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STUDIES ON THE BIOCHEMICAL MECHANISM OF THE GASTRIC EROSION CAUSED BY ASPIRIN

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Abstract—Aspirin in single doses had no specific effect on stores of pepsinogen or histamine in the stomach of fasted guinea pigs and the gastric erosions caused by the drug were not associated with hyperacidity or hyperproteolytic activity of the stomach content. There was a tendency for the excretion of 5HIAA to be reduced in the 24 hr following a large aspirin dose to animals but the effect did not achieve statistical significance at the 5 per cent level.

INTRODUCTION

PREVIOUS papers have described the gastric damage which followed the oral administration of aspirin[1] and modified forms of aspirin[2] to laboratory animals. The biochemical mechanism has been little explored although, in so far as could be judged by an analysis of the total gastric content, the tissue damage was not associated with hyperacid secretion.

The present paper examines the effect of aspirin on the gastric mucosal stores of pepsin and of histamine, and on the proteolytic activity of the gastric content, since one or all of these factors are known to be involved in some forms of drug-induced or spontaneous gastro-intestinal erosion.

It has been suggested that tissue damage might be due to the release of 5-hydroxytryptamine (5HT) from its gastrointestinal storage sites, since 5HT was known to cause acute gastric erosion and haemorrhage in laboratory animals.[3] The excretion of 5-hydroxy indoleacetic acid (5HIAA), the metabolite of 5HT, was therefore studied in fasted guinea pigs before and after oral doses of aspirin in an attempt to detect changes in the animal's turnover of the amine.

METHODS

Thirty female guinea-pigs (400–600 g) were fasted overnight as described[1] and randomised into groups. One group of twelve were orally dosed with 100 mg/kg of aspirin suspended in 0·25% gum tragacanth while another group of twelve were given the suspending vehicle only. Six animals received no treatment. At intervals of 1, 2 and 3 hr after dosing four animals from each group (two animals in the case of the untreated group) were killed and the stomachs rapidly removed. Pieces of tissue approximately 2 cm × 1 cm were cut from the body of each stomach, as far as possible in identical areas. After fixing in Zenker–Formol, sections of the mucosa were prepared and stained for pepsinogen granules with Crystal Violet–Orange G.

The extent of the pepsinogen staining in the sections was assessed by an experienced pathologist on a subjective basis using an arbitrary scale ranging from 0 to +++.

The gastric contents were collected at the time of death and stored overnight at 0°C. On the following day their peptic activity was determined by the method of Hunt.[4] Activity was expressed relative to ml of a Standard Pepsin Solution containing 0·66 mg/ml of Parke Davis Pepsin 1: 2,500 B.P. for which a calibration curve had been previously established.

The effect of aspirin on mucosal stores of histamine was examined in eight male guinea pigs (400–500 g) which had been fasted overnight as before and randomised into two equal groups. One group were orally dosed with 300 mg/kg of aspirin in 0·25% gum tragacanth and the other group given the suspending vehicle alone. All the animals were killed four hours later, their stomachs removed, opened out and washed with saline. After drying with filter paper, they were weighed and ground to a fine brei with 10% T.C.A. in an all glass homogenizer. Histamine was extracted by the procedure of Parrat and West,[5] and determined by conventional bioassay on guinea pig ileum.

The effect of aspirin on the excretion of 5HT was studied in sixteen male guinea pigs (350–450 g). Initially they were placed in individual metabolism cages and allowed food and water *ad lib*. After some days to let them become accustomed to the environment, a 24–hr collection of urine was made. The effect of ancillary treatments (fasting the animals and dosing them with the vehicle in which the drug would be suspended) on the excretion of 5 HIAA was initially determined one week later by removing food overnight (16 hr) and dosing the fasted guinea pigs with 5 ml/kg of 0·25% gum tragacanth before a further 24 hr collection of urine. The effect of aspirin was finally determined under similar conditions in the same animals one further week later by dosing eight fasted animals with 250 mg/kg, and eight with 500 mg/kg of aspirin suspended in 0·25% gum tragacanth.

The volumes of the 24-hr urines were recorded and their content of 5 HIAA determined on 6 ml aliquots by the procedure of Udenfriend *et al.*[6].

RESULTS

The data in Table I show the density of pepsinogen granules observed in tissue from the stomachs of fasted guinea pigs which had no subsequent treatment, and compares this with similar observations in animals given an oral dose of aspirin (100 mg/kg) or its suspending agent alone. The data suggest that the mere act of dosing with the inert vehicle was sufficient to release most of the pepsinogen over the following 60 min and no specific effect of aspirin on this release could be recognised.

The effect of aspirin and gum tragacanth on the proteolytic activity of the animal's gastric contents is shown in Table 2 where the six values from untreated animals were pooled to establish the mean basal level. The mean activity in both treated groups was higher than that found in untreated animals, but there was a large variation between individual animals and in this experiment the effect did not achieve statistical significance except at the two hour point after aspirin treatment. The difference between the treated groups did not achieve statistical significance at any time. Gastric erosion (which occurred in all aspirin-treated animals) was in some cases associated with lower levels of proteolytic activity than gum tragacanth-treated animals although the latter had no lesions. The results implied that proteolytic activity of the gastric juice in

TABLE 1. RELATIVE AMOUNTS OF PEPSINOGEN IN THE GASTRIC MUCOSA OF FASTED GUINEA PIGS BEFORE AND AFTER DOSING WITH GUM TRAGACANTH OR ASPIRIN

Treatment	Time (hr) 1	2	3
Nil	+++	+++	++
	++	+++	+++
Gum Tragacanth	+	Trace	Trace
	+	+	+
	+	+	0
	0	0	+
Aspirin	++	0	+
100 mg/kg	0	Trace	Trace
	+	0	+
	Trace	Trace	0

TABLE 2. PROTEOLYTIC ACTIVITY OF THE GASTRIC CONTENT OF FASTED GUINEA PIGS BEFORE AND AFTER DOSING WITH GUM TRAGACANTH OR ASPIRIN

Treatment	Number of animals	Time hours	Mean proteolytic activity units (see text)	Standard error of mean
Untreated	6		0·529	0·207
Gum Tragacanth	4	1	0·690	0·253
Gum Tragacanth	4	2	0·775	0·284
Gum Tragacanth	4	3	0·800	0·242
Aspirin 100 mg/kg	4	1	1·095	0·407
Aspirin 100 mg/kg	4	2	1·358*	0·257
Aspirin 100 mg/kg	4	3	0·885	0·082

* When the results were analysed by the *t*-test the only significant difference ($P=0·05$) observed was between the mean proteolytic activity of the untreated group and the 2 hr-aspirin group.

TABLE 3. THE HISTAMINE CONTENT OF THE STOMACHS OF FASTED GUINEA PIGS FOUR HOURS AFTER AN ORAL DOSE OF ASPIRIN OR GUM TRAGADANTH

Treatment	Number of animals	Histamine μg per g on wet basis. Mean ± S.E.
Gum Tragacanth	4	25·5 ± 3·02
Aspirin 300 mg/kg	4	23·1 ± 2·60

aspirin-treated animals was not itself a *prima facie* cause of tissue erosion though it may aid in the development of a lesion once the mucosal defence mechanism was disrupted.

The effect of aspirin on the mucosal stores of histamine in fasted guinea pigs is shown in Table 3. Four hours after an oral dose of 300 mg/kg the mean level of histamine in the stomach tissue was not significantly different from the level in animals dosed with gum tragacanth.

The amounts of 5HIAA and urine excreted before and after control and aspirin treatments are shown in Table 4 as the group means with their standard errors (s.e.).

There was a significant decrease ($P = <0{\cdot}05$) in the mean daily output of urine in fasted animals receiving tragacanth compared to non-fasted animals, although a similar effect was not seen when fasted animals were dosed with aspirin. This latter observation could be of some interest since it may indicate a diuretic effect of aspirin but since the consumption of water by the animals was neither controlled or recorded it is not possible to comment further.

TABLE 4. URINE OUTPUT AND 5HIAA EXCRETION OF GUINEA PIGS BEFORE AND AFTER DOSING WITH GUM TRAGACANTH AND ASPIRIN

Results are given as means ± standard errors

Treatment	Urine volume ml/24 hr.	5HIAA excretion μg/24 hr.
Untreated	28·8 ± 2·02*	177·8 ± 17·1
Fasted + tragacanth	19·4 ± 1·26*	155·5 ± 10·6
Fasted + Aspirin (250 mg/kg)	25·4 ± 3·85	143·9 ± 15·7
Fasted + Aspirin (500 mg/kg)	22·8 ± 3·42	129·1 ± 20·9

* When the results were anlysed by the *t*-test the only significant difference ($P=0{\cdot}05$) observed was between the urine volume of the untreated group and the fasted animals receiving tragacanth.

DISCUSSION

The administration to fasted guinea pigs of aspirin suspended in gum tragacanth, but not the suspending vehicle alone, caused acute mucosal erosions of the gastric mucosa.[1] Both the drug suspension and the vehicle alone caused a qualitively similar release of pepsinogen from storage sites in the gastric mucosa, which was probably a non specific 'feeding effect' as reported for fasted rats by Menzies.[7] Release of the pepsin precursor tended to increase the proteolytic activity of the animal's gastric content although in the experiments described here the increased activity was not statistically different from the activity of untreated animals at any time after the administration of gum tragacanth and only at one time interval (2 hr) after a suspension of aspirin. It seems highly unlikely therefore that hyperproteolytic activity of the gastric juice of aspirin-dosed animals was itself a primary cause of erosion—though it may aid in the development of secondary mucosal damage following disruption of the mucosal defence mechanism by the drug.

The administration of aspirin did not release histamine from mucosal storage sites in the guinea pig's gastric mucosa, which could perhaps have been anticipated since previous work[1] had shown that aspirin tended to diminish rather than increase the secretion of gastric acid by this animal.

Single large doses of aspirin tended to reduce the mean amounts of 5HIAA excreted per 24 hr although the effect failed to achieve statistical significance at the 5 per cent level in the present study. Since this study was completed, Kim and Shore[8] using spectro-photofluorimetric analysis of tissue extracts have reported that the treatment of rats with reserpine caused a lowering of the 5HT content of the stomach and this was associated with haemorrhagic gastric lesions in some 90 per cent of

the treated animals. However, the mean decrease in 5HT stores amounted to only 0·36 μg per g of tissue, so the total release of 5HT from its storage sites in the whole organ probably did not amount to more than 1–2 μg. It is clear that the normal variation in urinary output of 5HIAA was so large that changes of this order in the animal's turnover of 5HT would not be detected.

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