Salicylates and gastric juice

The suggestion that gastrointestinal bleeding resulting from oral administration of aspirin may be due in part to precipitation of a protective glycoprotein component of gastric juice by the aspirin has been made by Rainsford, Watkins & Smith (1968). This was supported by the observation that mucin extracts of pig stomachs were partially precipitated by 50 mM sodium salicylate at pH 3·6.

We have undertaken a series of experiments to establish whether salicylates cause any precipitation of glycoproteins in human gastric juice and saliva.

The conditions under which Rainsford & others (1968) observed the precipitation of pig mucins were used: pH 3·6 and a final salicylate concentration of 100 mM, which approximates to the concentration of salicylate in a human stomach after ingestion of 20 grains of sodium salicylate. Sodium salicylate, which at pH 3·6 is soluble up to concentrations of 0·5M, was used rather than acetylsalicylic acid which is insoluble.

The secretion was dialysed against 0·01M sodium acetate buffer, pH 3·6, in 0·15M NaCl. Samples (300 μl) of the dialysed secretion were mixed with 75 μl of the same buffer or with 75 μl of 0·5M sodium salicylate in the same buffer. The increase in absorbance at 420 nm after 10 min at 25° was taken as a measure of the precipitation. All measurements were done in duplicate.

Samples of gastric juice, both in the resting state and for 1 h after the injection of 50 mg of histalog were obtained from a normal volunteer (Group O, non secretor). During the aspiration of gastric juice all saliva produced was expectorated and collected. The gastric juice was neutralized with N NaOH immediately and both gastric juice and saliva were spun at 1800 *g* for 15 min. The supernatants were concentrated by ultrafiltration, dialysed against 0·1M sodium acetate buffer, pH 3·6, in 0·15M NaCl and again centrifuged at 1800 *g* for 15 min; a small amount of insoluble material separated in each case.

The interaction of the supernatants with sodium salicylate is shown in Fig. 1A. Both the resting gastric juice and saliva gave a precipitate with sodium salicylate

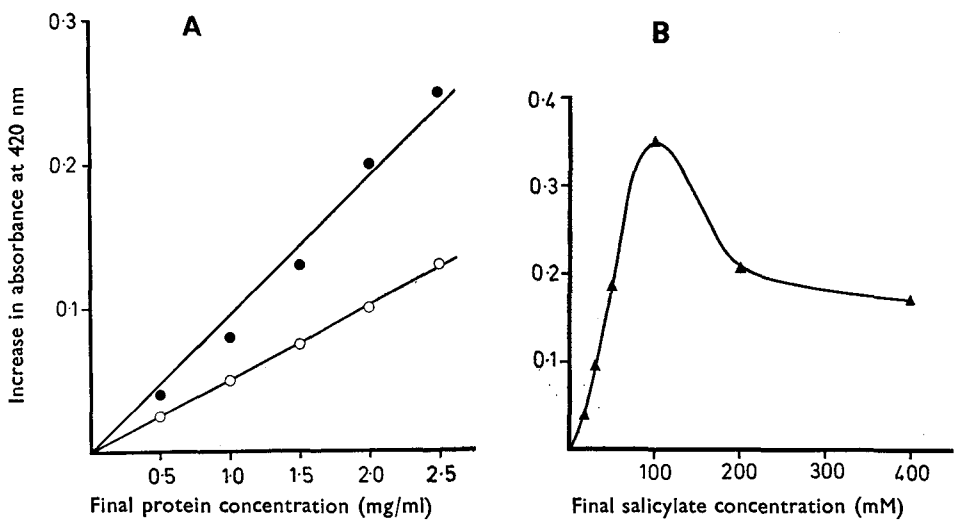

FIG. 1.A. Interaction of 50 mM sodium salicylate with gastric juice and saliva at pH 3·5. B. Effect of increasing concentrations of sodium salicylate on the precipitation of saliva at a concentration of 3 mg/ml protein.

over the concentration range tested, 0–2·5 mg/ml protein. Protein was measured by the method of Lowry, Roseborough & others (1951) as bovine serum albumin.

Increasing concentrations of sodium salicylate caused a linear increase in the precipitation of saliva at a protein concentration of 3 mg/ml. At higher salicylate concentrations there was less precipitation (Fig. 1B).

No interaction of post-histalog gastric juice over the range 1–4 mg/ml protein was detected with the final concentrations of 50, 100, 200 and 300 mM sodium salicylate.

It is possible that the precipitation of resting gastric juice, but not post-histalog gastric juice, with sodium salicylate was due to the presence in resting gastric juice of swallowed saliva which was found to interact strongly with sodium salicylate.

Once an interaction between sodium salicylate and both saliva and resting gastric juice had been demonstrated, it seemed important to determine which components of the secretions were involved in the reaction. Accordingly the same experiments were made on gastric juice and saliva samples from volunteers, X and Y, both Group O non-secretors, which were fractionated in a caesium chloride density gradient as described by Creeth & Denborough (1970).

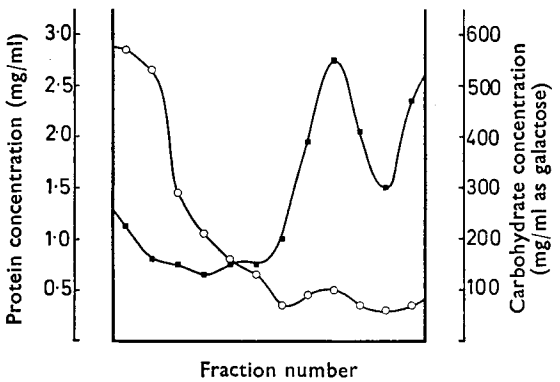

FIG. 2. Fractionation of resting gastric juice X, concentrated five-fold, in a caesium chloride density gradient. Protein ○——○. Carbohydrate ■——■.

In each case a good separation of the glycoprotein from the protein component was achieved. The fractionation of resting gastric juice X is shown in Fig. 2. Fractionation of other secretions gave similar patterns.

The fractions at the glycoprotein peak of each secretion were combined and

Table 1. *Interaction between sodium salicylate* (100 mM) *and fractions from saliva and gastric juice of volunteers X and Y.*

	Protein fraction		Glycoprotein fraction	
	Interaction with salicylate (Δ absorbance) 420 nm	Final concn (mg/ml protein)	Interaction with salicylate (Δ absorbance) 420 nm	Final concn (mg/ml protein)
X. Resting gastric juice	0·100	1·3	0·040	1·3
Y. Resting gastric juice	0·059	2·5	0·010	1·1
X. Gastric juice posthistalog ..	0·010	1·4	0·000	1·3
Y. Gastric juice posthistalog ..	0·000	1·3	0·000	1·4
X. Saliva	0·082	1·4	0·010	0·8
Y. Saliva	1·20	2·1	0·029	0·7

concentrated to give samples having >1·0 mg/ml protein. The fractions at the protein peak of each secretion were also combined and concentrated to give samples having similar concentrations of protein.

The concentrated combined fractions were separately dialysed against 0·01M sodium acetate buffer, pH 3·6 in 0·15M NaCl and the interaction with sodium salicylate at a final concentration of 100 mM was measured turbidimetrically as described. The results are shown in Table 1.

Neither the glycoprotein nor the protein fractions from either sample of post-histalog gastric juice interacted with sodium salicylate, confirming the observations with unfractionated post-histalog gastric juice. However, protein fractions of both resting gastric juice and saliva interacted strongly with sodium salicylate. There was also a very slight interaction with the glycoprotein fraction in both resting gastric juice and saliva.

Although little precipitation of the separated glycoproteins of resting gastric juice or saliva was detected, it is possible that in the whole secretions there may be some coprecipitation of the glycoprotein during precipitation of the protein.

However, to obtain any detectable precipitation of the protein from either gastric juice or saliva the secretion had to be concentrated at least 10 times, and even in the concentrated secretions, the maximum precipitation obtained represented less than 20% of the total protein and glycoprotein. Thus it seems unlikely that in man there would be any significant precipitation of glycoprotein of gastric juice by 100 mM sodium salicylate *in vivo*.

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Effects of desipramine, phentolamine and phenoxybenzamine on the release of noradrenaline from isolated tissues

Adrenergic nerves in isolated tissue incubated with [^{3}H]noradrenaline (^{3}H-NA) take up the amine by an active mechanism, the membrane pump, and incorporate it into the amine storage granules (Carlsson 1966; Hamberger 1967; Jonsson, Hamberger & others, 1969). Field stimulation of isolated tissue is known to cause release of ^{3}H-NA from the adrenergic nerves (Baldessarini & Kopin 1967; Farnebo & Hamberger 1970). The effects of membrane pump blocking and α-receptor blocking drugs on transmitter release and overflow have been examined in several experimental models with divergent results (see e.g. Brown & Gillespie, 1957; Blakeley, Brown & Ferry, 1963; Thoenen, Huerlimann & Haefely, 1964a, b; Boullin, Costa & Brodie, 1967). Field stimulation of isolated tissue was considered an appropriate model for such studies as the stimulation did not affect the circulation in the tissue. We now report the influence of drugs on the ^{3}H-NA release from central and peripheral tissues.

Isolated irides and cerebral cortex slices of standardized size (diameter 3 mm, thickness 0·5 mm) from untreated female rats (Sprague–Dawley, 180–200 g) were carefully prepared. The tissue was incubated at 37° in a modified Krebs-Ringer bicarbonate