Effect of intragastric bile salts on ionic movement across normal human gastric mucosa after intravenous atropine

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SUMMARY   The effect of intravenous atropine (2·0 mg/70 kg) and intragastric bile salts (5·0-5·5 mM) on ionic fluxes across the gastric mucosa was studied. Serial instillations of bile salts, in 200 ml 160 m-equiv/l HCl with ^{51}Cr as a non-absorbable indicator, were performed in eight normal subjects. Five subjects received a bile salt mixture of 84% taurocholic acid, 14% taurodeoxycholic acid, and 2% taurochenodeoxycholic acid. With HCl alone (controls) the mean net flux into the lumen was 1·4 m-equiv H^+, 2·9 m-equiv Cl^-, 1·5 m-equiv Na^+, and 0·26 m-equiv K^+ per 15 minutes after the first instillation. Where atropine plus bile salt was given the loss from the lumen was 5·4 m-equiv H^+ (P < 0·01) and 1·9 m-equiv Cl^- (P < 0·05) and movement into the lumen was 3·2 m-equiv Na^+ (P < 0·01) and 0·20 m-equiv K^+ in the corresponding period. Similar but smaller ion flux changes occurred in three subjects who received atropine and pure taurodeoxycholic acid. The net loss of H^+ from the gastric lumen was greater after atropine-bile salts than that shown in previous studies with bile salts alone. It is postulated that atropine reduced the volume of endogenous HCl secretion unmasking the loss of H^+.

Studies of ionic flux across human gastric mucosa have been hindered by spontaneous gastric secretion of H^+ and Cl^- ions.

In an earlier study (Ivey, Hubel, Clifton, and DenBesten, 1969; Ivey, DenBesten, and Clifton, 1970) we found that bile salts caused a net flux (loss) of H^+ from the gastric lumen. The mean volume of gastric secretion in these bile salt studies was 12% of the volume instilled. If some of this secretion were acid then the true loss of H^+ from the gastric lumen was even greater than that observed. To test the hypothesis that concurrent gastric secretion of HCl masks the true loss of H^+ and Cl^- from the gastric lumen after bile salt instillations, we repeated the studies of bile salt induced fluxes after intravenous atropine. In man, atropine reduces the volume of acid secreted without increasing ionic permeability of the gastric mucosa.

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Subjects and Methods

SUBJECTS

Eight healthy subjects (six men and two women) aged 21 to 31 years were studied. Each subject had two control studies and an atropine-bile salt study performed before, between, or after the control studies. Atropine sulphate, 2 mg/70 kg body weight, diluted to 10 ml with normal saline, was given as 0·4 mg/min intravenously. Half this dose was repeated one hour later.

TEST SOLUTIONS

The test solution was 160 m-equiv/l -HCl, 307 mOsm/kg, containing radioactive chromium chloride (^{51}Cr 25 μc/l as a non-absorbable indicator). In five bile salt studies a 5·5 mM mixture of taurine conjugated bile salts (84% cholic acid, 14% deoxycholic acid, and 2% chenodeoxycholic acid) was added to the test solution. In three studies 5·0 mM pure (gas chromatography) taurodeoxycholic acid was added. A wash solution contained 160 m-equiv/l HCl only.

EXPERIMENTAL TECHNIQUE

Subjects fasted 12 hours before an early morning study. A nasogastric tube was positioned under fluoroscopic control with the tip to the left of the vertebral column. Gastric contents were aspirated. (The pH was < 2.5 in all subjects at least once.) A 50 ml wash solution was instilled and aspirated. At this stage in the atropine studies atropine was injected and the time recorded. The wash was repeated three times (total 200 ml instilled) and the tube positioned so that 50 ml could be aspirated after each instillation ('free flow'). A 200 ml test solution was then instilled. Gastric contents were mixed thoroughly for 60 seconds by rapid withdrawal and reinstillation with a syringe. A 30 ml sample was aspirated. Fifteen minutes later the rest of the gastric contents were aspirated. The stomach was then rinsed four times with 50 ml of wash solution, and the four aspirates combined in one container and saved.

The entire procedure was repeated up to five times (periods I-IV in the section on results) per study. The patient maintained the left lateral decubitus position. Saliva was expectorated and cotton dental rolls, inserted between the upper and lower gums and cheeks, were changed as required. The position of the tube was rechecked by fluoroscopy if bile was present in the aspirates or if free flow could not be maintained.

LABORATORY MEASUREMENTS

The volumes of all samples were measured to the nearest 0.1 ml and duplicates saved for measurement of H^+, Na^+, K^+, and Cl^- concentrations, osmolality, and radioactivity. Bile-stained or Ictotest-positive samples were discarded. H^+ concentration was determined by titration to pH 7.4 with 0.1 N NaOH using a glass electrode. Na^+ and K^+ concentrations were determined with an atomic absorption spectrometer and Cl^- with a Buchler-Cotlove chloridometer. Osmolality was measured directly in milliosmoles with the Advanced Instruments Osmometer, model 31, LA. Radioactivity from ^{51}Cr was estimated by counting 5 ml aliquots in a nuclear Chicago Autogamma detector. Bile salt concentrations were measured in all the test solutions containing bile salts by gas liquid chromatography as described elsewhere (Ivey *et al*, 1970).

CALCULATIONS

The volume secreted and the volume passing through the pylorus (volume emptied) were calculated by the method of Hunt (1951), using ^{51}Cr as a non-absorbable indicator.

Ion fluxes were determined by adding the milliequivalent of each ion in the stomach at 15 minutes

(ion recovered) to the amount emptied through the pylorus (ion emptied) and subtracting the milliequivalent at the start (ion initial) (Overholt and Pollard, 1968a and 1968b):

$$\text{Ion net flux} = \text{ion recovered} + \text{ion emptied} - \text{ion initial}....(1)$$
$$\text{Ion initial} = \text{ion instilled} + \text{ion in residual volume} - \text{ion sampled at 60 sec.}.....(2)$$

On the basis of Hollander's two-component hypothesis (Hollander, 1932, 1952, and 1962 Altamirano, 1963), the gastric secretion was divided into a parietal and non-parietal component as described by Chapman, Werther, and Janowitz (1968). From this the amount of H^+ secreted and neutralized by bicarbonate per 15 minutes can be derived. The net flux value can then be corrected for this secretion and a corrected flux value for H^+ and Cl^- ions calculated for each period of 15 minutes by:

$$\text{Corrected flux } H^+ = \text{net flux } H^+ + H^+ \text{ neutralized} - H^+ \text{ secreted}....(3)$$
$$\text{Corrected flux } Cl^- = \text{net flux } Cl^- - Cl^- \text{ non-parietal secretion} - Cl^- \text{ parietal secretion}....(4)$$

A positive flux indicates a net addition of ions to the lumen. A negative flux indicates a net loss of an ion from the lumen.

STATISTICS

Student's t test was used to analyse paired observations. A 'P' value of < 0.05 was considered significant.

Results

REPRODUCIBILITY OF CONTROL STUDIES

The reproducibility for changes in ionic fluxes and volumes secreted and emptied were verified in five subjects. Mean $\pm$ SE net fluxes (m-equiv/15 min) respectively, for the first and second control studies, were: H^+ 1.0 $\pm$ 0.6 and 1.1 $\pm$ 0.6; Cl^- 2.8 $\pm$ 0.6 and 2.8 $\pm$ 0.6; Na^+ 1.8 $\pm$ 0.4 and 1.4 $\pm$ 0.2; K^+ 0.2 $\pm$ 0.03 and 0.2 $\pm$ 0.04. Volume changes (ml/15 min) were: volume secreted 25 $\pm$ 4 and 23 $\pm$ 4; volume emptied 23 $\pm$ 4 and 25 $\pm$ 6. There was no significant difference between any of these values.

ELECTROLYTE AND OSMOLAL CONCENTRATIONS

The mean electrolyte and osmolal concentrations in gastric contents in control and the studies using the atropine and bile salt mixture and atropine and taurodeoxycholic acid are given in Table I. In the studies with atropine and bile salt mixture H^+ and Cl^- concentrations and osmolality of gastric

	Period	No. of Subjects	H^+ (m-equiv/l)	Cl^- (m-equiv/l)	Na^+ (m-equiv/l)	K^+ (m-equiv/l)	Osmolality (mOsm/kg)
Controls[1]	I	5	144·3±3	152·1±1·8	9·3±1·2	1·9±0·2	293·6±3·8
	II-IV[3]	5	142·7±3·1	153·7±1·7	10·5±1·7	1·5±0·1	292·8±3·2
Atropine and bile salt mixture	I	5	115·9±2·8[3]	140·4±1·2[4]	25·9±1·8[3]	2·6±0·1	280·0±2·1[5]
	II-IV	5	137·3±2·4[5]	155·1±1·7	19·5±1·1[3]	1·8±0·1	307·4±1·7[4]
Controls[1]	I	3	141·0±2·5	151·5±1·6	11·0±0·6	1·7±0·1	289·2±3·2
	II-IV	3	141·8±1·9	152·7±0·8	11·5±1·0	1·2±0·1	289·8±2·1
Atropine and taurodeoxycholic acid	I	3	128·7±3·7	143·6±1·4	19·6±2·1	1·2±0·1	281·9±1·8
	II-IV	3	138·7±3·6	151·8±1·8	16·7±1·9	0·6±0·1	293·9±0·6

Table I *Electrolyte and osmolal concentration (mean ± SE) 15 min after intragastric instillation of 200 ml HCl (160 m-equiv/l, 307 mOsm) alone and with bile salts (bile salt mixture 339 and taurodeoxycholic and 316 mOsm) plus intravenous atropine*

[1]Mean of two studies.
[3]Combined means of periods II-IV.
[3]$p < 0.001$ ⎫
[4]$p < 0.01$ ⎬ for paired t test between control and studies where the atropine bile salt mixture was given.
[5]$p < 0.05$ ⎭

contents were significantly decreased and Na^+ concentrations significantly increased compared to controls. Ionic concentration and flux changes were significantly greater in period I than periods II, III, or IV of atropine and bile salt studies. The values for periods after the first did not differ significantly from one another hence they have been grouped together. Instillation of the taurodeoxycholic acid solution after intravenous atropine produced similar though less marked changes in osmolality and H^+, Cl^-, and Na^+ ion concentration. K^+ concentrations were not significantly altered in either study. The t test was not used in the atropine and taurodeoxycholic acid studies because of the smaller number of subjects (n = 3).

IONIC FLUXES AND VOLUMES[1]
Details of the net ionic fluxes, corrected fluxes for H^+ and Cl^- ions, and volumes secreted, and volumes emptied are given in Table II.

The Figure shows the net fluxes for H^+, Na^+, Cl^-, and K^+ ions for period I of the control (means of two studies), and the atropine and bile salt mixture studies. Individual net H^+ flux values for period I shown in Table III illustrate the relatively consistent loss of H^+ ions in the studies using the atropine

[1]Tables giving details of volumes (including residual volumes, volumes aspirated, volumes of parietal, and non-parietal secretion) and ionic changes (including amounts of H^+ ions present initially, recovered, secreted, and neutralized) are contained in document NAPS-00894 from ASIS-National Auxiliary Publication Service, C/P CCM-Information Corp., 909 Third Avenue, New York, NY 10022, USA.

	Period	No. of Subjects	Net Flux (m-equiv/15 min)				Volume (ml/15 min)		Corrected Flux (m-equiv/15 min)	
			H^+	Cl^-	Na^+	K^+	Secreted	Emptied	H^+	Cl^-
Controls	I	5	1·45±0·7	2·87±0·6	1·48±0·2	0·26±0·03	25·9±4·0	19·6±6·8	-0·86±0·3	-0·93±0·3
Atropine and bile salt mixture	I	5	-5·42±0·2[3]	Œ1·89±0·6[3]	3·18±0·4[3]	0·20±0·02	14·1±3·8	11·8±1·5	These values could not be calculated	
Controls	II-IV[4]	5	0·86±0·5	2·72±0·6	1·62±0·3	0·22±0·02	23·3±3·3	26·4±3·8	-0·88±0·1	-0·77±0·2
Atropine and bile salt mixture	II-IV	5	-1·28±0·1[3]	0·85±0·2[3]	1·89±0·3	0·08±0·01[3]	14·6±1·7	4·8±1·2[2]	-1·28±0·1[3]	-1·05±0·0
Controls	I	3	0·75±0·7	2·61±0·8	1·79±0·1	0·20±0·01	24·7±3·5	26·3±9·7	-0·99±0·2	-0·92±0·3
Atropine and taurodeoxycholic acid	I	3	-2·73±0·8	0·43±0·9	2·71±0·3	0·17±0·01	18·9±4·5	11·1±0·1	-2·41±0·2	-1·96±0·3
Controls	II-IV	3	0·16±0·3	2·04±0·4	1·62±0·2	0·16±0·0!	19·4±1·6	27·9±0·8	=0·96±0·2	-0·69±0·2
Atropine and taurodeoxycholic acid	II-IV	3	-0·90±0·2	1·55±0·5	1·78±0·6	0·09±0·02	18·5±0·02	1·9±3·2	-1·6±0·1	-0·92±0·2

Table II *Ionic movement and volumes secreted and emptied from the stomach contents (mean ± standard error) in response to replicate instillations of HCl (160 m-equiv/l) in control[1] and atropine and bile salt studies*

[1] = mean of two studies.
[2]$p < 0.01$ ⎫ for paired t test between controls and atropine and bile salt mixture.
[3]$p < 0.05$ ⎭
[4]combined means of periods II-IV.
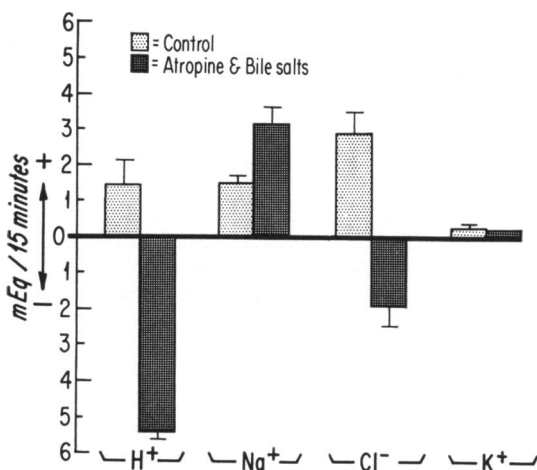minus sign = lumen to mucosa flux.

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Fig. *Net flux of ions, control, and atropine-bile salts.*

Subject no.	1	2	3	4	5
Control[1]	1·7	3·8	− 0·3	0·4	1·6
Atropine and bile salts	− 5·0	− 6·1	− 5·2	− 5·2	− 5·6

Table III   *Net H^+ fluxes in period I of control and atropine-bile salt mixture studies*

[1]Mean of two studies (m-equiv/15 min).

and bile salt mixture (range: − 5·0 − 6·1 m-equiv/ 15 min) when the volume secreted is reduced, compared to the marked variation between subjects (range: 3·8 − 0·3 m-equiv/15 min) in control studies.

Discussion

Spontaneous gastric acid secretion has been a major problem in studies of ionic fluxes across gastric mucosa in man. When Overholt and Pollard (1968b) instilled 110 ml of 20 mM acetylsalicylic acid with 100 mM HCl into the human stomach the mean volume of gastric secretion was 33 ml. These authors failed to demonstrate H^+ loss and attributed this to endogenous H^+ secretion masking H^+ loss. Subsequently, Overholt, Brodie, and Chase (1969) repeated the study in rats before and after reducing acid secretion with atropine. In the control studies no H^+ loss occurred but after reducing gastric secretion with atropine H^+ loss was demonstrated. This problem does not occur in experiments in dogs because the denervated Heidenhain pouch secretes little or no acid spontaneously. Davenport (1964) demonstrated increased back diffusion of H^+ after instillation of 20 mM acetylsalicylic acid with 100 mM HCl in dogs.

Our studies on the effect of bile salts on ionic fluxes across the gastric mucosa after intravenous atropine clearly demonstrate this masking effect of spontaneous acid secretion on H^+ ion losses in man. This effect is unmasked by atropine. Atropine reduces acid secretion without decreasing H^+ concentration and so does not alter ionic permeability in man (unpublished data) or dog (Janowitz and Hollander, 1956). In period I the mean volume secreted, 26 ml in control studies (Table II), was reduced to 14 ml in the studies with the atropine and bile salt mixture. Assuming that this difference of 12 ml was secretion HCl at a concentration of 160 m-equiv/l then atropine reduced the endogenous secretion of HCl into the lumen by 2 m-equiv/15 min. The mean flux from the lumen was 3·1 m-equiv H^+ and zero m-equiv Cl^- in the study using the bile salt mixture previously reported in these same five subjects (Ivey *et al*, 1969). Subtracting 2 m-equiv HCl from these fluxes results in values (− 5·1 m-equiv H^+ and −2·0 m-equiv Cl^-) almost identical with that obtained with the atropine and bile salt mixture (− 5·4 m-equiv H^+ and − 1·9 m-equiv Cl^-).

The importance of gastric secretion is clearly seen in subject 2 (Table III). This subject secreted considerable acid in two control studies resulting in a mean net flux of 3·8 m-equiv/15 min net H^+ flux in period I. Reduction of this secretion by atropine resulted in a net H^+ loss of 6·1 m-equiv H^+/ 15 min in the corresponding period after bile salt instillation. The relatively constant net H^+ loss in the atropine-bile salt mixture studies (range: 5·0-6·1 m-equiv/15 min; Table III) suggests that gastric mucosal permeability changes in a consistent manner after bile salt instillation. The data obtained from the atropine and bile salt study confirm our previous findings of the greatest ionic flux changes in period I with bile salts alone. It also confirms our previous findings that the effect of the bile salt solution (bile salt mixture = 84% taurocholic acid and 14% taurodeoxycholic acid) on ionic permeability changes is greater than that of taurodeoxy-cholic acid solution at *p*H 1 (Ivey *et al*, 1969).

We calculated our electrolyte flux data in terms of Hollander's (1932) two-component hypothesis ('corrected flux') in order to compare it with data on net flux in the presence of large amounts of H^+ ion loss. As determination of the values of net flux is based on direct data with fewer theoretical assumptions we prefer its usage. Calculating the data on the basis of the two-component hypothesis for period I with the atropine and bile salt mixture yielded a negative volume of parietal secretion in all five subjects (mean: 7 ml) and consequently an impossible value for H^+ 'secretion' of − 1·1 m-equiv/ 15 minutes. The corrected flux values for H^+ and

Cl⁻ ions could not therefore be calculated (Table II). This illustrates an important feature of the two-component hypothesis, namely, when diffusion of sodium ions occurs the corrected hydrogen loss from the lumen is underestimated. The larger the amount of sodium diffusing across the mucosa the smaller the corrected hydrogen ion loss (equation 3). Further, the mean volume of secretion in period I in these five subjects was 14 ml/15 minutes. The mean net Na^+ flux into the lumen was 3·2 m-equiv/15 minutes. Thus, according to the two-component hypothesis, the Na^+ concentration of this secretion was 227 m-equiv/l which is well above physiological concentrations in gastric secretion. We conclude that the theoretical and practical limitations of the two-component hypothesis limit its use in calculations of flux data. Teorell (1939) explains reduction in H^+ concentration of gastric contents on the basis of ionic diffusion. He postulated that H^+ ions diffusing out of the lumen were 'exchanged' for Na^+ ions moving into the lumen. Our data for H^+ and Na^+ fluxes and concentration changes support Teorell's theory. Nevertheless, the net H^+ flux was $-5·4$ m-equiv/15 min while net Na^+ flux was $+3·2$ m-equiv in period I in the atropine bile salt mixture study. Thus the 1:1 H^+ for Na^+ ion exchange originally postulated by Teorell does not occur. This conclusion is supported by Altamirano (1970) who, using a semi-isolated piece of the gastric corpus of anaesthetized dogs, found no relation between Na^+ inflow and H^+ outflow in various experimental conditions. We conclude that H^+ and Na^+ ions are capable of diffusing independently of each other across the gastric mucosa of man.

Finally, in our studies with bile salts alone (Ivey *et al*, 1969; Ivey *et al*, 1970) the possibility existed that the net loss of H^+ ions (3·12 m-equiv/15 min) could have been due to reflux of pure alkaline pancreatic juice with a bicarbonate concentration of 145 m-equiv/litre. If the stomach secreted no fluid during the study and the 'volume secreted' (mean 25 ml) in the bile salt study was all refluxed pancreatic juice this would account for a loss of 3·6 m-equiv/l by neutralization. No such argument can be applied in the study with bile salts and atropine. If the mean volume secreted (14 ml in period I) was all pancreatic juice it could account for a loss of only 2·0 m-equiv

H^+, whereas the actual loss was 5·4 m-equiv in this period. This study shows conclusively that back diffusion of hydrogen ions can be produced in man and can account for a large loss of H^+ ions.

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