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The action of analgesic substances on the gastric mucosa

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CLINICAL EFFECTS ON THE GUT OF INGESTION OF ASPIRIN, PHENYLBUTAZONE, INDOMETHACIN AND PARACETAMOL

THREE commonly used analgesics, aspirin, phenylbutazone and indomethacin, have undesirable effects on the gastrointestinal tract of man. Aspirin, the most widely used, causes upper abdominal discomfort and exacerbation of peptic ulcer symptoms in one of 20 subjects every time they take it by mouth (Muir, 1963) and French workers claim that there is a correlation between aspirin ingestion and the development of peptic ulcer (Levrat & Lambert, 1960). An association between aspirin and gastrointestinal bleeding has been demonstrated in two ways. Firstly, more patients admitted to hospital with a severe gastrointestinal bleed have been found to have taken aspirin during the preceding few days than patients admitted for other reasons (Alvarez & Summerskill, 1958; Muir & Cossar, 1959; Parry & Wood, 1963). Secondly, most subjects taking repeated doses of aspirin bleed slightly (Stubbé, 1958; Wood, Harvey-Smith & Dixon, 1962) and this may occasionally lead to iron deficiency anaemia (Summerskill & Alvarez, 1958).

Phenylbutazone also has upper gastrointestinal effects. Ten % of subjects taking this drug may develop nausea, vomiting or upper abdominal pain, 1% peptic ulceration and ½% gastrointestinal bleeding (Mauer, 1955). These effects may be largely a function of dosage.

Indomethacin, when first introduced, was in tablet form and caused perforated peptic ulceration and overt and occult gastrointestinal haemorrhage (Wanka, Jones, Wood & Dixon, 1964), particularly when given in large doses (Lövgren & Allander, 1964). On the introduction of powdered indomethacin in gelatin capsules, no serious gastrointestinal effects were found by Wanka & Dixon (1964) or by Hart & Boardman (1965). Of 137 patients with rheumatic disorders treated with indomethacin capsules by Thompson & Percy (1966) only one developed dyspepsia; one, with a previous history of peptic ulceration, had a slight melaena and one, without previous history of peptic ulceration, perforated an acute duodenal ulcer. These authors concluded that the incidence of indomethacin-induced side-effects originating in the gastrointestinal tract was small.

It is gratifying to find one commonly used analgesic, paracetamol, which does not cause abdominal discomfort, or overt or occult gastrointestinal bleeding (Wood & others, 1962; Goulston & Styring, 1964).

The incidence of dyspepsia, peptic ulceration and gastrointestinal bleeding associated with ingestion of these four analgesics has been assessed by studying groups of patients taking them. This approach has

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provided useful data on the incidence of pain, peptic ulcer or haemorrhage, but has been less helpful in explaining the mechanism whereby the drugs cause these undesirable effects. I wish to present evidence that aspirin is an irritant which causes cell exfoliation from the stomach and to suggest that this may be the basic mechanism whereby the aspirin-induced gastrointestinal bleeding lesion is produced.

SALICYLATE-INDUCED OCCULT BLEEDING INTO THE GUT

Occult bleeding into the gut after aspirin ingestion has received much attention recently and provides a tool with which to measure an irritant effect of this analgesic. Of 226 subjects with an apparently normal gastrointestinal tract, Wood and his colleagues found 78% bled more than 2 ml/day (Wood & others, 1962, Croft & Wood, unpublished). The bleeding was measured by the ^{51}Cr-labelled red cell technique and the volume of the blood lost was found to be relatively reproducible in an individual. Most subjects lost 2–5 ml/day and 10% lost more than 10 ml/day.

Apart from effervescent aspirin, which caused less bleeding, soluble and buffered aspirins, whether or not dissolved, caused the same amount of bleeding as plain aspirin tablets (Wood & others, 1962; Wood, 1963). However, these workers found that preparations which prevented release of aspirin in the stomach, by enteric coating or otherwise, significantly reduced blood loss. These data suggest that salicylate-induced occult bleeding is the response of the stomach to some physiological effect of repeated doses of the drug.

What physiological phenomenon can account for this effect? Roth (1963) has reviewed the various hypotheses that have been considered but none adequately explains the occult bleeding data. Mucosal irritants have been studied by Hollander who wrote in 1946 that, in the gut, surface epithelial "desquamation must be considered a normal physiological response to mild irritation" (Hollander, Stein & Lauber, 1946). In 1947, Wolf & Wolff, in their subject with a gastrostomy, found that this was so with irritants such as ethanol, clove oil, copper sulphate, hydrochloric acid and a suspension of mustard in water. Creamer, Shorter & Bamforth (1961) also observed it in the dog small intestine when the mucosa was perfused with physiological solutions which were too warm (40°–45°).

GASTRIC EPITHELIAL CELL TURNOVER AND MEASUREMENT OF CELL LOSS FROM GASTRIC MUCOSA

To appreciate the significance of increased epithelial cell loss after irritants it is necessary to consider the normal turnover of gastric mucosa. Surface epithelial cells are continuously formed by mitoses in the neck of the gastric glands (Bizzozero, 1893). After formation they migrate up the wall of the gastric pits to be extruded in a degenerate form into the lumen of the stomach (Stevens & Leblond, 1953). In man the life span or turnover of gastric surface cells is about two to six days, whereas

that of acid and pepsin secreting cells in the glands is much longer (MacDonald, Trier & Everett, 1964).

A clinical method has been developed for measuring the natural rate of cell loss from human gastric mucosa by estimating deoxyribonucleic acid (DNA) in gastric washings (Croft & Lubran, 1965). As fairly constant amounts of DNA are found in each human somatic cell irrespective of its type (Davidson, Leslie & White, 1951), the DNA content of a cellular specimen is proportional to the number of cells in it. It is in effect a cell count.

In the method, the stomach is emptied of fasting contents and the gastric mucosa is continuously perfused for 45 min with warm (29°–30°) saline. From the DNA content of the aspirate a rate of accumulation of DNA in the stomach is obtained. Under defined conditions in which the patients do not swallow or regurgitate duodenal fluid, the gastric DNA rate is obtained. In the four subjects on whom duplicate tests were made from 5–19 weeks apart, the gastric DNA rate was found to be reproducible within $\pm 10\%$ over a 13-fold range of values (Table 1). Twenty-eight

TABLE 1. REPRODUCIBILITY OF GASTRIC DNA RATE

Case No.	Weeks between tests	Gastric DNA rate (ng atoms DNA-P/min)	
		1st test	2nd test
13	19	6	5
4	11	12	12
11	5	52	42
24	15	73	71

By continuously perfusing human gastric mucosa with saline and estimating DNA in the aspirate, gastric DNA rates were measured in four subjects on two occasions 5 to 19 weeks apart. The DNA values, which are given in ng atoms DNA-P/min, indicated a reproducibility of $\pm 10\%$. The gastric DNA rate is considered to measure physiological cell loss from gastric mucosa and to be an index of gastric surface epithelial turnover. (Data abstracted from Croft, Pollock & Coghill, 1966.)

subjects have been studied by this technique and the gastric DNA rates indicate that under physiological conditions not only is there a continuous production of surface cells but a continuous loss of them into the gastric lumen (Croft, Pollock & Coghill, 1966). In the steady state these two parameters must be in equilibrium (Stevens & Leblond, 1953).

To determine what aspirin does to this equilibrium, seven volunteers were studied (Table 2). They were intubated with Ryle's tubes and their stomachs were cleaned of fasting contents. Fifty ml of saline was placed in each stomach for 14–60 min and a specimen of gastric washing obtained. Three soluble aspirin tablets (equivalent to 1 g of soluble aspirin) were then dispersed in 50 ml of saline, injected into the stomach and left for the same periods of time as saline alone. The gastric contents were then aspirated and cytological preparations made of pre- and post-aspirin specimens. These were inspected for the presence of undoubted gastric columnar cells (Croft, 1963a) which are rarely seen in gastric cytological specimens. These cells were observed much more commonly after aspirin than before (Table 2).

TABLE 2. GASTRIC EXFOLIATIVE CYTOLOGY BEFORE AND AFTER ASPIRIN

Case	Sex	Age	Diagnosis	Calcium aspirin (g)	Period of administration (min)	Gastric columnar cells	
						Pre-aspirin specimen	Post-aspirin specimen
1	M	31	Healthy volunteer	1·0	14	+	+++
2	M	54	Myocardial infarct	1·0	20	0	+
3	M	40	Hypertension	1·0	25	0	+
4	F	59	Cushing's syndrome	1·0	30	0	++
5	F	48	Depression	0·65	30	0	+++
6	F	66	Thyrotoxicosis	0·65	30	+	+++
7	M	59	Emphysema	1·0	60	0	+++

Seven volunteers were intubated. After removing fasting gastric contents 50 ml of saline was placed in stomach for 14–60 min and washings obtained for cytology. Soluble aspirin (0·65–1·0 g) was then suspended in 50 ml saline and placed in the stomach for equivalent periods and post-aspirin cytological specimens obtained. The specimens were assessed cytologically for the presence of undoubted gastric columnar cells. The frequency with which these cells were seen was indicated by +. Gastric columnar cells were found more frequently in specimens obtained after aspirin than in those obtained before it was given.

The technique was modified by washing with larger (500 ml) volumes of saline. The washings after aspirin were visibly more opaque than those before aspirin and commonly contained suspended particles (Croft, 1963a). This opacity was not due to blood and the particles were sheets of gastric surface cells and nuclei.

Four separate tests using 500 ml washings were made on a healthy medical student. In two of these no aspirin was used and, after the initial washing, fairly constant amounts of DNA were found in up to six washings. When 1 g of aspirin in 50 ml of saline was given and 5 min later the stomach was washed out and a further 1 g of aspirin given which was left in the stomach for 12 min before washing, more DNA was found in five of six subsequent washings (Fig. 1). Increased amounts of DNA were present 64 min after aspirin had been removed from the stomach. In the four tests on this student the mean DNA content of the 13 specimens obtained with no aspirin was 0·51 (s.d. 0·18) μg atoms DNA-P, and in the

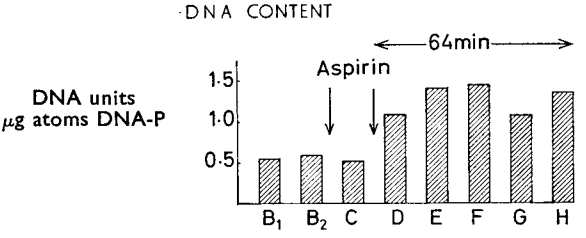

FIG. 1. A medical student was intubated with a Ryle's tube and fasting gastric contents removed with a 500 ml saline washing and discarded. 50 ml saline was placed in the stomach for 5 min and the stomach was then washed with 500 ml of saline to given specimen B_1. Following this washing 50 ml of saline was placed in the stomach for 12 min and a further 500 ml washing performed to give specimen B_2. 1 g soluble aspirin was then dispersed in 50 ml of saline placed in the stomach and left 5 min before performing a 500 ml washing (specimen C). This was followed by another 1 g dose of aspirin in 50 ml of saline which was left in for 12 min before gastric washing D. Subsequent washings E, F, G and H were obtained after placing 50 ml of saline in stomach for 5, 12, 5 and 5 min respectively. More DNA was found in five of the six washings obtained after the first dose of aspirin than was present before aspirin was administered.

nine specimens obtained after aspirin was 1·22 (s.d. 0·54) which is statistically significantly higher ($P < 0.001$).

ASPIRIN AND THE PRODUCTION OF GASTRIC EROSIONS

What effect does this excessive exfoliation have on the human stomach? Previous direct observation of the gastric mucosa, after single doses of plain aspirin tablets, by gastroscopy (Hurst & Lintott, 1939; Weiss, Pitman & Graham, 1961) and examination of the stomach after surgical excision (Muir & Cossar, 1955, 1959) indicated that the bleeding was from erosions in the mucosa. To determine the effect of repeated ingestion of soluble aspirin on human gastric mucosa, nine patients were studied at the time of elective partial gastrectomy for gastroduodenal disease (Croft & Wood, unpublished). Four patients were given three soluble aspirin tablets, dispersed in water, four times a day for 2–4 days before operation. Erosions were found in the mucosa of the resected specimens in all four patients. Erosions were found in only one of the five specimens from patients who were not given aspirin.

In the affected mucosae, one to five erosions were found, mainly along the lesser curvature and they were often joined by linear cracks which were apparent only when the specimens were stretched flat. Microscopically the erosions commonly penetrated the muscularis mucosa and blood vessels were seen in their base. When the mucosa was scraped, it stripped off readily from a specimen from a patient given aspirin, whereas the superficial layers were much tougher in the specimens from patients who had not received the drug. One patient bled post-operatively, probably from aspirin induced erosions, so it was decided not to give more patients aspirin before partial gastrectomies.

These data seemed to indicate that dispersions of soluble aspirin caused excessive exfoliation of gastric surface epithelial cells, and that if this exfoliation continued at a rate faster than the cells could be replaced an erosion would be formed.

GASTRIC SURFACE CELL TURNOVER AND OCCULT ASPIRIN BLEEDING

The relationship between cell loss and aspirin bleeding was further investigated in 15 subjects whose salicylate-induced occult bleeding had been measured by Wood (Croft, 1963b; Croft & Wood, unpublished). The technique used was based on the principles already described. Each subject was intubated with a Ryle's tube and the stomach cleaned of fasting DNA which was discarded. Fifty ml of saline was placed in the stomach which was again washed. The DNA content of these two specimens was converted to a rate by dividing the interval in minutes between each washing. Soluble aspirin (1 g) was then dispersed in 50 ml of saline and placed in the stomach for 5 min followed by another dose for 12 min, and at the end of each period a washing was made. The DNA content of these specimens was also converted to a rate of accumulation of DNA in the stomach.

Of the 15 patients, eight bled 2 ml or more per day and these were designated aspirin bleeders. Cytological preparations were not made of

specimens from one of these patients. The other seven patients bled less than 2 ml per day with repeated aspirin ingestion and these were designated aspirin non-bleeders. In one non-bleeder, only the pre-aspirin DNA rate was obtained. The results in these two groups appeared to differ in three respects (Table 3). Firstly, most bleeders appeared to have a lower

TABLE 3. THE EFFECT OF ASPIRIN ON GASTRIC SURFACE CELL EXFOLIATION IN PATIENTS SUSCEPTIBLE AND INSUSCEPTIBLE TO SALICYLATE-INDUCED OCCULT GASTRO-INTESTINAL BLEEDING

	Aspirin bleeders		Aspirin non-bleeders	
	No. patients	%	No. patients	%
Pre-aspirin gastric DNA .. .. .. >50 ng atoms DNA-P/min	2 (8)	25	6 (7)	86
Post-aspirin gastric DNA .. .. .. >100% change from pre-aspirin rate ..	6 (8)	75	1 (6)	17
Gastric cytology of Post-aspirin specimen				
Normal	6	86	1	17
Abnormal	1	14	5	83
	(7)		(6)	

Features of gastric washings obtained before and after aspirin from 15 patients, seven of whom bled less than 2 ml/day with aspirin (non-bleeders) and eight of whom bled 2 ml or more per day (bleeders). In one non-bleeder only the pre-aspirin DNA rate was obtained and in one bleeder cytological preparations were not made. Compared with non-bleeders most bleeders had lower pre-aspirin gastric DNA rate and a greater percentage change from pre-aspirin DNA rate after aspirin. Cytologically most bleeders had normal surface epithelial cells in the post-aspirin specimen, whereas most non-bleeders had abnormal cells and inflammatory cells which indicated that they may have had atrophic gastritis. The figures in brackets are the number of patients studied for each parameter. (Abstracted from unpublished data of Croft & Wood, and also reported in Croft, 1963b.)

pre-aspirin rate of accumulation of DNA in the stomach than most non-bleeders. The mean value of 52 ng atoms DNA-P/min in the bleeders was not, however, statistically significantly different from that of 112 in the non-bleeders ($P < 0{\cdot}1$).

Secondly, most of the bleeders increased their gastric DNA after the administration of aspirin by more than 50 or 100%. Most of the non-bleeders did not; four out of six actually showed a decrease compared with the pre-aspirin rate.

Thirdly, most of the bleeders had exfoliated normal gastric surface epithelial cells in the gastric washing obtained after aspirin. Most non-bleeders had cells of a very different appearance. Numerous abnormal gastric cells and inflammatory cells were seen. These cytological changes indicated that the non-bleeders may have had atrophic gastritis.

Thus the bleeders appeared to have cytologically normal gastric mucosa with a low pre-aspirin DNA rate. After aspirin they increased their DNA rate and cytologically the specimens contained normal surface cells. The non-bleeders on the other hand mostly began with a high resting DNA rate and this tended to fall after aspirin. The high resting or pre-aspirin value suggested that there might be a high natural turnover in the mucosa of these patients. The cytological features indicated that they might have atrophic gastritis, and of the three non-bleeders who had had gastric biopsies, two of these were found to have atrophic gastritis.

Atrophic gastritis is a condition in which there is atrophy of the gastric glands and some alteration in the histology of the surface epithelium.

It occurs both in the presence and absence of pernicious anaemia; in the latter instance it is simple atrophic gastritis. In normal gastric mucosa obtained by suction biopsy from 11 patients there was a mean of 0·63 (s.d. 0·19) mitoses per 100 surface epithelial cells. Statistically significantly higher counts were obtained in atrophic gastric mucosa of patients with pernicious anaemia (1·4, s.d. 0·42, $P < 0·001$) and simple atrophic gastritis (1·5, s.d. 0·63, $P < 0·001$). These values indicated a higher turnover in atrophic than normal gastric mucosa. This was confirmed by the gastric DNA rates which were also measured in these patients and found to be higher than normal per unit surface area (Croft & others, 1966).

CONCLUSIONS

Salicylate-induced occult bleeding after repeated ingestion of aspirin appears to be the response to a physiological effect of aspirin on the gastric mucosa. It occurs from gastric erosions which result when the rate of loss of surface cells exceeds the rate at which they are replaced. A hyperdynamic mucosa, with increased rate of production or turnover of surface epithelial cells, which is probably the situation in atrophic gastritis, may confer protection against salicylate-induced occult bleeding. The mucosa in atrophic gastritis appears to be in a maximally dynamic state as a result of which aspirin cannot increase the rate of loss of cells above the mucosa's ability to replace them. However, factors other than turnover, such as alteration of epithelial cell susceptibility to aspirin, alterations in mucus production (Menguy & Masters, 1965) or achlorhydria may also be relevant to the apparent resistance of atrophic mucosa to aspirin-induced occult bleeding.

The data presented suggest that aspirin-induced occult gastrointestinal bleeding occurs because, in irritating the gastric mucosa, the drug causes excessive loss of the cells which are required to protect it. Resistance to occult aspirin bleeding may be related to gastric conditions in which there is a high natural replacement or turnover of gastric surface cells. Factors enhancing gastric mucosal susceptibility to aspirin and thus leading to severe bleeding are unknown, but may be related to gastric surface cell turnover.

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Discussion

Dr. B. B. Newbould. What proportion of severe gastrointestinal side-effects, such as ulcers, can be attributed to local concentrations of anti-inflammatory analgesic drugs, and what proportion to systemic levels? In view of the fact that many analgesic anti-inflammatory agents can cause damage to the gastrointestinal tract of animals following parenteral administration, can different methods of presentation really reduce the incidence of side-effects in man or can the lower incidence of side-effects following, for example, the administration of enteric coated tablets, be attributed to more erratic absorption? Have any observations been made on the gastrointestinal irritant effects of aspirin in patients taking cytotoxic drugs? If Dr. Croft's hypothesis on cellular proliferation is correct then aspirin should have severe effects on the gastrointestinal tract of patients receiving therapy with cytotoxic drugs.

Dr. A. J. Hale. Mitotic counts of cellular turnover rate can be extremely misleading. For example, an apparent increased cell turnover rate can be caused by arresting cells in mitosis; quite a number of agents