Comparison of the effects of diflunisal and other salicylates on the intragastric electropotential

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The integrity of the gastric mucosal barrier in animals and man is disrupted by salicylates and other agents that damage the mucosa with a resulting loss of H^+, an increase in gastric luminal Na^+ and mucosal bleeding (Davenport 1964, 1967; Ivey et al 1972). These ionic shifts have been associated with a decrease in the intragastric electropotential difference (PD) (Davenport et al 1964; Chvasta & Cooke 1972). In man a correlation exists between aspirin-induced alterations in PD and ultrastructural changes in the gastric mucosa (Ivey et al 1975).

We have compared the effect of a salicylic acid derivative diflunisal with that of other salicylate preparations on the PD in anaesthetized cats.

Adult cats of either sex, fasted for 18–20 h, were anaesthetized with pentobarbitone (25 mg kg^{-1} i.v.) and placed in a supine position and maintained at 37 °C with a heat lamp. Electrodes were prepared by filling polyethylene tubing (PE 190, Clay Adams) with an agar (3%)-KCl (saturated) solution. One electrode was positioned in the pyloric region of the stomach and the indifferent electrode was placed in a femoral vein. The potential difference between the intragastric and indifferent electrode was measured by connecting the output of an mV meter (Beckman SS-1) to a calibrated chart recorder. After occlusion of the pylorus and lower esophagus, steady state potentials were recorded in the presence of a solution of HCl-NaCl (50 ml of 100 mM HCl containing 54 mM NaCl) introduced into the stomach. Test compounds, finely ground and suspended in the HCl-NaCl solution, were introduced into the stomach in the second test period (30–60 min); the succeeding two periods were 60–90 and 90–120 min, as placebo, the HCl-NaCl solution was used alone.

Drugs used were: aspirin, U.S.P., choline-magnesium trisalicylate (Trilisate), Purdue Frederick, Norwalk, Conn., diflunisal (MK-647) 5-(2′,4′-difluorophenyl)-salicylic acid, Merck Sharp & Dohme, West Point, Penna., and salicylic acid (reagent grade) J. T. Baker Chemical Co., Phillipsburg, N.J.

PD ranged between −50 and −60 mV. Diflunisal (250 and 500 mg, a single therapeutic dose), did not significantly modify this (Table 1) while aspirin (180–600 mg), salicylic acid (125 and 250 mg), and choline magnesium trisalicylate (250 and 500 mg) caused a pronounced decrease which persisted throughout the post-treatment periods when the drug had been removed from the stomach. The decrease in PD was greatest in cats given 500 mg of choline-magnesium trisalicylate and was similar to that observed by us with buffered aspirin (unpublished).

Table 1. Intragastric potential (−mV) in anaesthetized cats (mean ± s.e.)* n = no. of animals

Comp. mg	N	Pretreat. 0–30	Treat. 30–60	Post-Treat. min 60–90	90–120
Placebo	(17)	60 ± 1	61 ± 1	62 ± 2	61 ± 1
Diflunisal†					
500	(6)	55 ± 3	55 ± 3 *P* n.s.	54 ± 2 n.s.	55 ± 2 n.s.
250	(4)	61 ± 3	57 ± 5 *P* n.s.	56 ± 5 n.s.	57 ± 4 n.s.
Aspirin					
600	(4)	57 ± 5	25 ± 0 *P* <0·001	21 ± 1 <0·001	20 ± 1 <0·001
300	(10)	58 ± 4	28 ± 4 *P* <0·001	28 ± 4 <0·001	26 ± 4 <0·001
180	(6)	58 ± 3	30 ± 5 *P* <0·005	34 ± 3 <0·005	35 ± 4 <0·025
50	(4)	58 ± 4	53 ± 2 *P* n.s.	54 ± 2 n.s.	56 ± 3 n.s.
Salicylic acid					
250	(4)	56 ± 2	22 ± 5 *P* <0·01	29 ± 4 <0·01	33 ± 5 <0·05
125	(6)	52 ± 3	43 ± 2 *P* <0·05	48 ± 2 n.s.	48 ± 2 n.s.
Choline-magnesium trisalicylate‡					
500	(4)	54 ± 3	5 ± 2 *P* <0·001	20 ± 1 <0·005	23 ± 1 <0·005
250	(4)	55 ± 3	23 ± 4 *P* <0·001	32 ± 8 <0·025	35 ± 9 <0·05

* During each period 50 ml of 100 mM HCl containing 54 mM NaCl was introduced into the stomach; test compounds were suspended in the HCl-NaCl solution.

† Diflunisal: 5-(2′,4′-difluorophenyl)salicylic acid (MK-647), Merck Sharp & Dohme, West Point, Penna.

‡ *Choline-magnesium trisalicylate: Trilisate*, Purdue Frederick, Norwalk, Conn. Statistical evaluation by paired data analysis comparing each period with the pretreatment (0–30 min) period; N.S. indicates a *P* value >0·05.

PD in the anaesthetized cat is similar to that observed in the dog (Davenport et al 1964; Chvasta & Cooke 1972) and man (Geall et al 1970; Murray et al 1974) and we found in cats that a single therapeutic dose of diflunisal (250 or 500 mg) did not significantly modify this potential while aspirin did so. Since it has been shown that a correlation exists between aspirin-induced PD alterations and ultrastructural changes in the gastric mucosa (Ivey et al 1975), it is possible to infer that diflunisal did not disrupt the integrity of the gastric mucosal barrier.

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Transport of cycloserine across the mucosae of rat colon and the human mouth

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The permeability of the mucosa of the mammalian colon to several acidic and basic drugs was increased by the percentage of each compound present in the unionized form and was also proportional to the degree of lipid solubility (Schanker 1959). Similar considerations apply to the passage of a much wider range of compounds across the mucosa of the human buccal cavity (Beckett & Hossie 1971). Moreover, results of these buccal absorption studies have been used to predict the passage of drugs across other biomembranes, for example, the kidney tubule (Beckett & Triggs 1967) and the small intestine (Beckett & Pickup 1975). A parallelism has been indicated between the absorptive properties of the mouth and colon with reference to certain drugs and nutrients (Evered 1973).

D-Cycloserine, a 'broad spectrum' antibiotic, and its inactive L-isomer pass across the mucosa of mammalian small intestine (Wass & Evered 1971). Both isomers have low solubilities in lipid and are highly ionized at physiological pH values. D-Cycloserine has been shown to pass across the human buccal mucosa (Evered 1972). In the present study some of the transport characteristics of mucosa from both colon and the buccal cavity were compared with respect to D- and L-cycloserine.

Everted sacs of muscle-free colon from female Wistar albino rats were prepared and used in vitro by the method of Parsons & Paterson (1960) with modifications (Evered & Nunn 1968). After incubation 0·2 ml aliquots of serosal and mucosal fluids were deproteinized with 1·8 ml of 5% w/v aqueous trichloroacetic acid and centrifugation at 3000 *g* for 10 min. Since cycloserine is relatively unstable in acid solutions, assays were completed immediately after deproteinization (Wass & Evered 1971).

Absorption from the human mouth in vivo was by the method of Beckett & Triggs (1967) with modifications (Manning & Evered 1976) entailing the use of a buccal 'blank', a pre-incubation period and the use of a physiological buffer solution. Portions of the buccal washes, suitably diluted, were centrifuged at 3000 *g* for 10 min to sediment any debris before analysis. Most of the experiments on buccal absorption were carried out with a female Caucasian subject aged 25 years.

Both cycloserine isomers were assayed spectrophotometrically by the method of Jones (1956) using a Pye-Unicam SP1800 spectrophotometer.

The relationship between the rate of transport of D- and L-cycloserine against initial concentration for both the everted sacs of rat colon (Fig. 1) and the buccal mucosa (Fig. 2) indicated that the process involved was passive diffusion. These results correlated with those of a similar study using rat small intestine (Wass & Evered 1971). The curvilinear relationship shown by the buccal absorption of these compounds should not be taken to indicate carrier-mediated transport as the buccal absorption of a number of drugs has shown this response (Beckett & Hossie 1971). This effect is presumably due to limitations of blood flow or permeability barriers to polar molecules. There was no evidence of stereospecificity for the transport rates in both systems which again is compatible with passive diffusion.

Changes in the initial pH of the D-cycloserine solutions had little effect on its rate of transport in both systems (Table. 1) Assuming that only the unionized species of the molecule will readily cross biological membranes, this result was as expected. Calculations of the extent of ionization of each ionizable group over the pH range studied (Albert & Serjeant 1962) showed that the acidic hydroxy group was almost 100% ionized at pH 7·5 and the basic amino group was almost 100% ionized at pH 4·5. At pH 6·0 each group was approximately 97% ionized. Thus, over the pH range of the study at least one group is almost fully ionized and the molecule exists mainly as a zwitterion.

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