7 ± 6.9	35.4	1.13
	—with sodium acetate	9.0 ± 5.0	20.0	1.00
	—with sodium acetate and:			
	glucose	0.7 ± 0.3†	8.1	1.18
	N-acetylglucosamine	4.7 ± 2.9†	12.4	1.43
	fructose	2.0 ± 1.0†	8.7	1.12
	ribose	14.7 ± 3.7	26.7	1.25
	lactose	4.7 ± 3.3†	12.4	1.20
	galactose	8.3 ± 5.5†	19.6	1.38
	β-hydroxybutyrate	30.3 ± 14.9	42.0	ND
	alanine	30.0 ± 4.6	42.0	ND
	glycerol	9.0 ± 1.0	21.3	1.48
	sucrose	1.1 ± 1.0†	4.6	1.22

* Experimental details as described in Table 1 except that in some experiments the animals were pre-dosed with metabolites at times stated in the table. Where no timing is indicated, the metabolites were co-dosed with aspirin.

† Statistically significant reduction in numbers of lesions ($P < 0.05$, Student's *t*-test) compared with aspirin alone.

‡ Statistically significant reduction in lesion numbers ($P < 0.05$, Student's *t*-test) compared with 3,5-dibromo-aspirin group.

§ Statistically significant reduction in lesion numbers ($P < 0.05$, *t*-test) compared with aspirin + glucose treatment.

ND = not determined.

ammonium chloride (a procedure commonly employed for urinary acidification) enhanced the gastrotoxicity of aspirin. Aspirin (150 mg/kg p.o.) + ammonium chloride (in 1:3 molar proportions) produced 64.0 ± 5.0 (N = 5) lesions (LI = 77.0), whereas aspirin (150 mg/kg p.o.) alone gave 34.7 ± 6.3 (N = 5) lesions (LI = 48.4). Sodium chloride co-administered with aspirin under the same conditions does not produce any statistically significant difference in the number of lesions or their severity compared with that obtained from aspirin alone (data not shown). These results reinforce the role of physiological acidification as exemplified from effects of NH_4^+ ions in the development of gastric mucosal damage.

Of the sugars tested, D-glucose, D-fructose and sucrose were most effective when given orally in combination with sodium acetate in reducing the gastric mucosal damage induced by aspirin (Table 2). The other sugars or metabolites tested, some of which could be gluconeogenic (e.g. glycerol, L-alanine, D-galactose), were clearly less effective (Table 2). In fact, DL-β-hydroxybutyrate and L-alanine appear to reverse the gastroprotection afforded by sodium acetate alone (Table 2). Alanine alone was ineffective in reducing the gastric damage induced by aspirin (Table 2). The other gluconeogenic amino acids, glutamate and aspartate [which are metabolized to tricarboxylic acid (TCA) cycle intermediates], are orally effective when given either alone or

in combination with glucose (Table 2). The prior oral or parenteral administration of glucose and these amino acids is particularly effective in reducing the gastric damage by aspirin and a new derivative of aspirin, 3,5-dibromoaspirin, recently introduced [25]. The same combination of nutrients was also effective in reducing the ulcerogenic effects of other NSAI drugs (data in part not shown). For instance, indomethacin (10 mg/kg p.o.) with 1:3:3 molar proportions of glucose + L-aspartate. Na (i.p., 10 min before indomethacin) produced 15.3 ± 1.0 (N = 5) lesions (LI = 18.5), cf. indomethacin alone which induced 20.5 ± 8.7 (N = 5) lesions (LI = 32.5). Likewise, diclofenac (10 mg/kg p.o.) with 1:3:3 molar proportions of glucose + L-aspartate . Na (i.p., 10 min before drug) produced 1.3 ± 1.2 (N = 5) lesions (LI = 4.9), whereas the drug alone caused 30.5 ± 3.8 (N = 5) lesions (LI = 44.2). The glucose + aspartate mixture given systemically was equally effective when given 60 min prior to the oral administration of these NSAI drugs (data not shown).

These results suggest that the gastroprotective actions of these nutrient/buffer combinations may be due to their stimulation of glucose metabolism and/or the TCA cycle (and concomitant ATP synthesis). The presence of glucose in the mixture may reduce acid secretion [26] and so reduce the possibility of any drug-induced stimulatory effects on acid secretion, e.g. via histamine release [2], contributing to the development of gastric damage. However, this may only be part of the effects of glucose in the gastroprotective nutrient mixtures.

The non-metabolizable analogue of glutamic acid, *S*-carboxymethyl-L-cysteine (='thiohomoglutamic' acid), while of comparable buffering capacity to glutamate, could not replace glutamic acid to afford gastroprotection when given with aspirin either alone or in combination with glucose (data not shown). This emphasizes the metabolic, as opposed to merely buffering, contribution of glutamic acid in the nutrient mixture.

Glutamine and asparagine alone were found to exert some gastroprotective effects (Table 2). This confirms the results of Okabe and co-workers [14, 27] who attributed the gastroprotective effects of glutamine to an inhibition by this amino acid to the back-diffusion of acid generated by the irritant actions of aspirin. However, it should be noted that recently Leeling *et al.* [28] have shown that glutamine, when co-administered with low doses of aspirin (approx. 65 mg/kg), failed to reduce the loss of blood from the gastrointestinal tract of dogs, as measured by the radioactive iron technique. It is possible that the effects of glutamine alone (which are not quite as effective as other gastroprotective mixtures observed in the present work) may not be so effective when given to non-stressed and/or non-starved animals (such as were used in the study by Leeling and co-workers). As well, the technique for measuring the gastrointestinal blood loss employed by these workers will not discriminate gastric mucosal damage *per se* (including the development of non-, or slow haemorrhaging ulcers) and may have some of the deficiencies inherent in the radiochromium technique [2, 29]. Thus, the more sensitive and specific gastric assays may reveal gastroprotective effects of glutamine and other amino acids which have been found to be gastroprotective (see refs. 14, 27, 30, together with present studies).

It is curious that in comparison with the corresponding dicarboxylic amino acids, these ω-amides (i.e. glutamine, asparagine) are not particularly effective (given orally with glucose) in reducing gastric damage by aspirin (Table 2). This is surprising because it might be expected that the ω-amides would be metabolized to the corresponding acids and then to TCA cycle intermediates via aminotransferase reactions, especially under the conditions of starvation employed in the present study [31].

It was also found that D-aspartate was as effective as the L-isomer in reducing the gastric damage by aspirin when given parenterally with glucose (data not shown). However, polarographic assays of D-amino acid oxidase in mucosal homogenates [17] suggested that this enzyme is probably present in the mucosa and therefore could generate the corresponding keto acid, i.e. oxaloacetate, to stimulate the tricarboxylic acid cycle (in gastric mucosa at least). While stomachs used for assays were carefully washed free of all adherent debris, it is not possible to totally eliminate the possibility of traces of contaminating bacteria being a source of the D-amino acid oxidase activity, detected under these non-sterile conditions.

Oral administration of L-aspartic acid with the non-metabolizable sugar, 2-deoxy-D-glucose, was much less effective in reducing the gastric mucosal damage by aspirin compared with glucose and L-aspartic acid (data not shown). While some protection might be expected from the high osmotic pressure of such solutions instilled into the stomach, it would seem from analysis of the results that this effect would be relatively small, in comparison with the metabolic and buffering effects discussed earlier. Also, the timing of the prior oral administration (i.e. 10 min prior to drug) of the 2-deoxy-D-glucose or glucose solutions would be expected to minimize the possibility of solutions of high osmotic pressure being present in the gastric lumen at the time of aspirin administration.

There are several nutrient–buffer mixtures which might afford possible therapeutic benefit (when co-administered with aspirin) and the consequent anti-inflammatory potency and gastric ulcerogenic activity is compared with that of aspirin alone in Tables 1 and 2. Among these, glucose with either acetate, L-aspartate or L-glutamate, or glucose with sodium aspirin, seem among the most promising. Both acute and chronic oral administration of aspirin with glucose and sodium acetate (1:3:3 molar proportions) to domestic pigs caused less gastric mucosal damage than observed from administration of aspirin alone (Table 3). The effectiveness of this combination in this species, in which gastrointestinal physiology and structure resembles that of man [32], suggests that the combination may have therepeutic potential as a less ulcerogenic formulation. The reduced ulcerogenic activity of this combination compared with that of an identical dose of aspirin (200 mg/kg p.o.) was also confirmed in a limited acute (2.5 hr) study in two ketamine-anaesthetized pig-tail monkeys (data not shown).

While acetic acid is known to damage the gastric

Table 3. Effects on the pig gastric mucosa of acute and chronic administration of the combination of sodium acetate (Ac) plus glucose (Glc) with aspirin compared with aspirin alone

Group	Treatment	Gastric mucosal damage		
		Number of lesions (mean ± S.D.)	Average severity	Lesion index
I. Acute				
	H_2O (control)	0	0	0
	Aspirin (200 mg/kg)	43.7 ± 4.5	3.3	57.0
	Aspirin (200 mg/kg) + Glc + Ac (1:3:3 molar proportions)	11.3 ± 9.0*	0.7	18.7
II. Chronic				
	H_2O (control)	0	0	0
	Aspirin (30 mg/kg/day) for 10 days	32.0 ± 5.0	1.7	43.7
	Aspirin (30 mg/kg/day) + Glc + Ac (1:3:3 molar proportions) for 10 days	19.7 ± 10.7*	2.3	32.0

* $P < 0.05$ Students 't' test c.f. aspirin.

mucosa [33, 34], these studies show that addition of the sodium salt, i.e. sodium acetate, to solubilize aspirin does not engender a particularly irritant mixture, but in fact a mixture of low gastric irritancy. No enhanced gastric emptying, as assessed in studies of radioactive acetate absorption (see Methods—Biochemical Assays), was evident after administering aspirin buffered with sodium acetate. These aspirin–acetate mixtures do not appear to be emptied from the stomach at an appreciably higher rate than that with aspirin alone.

The addition of sodium salts of citric acid, as in some commercial aspirin formulations (to buffer and solubilize the aspirin) also affords some gastroprotection, but high concentrations of sodium (i.e. using trisodium citrate) are required to achieve this gastroprotection (Table 1). Some gastric absorption of the citrate anion may also occur [35]. A high concentration of sodium salts of citric acid may have marked effects on gastric emptying [36]. Thus absorption of aspirin by the gastric mucosa might be simultaneously effected and hence interpretation of the data for trisodium citrate–aspirin mixtures is more complex.

Metabolite concentrations in blood and gastric mucosa induced by nutrient mixtures in aspirin + stress treated rats. The gastric mucosa is normally dependent for its energy production upon either the metabolic reserves in liver and muscle or on intestinally absorbed nutrients, both being provided via the blood. In addition, any nutrients absorbed directly from the gastric lumen may fortify the blood-borne supply of metabolic fuels.

Under the conditions of starvation employed, no detectable glycogen was present in the gastric mucosa. This apparent absence of glycogen in the mucosa has also been noted by Menguy and Masters in rabbits [37]. Little or no food was present in the stomach and consequently the blood glucose levels should indicate the glucose available from body reserves to produce energy (ATP) in the gastric mucosa. The results (Table 4) show that a significant ($P < 0.05$) decrease in blood glucose occurred 0·5 hr (but not at later time intervals) after initiation of stress treatment alone. A significant ($P < 0·05$) increase in blood glucose concentration was evident 2 hr after initiation of aspirin plus the stress treatment. This probably reflects the metabolic effects of aspirin (not the stress treatment), since aspirin administration alone increases blood glucose over this time period (data not shown).

The increase in blood glucose was accompanied by an increase in mucosal concentrations of glucose and lactate 2 hr after aspirin + stress treatment compared to that in control or control + stress groups (Table 5). Oral co-administration of glucose and

Table 4. Blood glucose concentrations (mg/100 ml blood) in aspirin + stress-treated rats

Time (hr)	Treatment			
	Control	Control + stress	Aspirin + stress	Aspirin + glucose + NaAc + stress
0.5	90.0 ± 4.5 (3)	72.0 ± 11.7 (3)*	63.0 ± 8.5 (3)	
1.0	90.5 ± 5.1 (3)	86.3 ± 17.8 (4)	69.0 ± 8.1 (4)†	67.3 ± 5.4 (4)†
2.0	80.8 ± 5.1 (3)	89.8 ± 8.5 (3)	108.0 ± 5.6 (3)†	66.2 ± 7.4 (6)*†‡
6.0	97.3 ± 6.9 (3)	89.3 ± 14.4 (3)	98.1 ± 6.7 (3)	
20.0	56.5 ± 9.1 (7)	51.1 ± 5.7 (7)	57.2 ± 8.8 (7)	

Statistically significant difference (Student's *t*-test, $P < 0.05$) from * control, † control + stress or ‡ aspirin + stress treatments. Values in parentheses denote number of animals in each treatment group.

Table 5. The effects of glucose and sodium acetate on blood and gastric mucosal metabolite concentrations and plasma corticosterone levels in aspirin + stress treated rats

Treatment	Gastric mucosa			Blood		
	Glucose (μg/g wet wt)	Lactate (nmoles/g wet wt)	ATP (μg/g wet wt)	Glucose (mg/100 ml)	Lactate (nmoles/100 ml)	Corticosterone (μg/ml plasma)
Control	0	0.17 ± 0.03	252 ± 48	81.5 ± 5.6	2.83 ± 0.11	0.93 ± 0.17
Control + stress	0	0.17 ± 0.03	132 ± 41*	82.8 ± 3.7	4.45 ± 0.45	1.34 ± 0.3*
Aspirin + stress	60.8 ± 19.3	0.20 ± 0.02†	129 ± 63	99.7 ± 1.4†	6.00 ± 1.70	0.68 ± 0.11†
Aspirin + glucose + sodium acetate + stress	30.6 ± 36.7	0.20 ± 0.02†		61.3 ± 6.1†‡	3.18 ± 0.69†‡	

All values are mean ± S.D. of three to eight determinations 2 hr after treatment. Statistically significant difference (Student's *t*-test, $P < 0.05$) from * control, † control + stress, or ‡ aspirin + stress treatments.

sodium acetate with aspirin (1:3:3 molar proportions with respect to aspirin) caused a statistically significant decrease (relative to aspirin plus stress-treated groups) in both the blood and mucosal concentrations of glucose (Table 4).

An increase in the lactate:glucose ratio in the gastric mucosa and ATP levels after administering aspirin + glucose + acetate c.f. aspirin plus stress group indicates that there is enhanced (i.e. protective) mucosal metabolism in animals given to glucose + acetate combination. Similar effects were noted after prior administration (10 min) of D-glucose + L-glutamic acid to aspirin plus stress-treated rats (data not shown). The mucosal ATP concentrations were also lower in aspirin plus glucose treated groups (data not shown) compared with other aspirin or control groups.

Thus the beneficial gastroprotection afforded by administering acetate or glutamic acid (or sodium glutamate) plus glucose, compared with that from glucose alone (Tables 1–3), may be due to effects of acetate or glutamate unblocking the aspirin- and salicylate-inhibited glucose oxidation [38], probably by stimulating the tricarboxylic (TCA) acid cycle, and hence enhancing ATP production. Co-dosing with glucose alone is clearly insufficient to enhance energy production in the gastric mucosa of aspirin plus stress-treated animals, because it cannot 'spark' its own complete metabolism (and optimal ATP synthesis) via the TCA cycle.

Experiments in both pyloric-ligated and intact rats show that direct gastric absorption of (1-^{14}C)-acetate occurs relatively rapidly, although its uptake from the stomach of pyloric-ligated rats is slower than in intact animals. The time for removal of 50 per cent of the instilled dose of (1-^{14}C)-acetate in pyloric-ligated rats was approximately 170 min. The ^{14}C content in the blood of these rats fell rapidly to about half the initial (maximal) levels at about 40 min and then levelled out. No significant differences were observed in the quantity of ^{14}C-acetate recovered from blood or gastrointestinal contents of aspirin-treated or non-dosed rats.

Possible mechanisms of gastroprotection by nutrients/buffers. The results indicate that a variety of mechanisms may be involved in attaining the gastroprotection (in the presence of aspirin or NSAI drugs) realised following oral administration of glucose together with either acetate, aspartate or glutamate. Proton buffering (by organic anions), solubilization of the drug (afforded by these buffers), the osmotic effects of the drug–buffer solutions, as well as direct metabolic effects of the nutrients in stimulating mucosal (oxidative) metabolism, are all important mechanisms that might individually contribute to the overall gastroprotection afforded by these mixtures. Since even parenterally administered glucose + aspartate or glucose + acetate mixtures were found to protect the stomach from damage by orally administered aspirin, the metabolic protection may well be more dominant than the physical protection (i.e. due to buffering or solubilization of the drug itself).

There are three interrelated factors which should be considered in any analysis of the gastroprotective actions of these nutrients/buffers. Firstly, there is

the influence of starvation and/or stress treatments, as it affects mucosal glucose metabolism (low body glycogen, low blood glucose) of the rats under the conditions employed in this study. The second factor is the considerable anoxia following mucosal ischaemia produced by the drugs as a consequence of their profound effects on local prostaglandin synthesis [39]; prostaglandins having potent effects on blood flow and vasodilatation in the gastric mucosa [40, 41]. The third factor is any effects of the drugs on glucose metabolism and the TCA cycle. The importance of considering their interrelated aspects is illustrated in the studies of bicarbonate and glucose as gastroprotectants. Bicarbonate production by a gastric mucosal HCO_3^-–ATP-asc [42] may act as means for neutralizing hydrogen ions in the immediate vicinity of the mucosal cells and this process appears to be inhibited by some ulcerogenic NSAI drugs [43]. Administration of bicarbonate may partially overcome the inhibitory effects of aspirin or bicarbonate production. However, it may not be sufficient to account for the potentially deleterious effects of depleting intracellular ATP levels, especially in more severe conditions of stress or high drug dosage. Addition of glucose (or other metabolic precursors of its oxidative metabolism) to aspirin–bicarbonate mixtures may overcome the intracellular utilization of energy reserves and hence protect the stomach against damage by aspirin or other NSAI drugs.

Some of the metabolic effects of aspirin on oxidative metabolism of glucose *in vitro* and *in vivo* [38] are summarised in Fig. 1, together with the possible influences of starvation and/or stress on the oxidative metabolism of glucose. It is clear that the principal

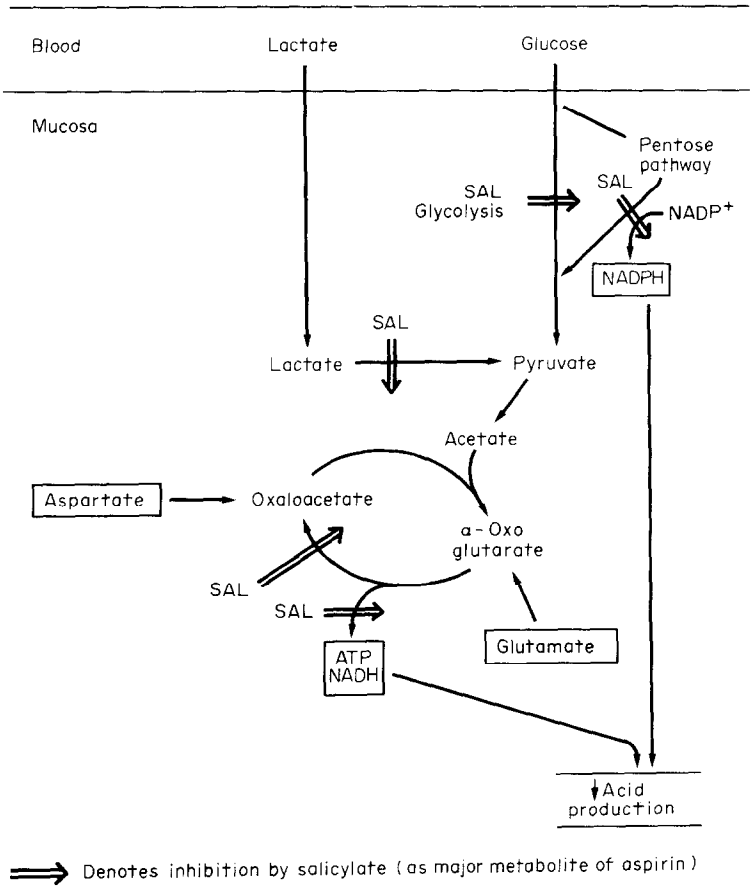

Fig. 1. Inhibitory effects of aspirin (salicylate) and possible consequences on oxidative metabolism of glucose in the gastric mucosa of starved rats. Glucose oxidation in the gastric mucosa of starved (ketotic) rats would depend on a supply of gluconeogenic amino acids entering the mitochondria, since in this ketotic state acetate cannot be oxidized without TCA cycle intermediates [31, 44–48]. Resting acid production depends on the pentose pathway of glucose metabolism; whereas stimulated acid secretion depends on activity of the TCA cycle [44]. Decreased availability of TCA cycle intermediates or precursors will lead to decreased acid and ATP production; the latter being critical for cellular synthesis and metabolism in a tissue with high cell turnover. Inhibition of glucose oxidation [38] by salicylate (from aspirin) impairs mucosal cell biosynthesis (e.g. of protective mucus) and general viability. *Hypothesis:* Administration of acetate, aspartate or glutamate (with glucose) provides a ready source of α-oxo acids and other intermediates in the TCA cycle, so overcoming the inhibitory effects of aspirin (salicylate), enhancing utilization of glucose with consequent increased cellular viability and mucosal defensive capacity.

metabolic effects of both aspirin (or its metabolite, salicylate) and of starvation–stress combine to retard glucose catabolism and the TCA cycle—thus reducing ATP synthesis in the gastric mucosa. Both the pentose pathway for glucose oxidation and the TCA cycle are essential for mucosal functions; the former being especially involved in the 'resting' phase of gastric acid secretion, while the latter is involved in 'stimulated' secretion [44, 45]. Considerable energy must be consumed by the gastric mucosa in the biosynthesis of the major gastroprotective agent, the (constantly shed) mucus lining, as well as in the maintenance of high cell turnover with repair and regeneration of the mucosa.

As shown in Fig. 1, a balance between glucose and TCA cycle intermediates or precursors may be required to (i) achieve maximal generation of energy (ATP) in the gastric mucosa, and (ii) overcome the metabolic consequences of the absorbed aspirin/NSAI drugs inhibiting glucose and amino acid catabolism (e.g. aminotransferases, dehydrogenases) and uncoupling of oxidative phosphorylation [38]. Thus co-administered acetate or the amino acids, glutamate and aspartate (which can fuel the TCA cycle), together with glucose can profoundly stimulate and effectively over-ride NSAI drug-induced inhibition of glucose oxidative metabolism in the gastric mucosa when it is adversely affected by (local) absorption of lipophilic drugs from the gastric lumen.

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