Aspirin and mucus

SIR,—Bleeding from the stomach may occur in about half the general population who take aspirin (Smith & Smith, 1966). The site of this untoward action of the drug has not been established, although several mechanisms have been suggested (Roth, 1963). One of these is that the drug diminishes the protective mucus barrier of the stomach, thus allowing abrasive food particles and the proteolytic enzymes and acid of the gastric juice to come into direct contact with the gastric mucosa. Erosions resulting in haemorrhage would then ensue. Aspirin could interfere with gastric mucus in one or more of several ways. The rate of secretion of mucus by the stomach may be affected and it has been reported (Menguy & Masters, 1965) that the administration of aspirin to the intact rat and to the dog with a denervated antral pouch resulted in a lowered volume of mucus. A decrease in the polysaccharide, particularly the sialic acid, content of the mucoproteins, was also observed, suggesting that aspirin inhibited the *in vivo* biosynthesis of these substances. Analogous experiments with an epithelial glycoprotein obtained from sheep colonic mucosa have shown that salicylic acid inhibits the *in vitro* biosynthesis of the protein (Kent & Allen, 1968). A further possible area of interaction is between aspirin and the formed mucoproteins. The topical application of the drug to the gastric mucosa of the cat has been stated to cause chemical denaturation of the mucus (Roth & Valdes-Dapena, 1963).

During the course of some experiments concerned with the incorporation of radio-active amino-acids into the proteins of gastric mucosa, we observed that the oral administration of aspirin caused an apparent aggregation and subsequent sloughing of gastric mucus in the rat and the rabbit. For this reason the effects of aspirin and salicylate on mucus were studied in mucoproteins separated by ultracentrifugation from pig mucus.

Mucin extracts were prepared by lightly scraping mucus with a glass microscope slide from the stomachs of freshly killed pigs, followed by gentle homogenization of the mucus in approximately four times by volume of 0·9% NaCl (pH 4·8) using an all-glass mortar and pestle. All operations were at 4°. The mixture was centrifuged at 3000 *g* for 10 min, the supernatant removed and concentrated twentyfold by ultrafiltration for 12 hr and the centrifugation repeated. The extracts were then dialysed for 10 hours against 0·15 M NaCl dissolved in either 0·01 M sodium phosphate buffer, pH 7·3, or 0·01 M sodium acetate buffer, pH 3·6. Portions (0·7 ml) of the dialysed extracts were mixed with 0·3 ml of the appropriate buffer-salt solution (control samples) or the buffer-salt solution to which either aspirin (acetylsalicylic acid) or sodium salicylate had been added in amounts to give final concentrations in the reaction mixtures of 50 mM. This concentration was chosen to approximate that expected to occur if 10 grains (equivalent to 650 mg) of aspirin was dissolved in 50 ml of water and mixed with 25 ml of human residual gastric juice. The sodium salicylate was easily soluble but the acetylsalicylic acid remained as a fine suspension in the reaction mixtures. In the salicylate-treated samples at pH 3·6 a large quantity of precipitate was observed. Sedimentation velocity experiments were made on these samples in single sector cells at 60,000 rev/min (250,000 *g*) and at 16·7 ± 0·1° using an Analytical Ultracentrifuge (Measuring and Scientific Equipment Ltd., London) fitted with a Schlieren optical system.

The results (Table 1 and Fig. 1) show that in the control samples, two components with approximate sedimentation constants of 10S and 4S were present. Salicylate at pH 7·3 caused no change but salicylate and acetylsalicylate at pH 3·6 caused a marked decrease in the 4S component and a slight increase in the 10S fraction.

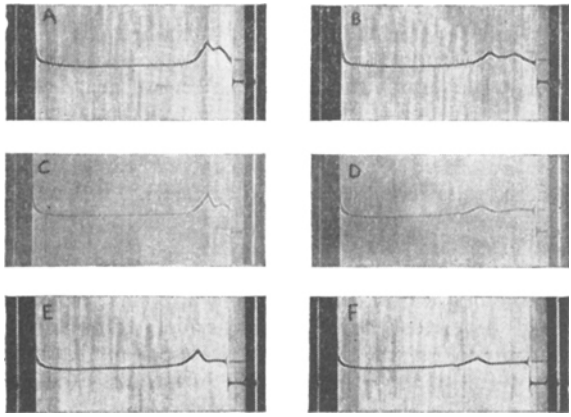

FIG. 1. Sedimentation patterns obtained of pig mucus pH 3·6 at constant speed of 60,000 rev/min. A, control at 750 sec; B, control at 1440 sec; C, with acetylsalicylate at 750 sec; D, with acetylsalicylate at 1440 sec; E, with salicylate at 750 sec; F, with salicylate at 1440 sec. Temperature 16·7 ± 0·1°. Phase angle 20° (M.S.E. Schlieren system). Protein concentration 10–12 mg/ml.

TABLE 1. SEDIMENTATION CONSTANTS OF PIG MUCUS FRACTIONS

pH of buffer-salt mixture	Treatment	Sedimentation constants ($S_{20W} \times 10^{13}$)	
		Slow component	Fast component
7·3	Control	3·59	Not determined
	Sodium salicylate	3·82	10·5
3·6	Control	4·48	9·62
	Sodium salicylate	Precipitated by salicylate	9·32
	Control	4·48	9·62
	Aspirin	Precipitated by acetylsalicylate	9·87

The sedimentation coefficients were calculated from the linear graphs of ln(x), where x is the distance between axis of rotation and the Schlieren peak plotted against time at constant speed. The effects of the salicylates on the viscosity of the solvents were measured and used in correcting the results to water values (S_{20W}) at 20°.

These data show that salicylate and acetylsalicylate change the sedimentation patterns of pig mucus preparations *in vitro* at pH 3·6. The principal effect is an aggregation of the 4S component to insoluble molecular species. If this effect occurs in man after the oral administration of aspirin, then the altered mucus may lack its normal protective properties. The cells of the gastric mucosa would then be directly vulnerable to toxic effects of the drug itself, as well as to damage caused by normal constituents of the gastric contents. Thus a physical interaction of aspirin and gastric mucus may contribute, at least in part, to the mechanism of gastrointestinal bleeding. Salicylate has no effect on the components of pig mucus at pH 7·3, and it may be relevant that either the incorporation of an excess of antacid, in the region of 20 m-equiv. per aspirin tablet (Wood, Harvey-Smith & Dixon, 1962) or the continuous administration of

calcium carbonate or aluminium hydroxide gel by mouth (Matsumoto & Grossman, 1960) reduces the gastrointestinal blood loss caused by oral aspirin in man.

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Distribution and metabolism of dopamine in guinea-pigs

SIR,—While the tissue distribution and metabolism of noradrenaline after intravenous injection has been extensively investigated, relatively little information is available concerning the fate of its precursor dopamine shortly after injection. While investigating the possible formation of vasoactive metabolites from [2-^{14}C]dopamine in guinea-pigs, we observed that the kidneys contained a proportionately larger amount of dopamine (μg/g) than all other tissues investigated in the 5 min after its intravenous administration. In those tissues examined, more than 50% of all the dopamine was metabolized to acidic metabolites within 1·5–2 min after its injection.

Guinea-pigs anaesthetized with urethane were killed either 1·5–2 min (maximum depressor effect) or 5 min (response completed) after the intravenous injection of 250 μg/kg (5 or 10 μc) of [2-^{14}C]dopamine (Halushka & Hoffmann, 1968). Tissue extracts of samples of the heart, liver, lungs, kidneys, spleen and plasma were chromatographed on thin-layer plates for separation of the radioactive components. The procedure involved an acidified acetone precipitation followed by a butanol–heptane extraction, drying of the final acid extract and the reconstitution in 250 μl of 0·01 N HCl–acetone (1 : 5). Fifty μl of the reconstituted solution was spotted onto either phosphate buffered silica gel or cellulose plates and developed with either water-saturated n-butanol–glacial acetic acid (6 : 1) or butanol–formic acid–water (15 : 3 : 2) (Holtz, Stock & Westerman, 1963).

At death the kidneys contained the highest concentration (μg/g wet weight) of radioactivity without regard to chemical species (dopamine equivalents) (Table 1). At the 1·5–2 min time interval this value was about three times greater than that in the tissue exhibiting the next highest concentration (lungs). The third highest concentration was in the liver, followed by the heart and then the plasma. In the animals killed at 5 min, the kidneys contained 6 times as much radioactivity as the lungs and the total radioactivity in the lungs and liver