the sink condition (compared with M_1 and M_2 in Table IV). The deviation from the slope of unity existed as long as some degree of the first term, $(D_A/X_L)A_0$, affected the dissolution rate. The break point difference can be explained by the differences between $k_{OH^-}[OH^-] - k_0$ and $(D_B/nX_L)B_L - (D_A/X_L)A_0$.

SUMMARY AND CONCLUSION

The prodrug, 7,7′-succinylditheophylline, was hydrolyzed with a half-life of about 10 sec in the physiological pH range. These hydrolysis studies suggested that the prodrug would hydrolyze very rapidly and release the parent molecule, theophylline, once it was dissolved.

The dissolution rate of 7,7′-succinylditheophylline was 35 times slower than that of theophylline under the same conditions, and its dissolution rate was independent of pH within the physiological pH range. Dissolution rate studies coupled with hydrolysis rate studies suggested that the dissolution process would be the rate-determining step to keep the steady-state release into solution (Scheme III).

$$\text{prodrug in solid} \xrightarrow[\text{slow process}]{\text{dissolution}} \text{prodrug in solution} \xrightarrow[\text{rapid process}]{\text{hydrolysis}} \text{parent drug in solution}$$

Scheme III

The saturation solubility of 7,7′-succinylditheophylline at 25° in water was estimated to be 1.63×10^{-3} M from the rate dissolution data. Under the same conditions, theophylline solubility was 4×10^{-2} M. From these results, it appears that 7,7′-succinylditheophylline might be a valuable candidate as a prolonged-release theophylline prodrug.

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Beneficial Effects of Methionine and Histidine in Aspirin Solutions on Gastric Mucosal Damage in Rats

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Abstract □ Amino acids methionine and histidine, which are soluble in propylene glycol, were investigated for their purported beneficial effects on aspirin-induced gastric mucosal damage in the rat. The pathognomonic changes observed microscopically in the fundic region of the stomach of animals administered daily doses (100 mg/kg), for up to 15 days, of aspirin solutions (0.36 M) in propylene glycol incorporated with the amino acids were compared with those of animals given equivalent quantities of aspirin in an aqueous suspension combined with an aluminum hydroxide antacid. A "delayed" onset of aspirin-induced cellular damage due to the presence of amino acids, analogous to that associated with the use of antacids, was found as determined partly by differences in the staining ability of injured cells with hematoxylin and eosin.

Keyphrases □ Methionine—effect on aspirin-induced gastric mucosal damage in rats □ Histidine—effect on aspirin-induced gastric mucosal damage in rats □ Aspirin—induction of gastric mucosal damage in rats, effect of methionine and histidine □ Amino acids—methionine and histidine, effect of aspirin-induced gastric mucosal damage in rats □ Gastric mucosal damage—induced by aspirin in rats, effect of methionine and histidine

GI bleeding in normal subjects, but especially in patients afflicted with chronic GI lesions, can accompany aspirin ingestion (1, 2). Several mechanisms reported for the pathogenesis of these lesions involve aspirin administration by the intra- or extragastric route (3–5). Among the more significant factors in aspirin-induced gastric hem-

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Table I—Composition of Various Aspirin Formulations Administered to Rats

Formulation	Aspirin, 6.5% (w/v) (0.36 *M*)	Histidine, 0.32% (w/v) (0.02 *M*)	Methionine, 0.30% (w/v) (0.02 *M*)	Propylene Glycol	Water	Antacid
I	+			+		
II	+	+		+		
III	+		+	+		
IV	+				+	
V[a]					+	+

[a] Contained 3.63% aspirin with 10 ml of antacid (60 mg/ml) and 1 ml of water.

orrhages were area and duration of contact of solid drug particles with mucosal cells (6). Consequently, efforts toward reducing these undesirable effects with solid or crystalline aspirin frequently have led to the concomitant use of buffering agents (7, 8) and the administration of freshly prepared solutions of aspirin in water (9).

The objective of the present study was to determine the advantages of a solution form of aspirin with and without "buffers" on experimentally induced gastric lesions in the rat. For stability reasons, a nonaqueous medium, propylene glycol[1], was used as the solvent. Methionine[2] and histidine[1] were investigated because of their solubility in propylene glycol. Furthermore, encouraging results have been ascribed to amino acids generally for reducing aspirin-induced gastric lesions (10–12).

The extent of gastric mucosal damage was examined carefully by light microscopy similar to that previously

Figure 1—*Fundic region of stomach of rat dosed daily with 100 mg of aspirin/kg in Formulation III for 15 days. Shown are intact epithelial lining cells (a), chief cells (b), and parietal cells (p). Overall gastric histology appears normal.*

[1] Fisher Scientific Co.
[2] Mann Research Laboratory.

Table II—Pharmacokinetic Parameters Estimated from Blood Plasma Salicylate Levels following Intubation of a Single Aspirin Dose (100 mg/kg) to Fasting Rats

Formulation	Peak Concentration, mg/ml	Peak Time, min	K_a, hr^{-1}	K_e, hr^{-1}	$t_{1/2}$, min	$AUC_{0-\infty}$, mg hr/ml
I	0.271	30	5.94	0.3080	135	0.6960
II	0.246	30	5.21	0.3035	137	0.6871
III	0.286	30	5.54	0.1979	210	1.0450
IV	0.126	45	3.78	0.3465	120	0.2828
V	0.120	48	2.19	0.2665	156	0.3560

described for the loss of staining intensity by the cytoplasmic matrix of injured epithelial cells with hematoxylin and eosin (13). For comparison, freshly prepared suspensions of aspirin[3] in water and of aspirin combined with a commercially available antacid, aluminum hydroxide suspension[4], were administered.

EXPERIMENTAL

Fifty-four male Wistar rats[5], 300–350 g, were divided into six groups of nine animals. Five groups each received one of the five formulations (Table I), and one group served as the control. At the outset, three animals were randomly selected from within a group and staggered for blood withdrawals to study the rate and extent of aspirin absorption following administration of only a single dose of each formulation. These partially bled animals were then returned to their original groups for further daily dosing with the respective aspirin formulations.

The pathognomonic changes resultant from the daily oral intubation

Figure 2—*Fundic region of stomach of control rat dosed daily with 0.5 ml of water/0.3 kg for 15 days. Shown are intact epithelial lining cells (a), chief cells (b), and parietal cells (p).*

[3] Amend Drugs and Chemicals, Irvington, N.J.
[4] Amphojel Suspension, Wyeth Laboratories, Philadelphia, Pa.
[5] Hilltop Laboratories, Scotsdale, Pa.

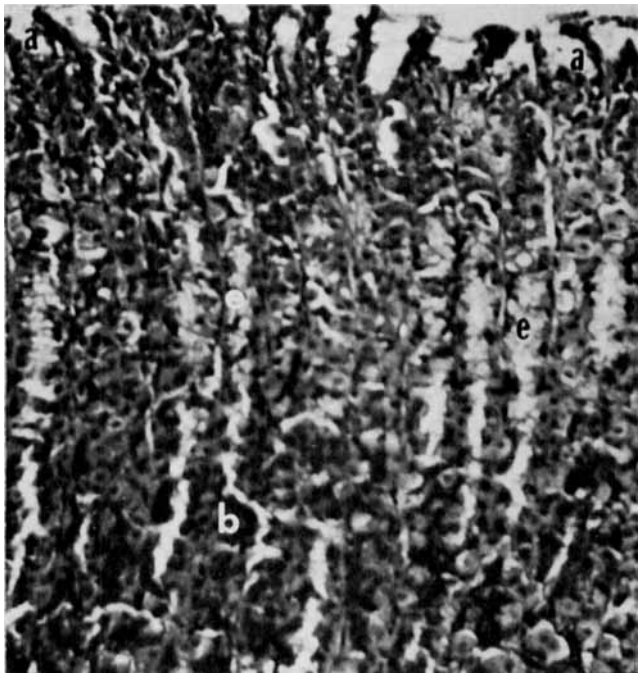

Figure 3—*Fundic region of stomach of rat dosed daily with 100 mg of aspirin/kg in Formulation II for 12 days. Shown are normal epithelial cells (a) and chief cells (b) but very few pale, unstained cells (e). General histology appears normal.*

of single doses of aspirin (100 mg/kg) for 7, 12, or 15 consecutive days (arbitrarily chosen) were derived from three animals sacrificed from each group, including controls, at the end of these designated periods. Routinely, all animals were lightly anesthetized with ether before intubation with the aspirin formulations and also prior to blood withdrawals by the orbital sinus puncture method (14). Animals were kept on regular chow diet and fasted 20–24 hr before the absorption experiment or prior to their sacrifice with a lethal dose of ether; they were allowed access to drinking water at all times.

At autopsy, after both the pylorus and esophagus were securely tied, a fixative solution was injected into the gastric cavity to prevent excessive wrinkling of tissues. With the stomach completely removed, small sections of its lesser and greater curvatures were obtained and dehydrated to be imbedded in paraffin. Sections from these tissues, 5–7 μm thick, subsequently were prepared for microscopic examination.

In the absorption studies, approximately 1 ml of blood was collected in heparinized centrifuge tubes from animals staggered at 10–180 min after oral intubation. These samples were stored at refrigeration temperature. For the analysis of aspirin, 0.5 ml of thawed blood was mixed with 6 ml of Trinder's reagent (15) and immediately centrifuged at 3000 rpm for 10 min to remove the precipitated proteins. A sufficient volume of the clear supernate was used for the absorbance measurement at 540 nm. The respective aspirin concentrations then were derived from a calibration graph prepared with 0.05–0.80 mg of sodium salicylate[1]/ml of deproteinized blood. All aspirin solution formulations were prepared by continuous agitation for 4–6 hr on a mechanical shaker[6] at ambient room temperatures until complete solution of the drug.

RESULTS

Histology of Gastric Mucosa—Figure 1 presents the typical normal appearance of a section in the fundic region of the stomach of rats dosed daily with Formulation III (Table I) for 15 consecutive days. It shows intact gastric epithelial lining cells, chief cells, and parietal cells analogous to those observed for control animals fed plain water (Fig. 2). Similarly, the general histology of animals given Formulation II daily for 12 days appeared normal, with only a few cells losing the ability to pick up the hematoxylin and eosin stain (Fig. 3).

In contrast, the fundic region of stomachs of animals dosed daily with IV for 7 (Fig. 4) and 15 (Figs. 5 and 6) days exhibited not only numerous pale cells but also engorged capillaries containing blood cells, rupturing lining epithelial cells and gastric glands in the later stages. A vast improvement was observed in the general histology of animals administered V. Numerous pale cells remained, but the lining gastric epithelial cells generally appeared intact (Fig. 7). A careful examination of corresponding sites in animals fed a single daily dose of a simple solution of aspirin in propylene glycol (I) for up to 12 days showed generally intact epithelial lining cells and some pale cells, closely analogous to those of Fig. 3.

[6] Model S3, Gyrotory, New Brunswick, N.J.

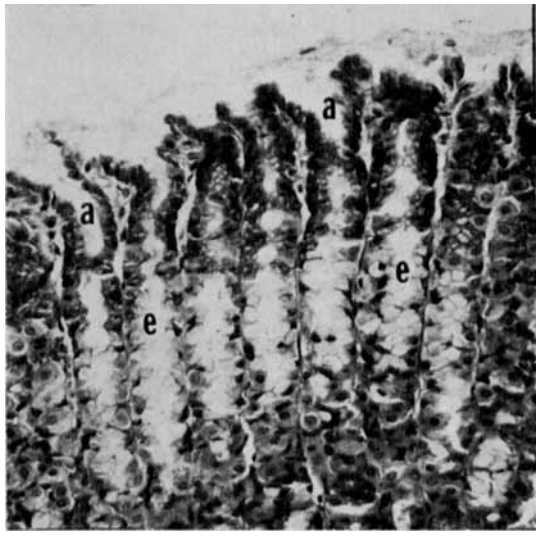

Figure 4—*Fundic region of stomach of rat dosed daily with 100 mg of aspirin/kg in Formulation IV for 7 days. Shown are normal epithelial lining cells (a) and numerous pale cells (e) that did not pick up the stain.*

Aspirin Absorption—Figure 8 illustrates the blood plasma salicylate concentrations reached at 10–180 min following administration of a single dose of aspirin in Formulations I–V. With solutions I–III, the salicylate levels peaked at about 30 min after oral intubation. In comparison, the salicylate peaks occurred after approximately 45–50 min with ingestion of suspensions IV and V.

On the basis of the area under the curve, *AUC*, the amounts of drug absorbed appeared significantly different between solutions and suspensions. The total amounts of aspirin "apparently" absorbed with I–III were three to four times greater than those obtained with IV and V. Again, the *AUC* of III, about 1.5 times greater than those of I and II, was relatively the largest because of a low elimination rate (Table II).

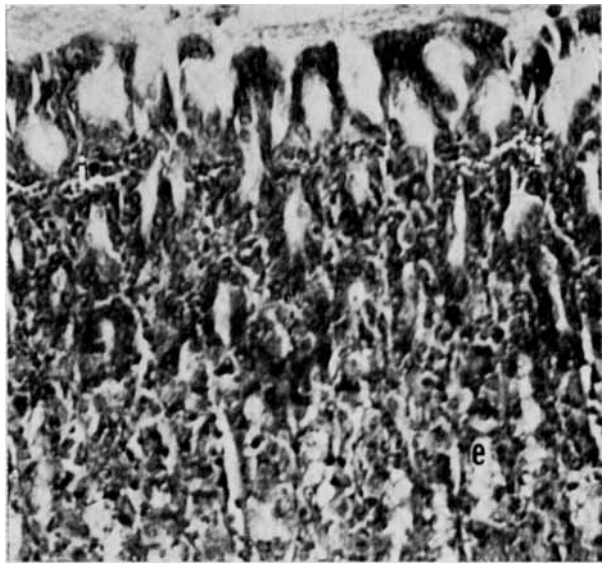

Figure 5—*Fundic region of stomach of rat dosed daily with 100 mg of aspirin/kg in Formulation IV for 15 days. Shown are pale cells (e) and numerous capillaries engorged with blood cells (i).*

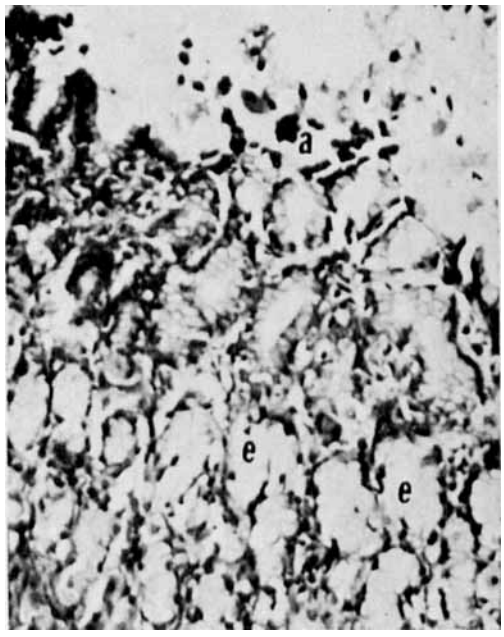

Figure 6—*Fundic region of stomach of rat dosed daily with 100 mg of aspirin/kg in Formulation IV for 15 days. Shown are broken and ruptured lining epithelial cells and gastric glands (a) and also pale cells (e).*

DISCUSSION

On the basis of the loss of staining ability by injured epithelial cells, described by Hingson and Ito (13) as the preliminary diagnostic stage leading to gastric ulceration, a histologic examination of the gastric mucosa tissue sections revealed that the daily oral administration for up to 15 days of single doses of an aspirin solution, aqueous or nonaqueous, produced comparatively less damage than the same dose given as a suspension in the rat. An enhanced beneficial effect due to the incorporation of methionine or histidine was seen with the solutions of aspirin in propylene glycol. This result parallels the observation of Aron *et al.* (16) on reduced fecal blood loss in humans and rats following ingestion of aspirin

Figure 7—*Fundic region of stomach of rat dosed daily with 100 mg of aspirin/kg in Formulation V for 15 days. Shown are intact epithelial lining cells (a), numerous pale cells (e), and an engorged capillary (i).*

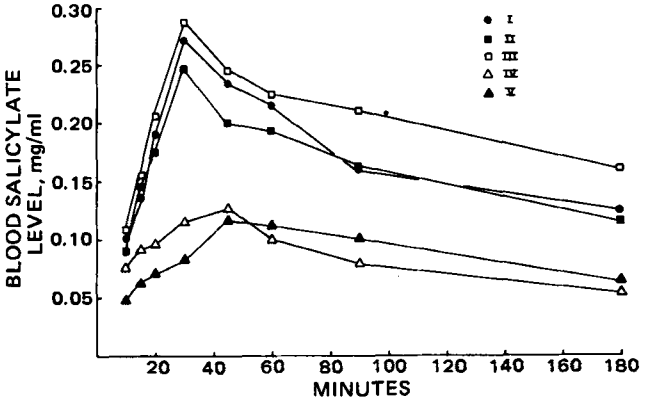

Figure 8—*Single-point plasma salicylate levels obtained at 10–180 min after administration of aspirin Formulations I–V.*

with another amino acid, lysine, as soluble lysine acetylsalicylate.

No data are available at present to elucidate the probable mechanism associated with the use of these amino acids. However, since amino acids represent "weak buffers," an analogous process attributed to the general use of antacids (8) probably occurred. Cooke (17) recently strongly suggested that hydrogen ions from unbuffered aspirin were responsible for the breakdown of the gastric mucosal barrier. A decrease in transmucosal potential difference attributed to an increase in the net passive fluxes of both hydrogen and sodium ions (18) was observed following aspirin-induced gastric mucosal damage. The present data support previous findings (19, 20) that the unionized aspirin molecule, not its ionized or salt form, is mainly responsible for these gastric injuries. In addition, the apparent "delay" in the onset of gastric damage in the rat from administration of an aspirin suspension with an antacid (≃2 mEq) resembles results with humans.

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