Br. J. Pharmac. (1977), **61**, 579–586

ESTIMATION AND CHARACTERIZATION OF PROSTAGLANDINS IN THE HUMAN GASTROINTESTINAL TRACT

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1 Prostaglandin-like material was extracted from muscle and mucosa of surgically removed human stomach, ileum and colon and assayed against prostaglandin E_2 on strips of rat gastric fundus. Superfused human isolated gastric mucosa released prostaglandin-like material and release was increased by stretching or clamping the tissue.

2 The relative amounts of extracted biological activity were broadly as follows: gastric antral mucosa > colon muscle > gastric body mucosa ≈ ileal mucosa > colon mucosa ≈ gastric muscle ≈ ileal muscle.

3 Prostaglandin E and F were tentatively identified by chromatography and sensitivity to inactivation by alkali.

4 Prostaglandin E apparently contributed most to the biological activity, possibly because the assay tissue is more sensitive to prostaglandin E than to F. Chromatography of gastric body mucosal extracts located material running with prostaglandin E_2 and a little with E_1. Colonic muscle and mucosal extracts contained material with R_F values of prostaglandins E_1, E_2, E_3 and $F_{1\alpha}$, whereas $F_{2\alpha}$ and $F_{3\alpha}$-like substances were found only in the mucosa. The proportions of prostaglandin F varied between specimens.

5 The amount of extracted prostaglandin-like activity was increased by adding cofactors and arachidonic acid, and lessened by homogenization with acid-ethanol.

6 The type and amount of activity generated from arachidonic acid by partly purified colonic mucosal prostaglandin synthetase depended on the substrate concentration.

7 The possible relationships of prostaglandins to mucus secretion and other physiological and pathological gut functions are discussed.

Introduction

The discovery of prostaglandin-like material in extracts of human tissues and gastric juice (Bennett, Murray & Wyllie, 1968) has been confirmed by several authors (for references see Bennett, 1976a, b). Prostaglandin-like material is released from incubated colonic cells (Jaffe, Parker & Philpott, 1971) and is present in extracts of homogenized colon (Bennett, del Tacca, Stamford & Zebro, 1977). The possible contributions of prostaglandins to gastrointestinal functions and disorders have been discussed by Bennett & Fleshler (1970) and Bennett (1976a, b). The object of the present work was to characterize the prostaglandin extracted from muscle and mucosa of various regions of the human gut, and to study prostaglandin synthetase extracted from colonic mucosa.

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Methods

Extraction of prostaglandin like material

Apparently normal gastrointestinal tissue (at least 6 cm from any macroscopic lesion) was obtained from patients undergoing surgery, mainly for benign gastric ulceration or colonic carcinoma. The specimens were placed in Krebs solution as soon as possible after surgical removal (within 1 h) and either extracted immediately or stored overnight at 4°C, or frozen where stated. Mesentery and fat were removed and the muscle and mucosal layers ('mucosa') were separated along the submucosal plexus. The tissue was cut into small pieces and washed on filter paper with Krebs solution. Weighed samples were homogenized for 30 s in Krebs solution or in acid-ethanol (Krebs solution : ethanol, 1 : 1, acidified to approximately pH 3 with formic acid). As discussed later, the acid-ethanol values indicate 'basal' levels; the Krebs solution values indicate 'total' levels (formation of prostaglandin-like

material from endogenous precursors plus 'basal' levels, less any prostaglandin inactivated; Bennett, Stamford & Unger, 1973). Prostaglandin-like material was extracted into chloroform as described by Unger, Stamford & Bennett (1971).

Some samples were homogenized and then incubated at 37°C for 30 min, before extraction, in 5 ml of either Krebs solution or acid-ethanol with or without added arachidonic acid and/or reduced glutathione and adrenaline (Flower, Cheung & Cushman, 1973).

Bioassay of prostaglandin-like material

The extracts were dissolved in Krebs solution and assayed against prostaglandin E_2 on rat gastric fundus strips (Vane, 1957) in the presence of drugs which increase assay selectivity and sensitivity (Gilmore, Vane & Wyllie, 1968, as modified by Bennett *et al.*, 1973).

Characterization of prostaglandins

Aliquots of some extracts from homogenates in Krebs solution were subjected to alkaline hydrolysis to inactivate prostaglandin E but not F compounds (Bennett *et al.*, 1968). The method does not discriminate between prostaglandins E_1, E_2 and E_3 or other susceptible prostaglandins.

Silicic acid column chromatography (Stamford & Unger, 1972) was carried out on many samples to remove impurities before further separation. When sufficient prostaglandin-like activity was present, it was subjected to chromatography (thin-layer plates with AI and AII solvent systems, Gréen & Samuelsson, 1964; or silica-gel-impregnated paper, AII and the solvent system described by Stamford & Unger (1972), here called XI). Individual zones were eluted with Krebs solution before bioassay (AI and XI systems) or first extracted into chloroform as above to remove silver nitrate (AII system). Amounts of prostaglandin-like material running at the R_F values of authentic prostaglandin E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$ or $F_{3\alpha}$ are expressed as prostaglandin E_2 equivalents and calculated as % total biological activity.

Location of prostaglandin formation in the gut wall

A rectangular piece of tissue approximately 3 x 4 cm was placed mucosal side uppermost on a freezing stage (Frigistor FS13 Reichart Co.). During freezing it was covered by a weighted glass slide which was supported on either side so that the tissue was only slightly compressed. Sections 20 μm thick were cut parallel to the mucosal surface with a sledge microtome having a Frigistor module attached to the knife. Alternate groups of five sections (100 μm) were homogenized for 30 s in 5 ml Krebs solution or acid-ethanol with a glass piston homogenizer, and extracted for prostaglandins. Every sixth section was stained with haematoxylin and eosin for histological examination.

Prostaglandin release from superfused gastric mucosa

A strip of fresh human gastric mucosa (approximately 5 x 0.5 cm, weighing about 4 g) and a rat stomach strip were superfused in series with Krebs solution containing hyoscine and mepyramine (both 0.2 μg/ml), methysergide and phenoxybenzamine (both 0.1 μg/ml) and pronethalol (1 μg/ml) to improve tissue selectivity and sensitivity to prostaglandins. Constant submaximal responses of the assay tissue were obtained with prostaglandin E_2 passed over the rat fundus, and the loads of 2 to 50 g or a small artery clamp (area of each face 50 mm; closing tension 750 g), were applied to the human gastric mucosa for 2 or 4 min to cause mechanical trauma. The superfusate was collected, extracted for prostaglandins, and subjected to either alkaline hydrolysis or chromatography in the AII solvent system.

Preparation of prostaglandin synthetase

Prostaglandin synthetase was prepared from human colonic mucosa following the method of Flower *et al.* (1973). Fresh tissue was washed with Krebs solution, cut finely with scissors, homogenized for 1 min with two volumes of 100 mM Tris-HCl buffer pH 8.2 and centrifuged for 10 min at 12,000 *g*. The supernatant was poured through two layers of cheesecloth and centrifuged at 80,000 *g* for 2.5 h, and the precipitate was smeared inside a round-bottomed flask. After lyophilizing, the material was pulverized and stored at −20°C. Samples of 5 mg mucosal synthetase preparation were incubated (37°C for 30 min) with cofactors (reduced glutathione and adrenaline, both 2.5 mM, Flower *et al.*, 1973) and 0.5 to 3.0 mM arachidonic acid in 1 ml Tris buffer. Matched control samples without enzymes were completed for each substrate concentration. The samples were extracted and bioassayed for prostaglandins, and the levels (test-control) were plotted against substrate concentration.

Drugs

The following drugs were used: (−)-adrenaline bitartrate (BDH), arachidonic acid (Sigma), reduced glutathione (Sigma), (−)-hyoscine hydrobromide (BDH), indomethacin (Merck, Sharpe & Dohme), methysergide bimaleate (Sandoz), mepyramine maleate (May & Baker), phenoxybenzamine hydrochloride (Smith, Kline & French), pronethalol hydrochloride (ICI), prostaglandins E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$ tromethamine salt and $F_{3\alpha}$ (Upjohn).

The Krebs solution contained (g/l): NaCl 7.1, $CaCl_2.6H_2O$ 0.55, KCl 0.35, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29, $NaHCO_3$ 2.1 and dextrose 1.0.

Prostaglandin levels are expressed as mean ± s.e. mean and differences were analysed with *t* tests for paired or unpaired data. All *P* values refer to two-tailed tests.

Results

Prostaglandins extracted from gastrointestinal tissue

The results are summarized in Table 1. In each case more biological activity was extracted from homogenates in Krebs solution ('total' amounts) than from acid-ethanol ('basal' amounts) ($P<0.05$ to <0.001).

Stomach. Muscle and/or mucosa from 28 stomach specimens (14 corpus, 14 antrum) were studied. The tendency for antral 'basal', 'total' and 'synthesized' ('total−basal') prostaglandin amounts to be higher than in corpus was not statistically significant (all $P>0.14$).

Terminal ileum. In 13 specimens of terminal ileum the tendency for total or synthesized prostaglandin amounts to be higher in the mucosa than in the muscle was not statistically significant ($P=0.076$ and 0.081 respectively). Basal levels were similar.

Colon. Table 1 shows separate results for ascending, descending and sigmoid colon (10, 5 and 15 specimens respectively) but the values are similar and have been calculated together. The muscle total, basal and synthesized amounts were higher than in mucosa (all $P<0.001$). In 6 further specimens of colon (5 sigmoid, 1 ascending) frozen at −20°C for 7 to 21 days before prostaglandin extraction, only the total prostaglandin amounts were measured. These too were higher in muscle compared to mucosa (respectively 100 ± 26 and 63 ± 21 ng prostaglandin E_2 equivalents/g, $n=5$, $P<0.01$), but lower ($P<0.001$) than values for the fresh tissue in Table 1.

Other regions. Proximal small intestine is difficult to obtain and only single specimens of distal duodenum and jejunum were examined. There was also only one specimen of rectum.

Comparison of prostaglandin amounts extracted from different regions

The prostaglandin values in Table 1 reveal substantial differences between regions. Total, basal and synthesized amounts generally varied in parallel; for clarity and because of the larger numbers, only total amounts were compared. Mucosal values for colon were lower than for gastric antrum ($P<0.01$), with ileal values intermediate but not significantly different from either. Extracts of muscle from stomach and terminal ileum contained similar quantities which were less than in the colon ($P<0.005$).

Characterization of prostaglandin-like material

The detailed results are shown in Table 2 (alkaline hydrolysis, XI and AI chromatography) and Table 3 (AII chromatography).

Stomach. Alkaline hydrolysis of extracts from corpus and antral muscle removed various proportions of the prostaglandin-like activity 'prostaglandin E'. In different specimens, however, thin layer chromatography (t.l.c.) with the AI solvent system indicated only prostaglandin E in gastric muscle and mucosa. AII t.l.c. of corpus mucosal extracts indicated mainly prostaglandin E_2 and material running at the R_F of prostaglandin E_1.

Duodenum. XI chromatography of muscle and mucosal extracts indicated prostaglandins E and F.

Terminal ileum. Alkaline hydrolysis of ileal muscle extracts removed various proportions of the prostaglandin-like activity. AI chromatography of samples from 3 other specimens indicated prostaglandin E- and F-like activity in the muscle and mucosa of two, but only prostaglandin-like material in another.

Colon. Alkali inactivation and XI chromatography indicated predominantly prostaglandin E in the mucosa and muscle. AII chromatography indicated predominantly prostaglandin E_2, with some material running at the R_F values of other prostaglandins.

Rectum. In the single specimen of rectum, the material from both muscle and mucosa was totally inactivated by alkali ('prostaglandin E').

Formation of prostaglandin-like material in sections of colon cut parallel to the mucosal surface

Three fresh specimens of colon (1 ascending, 2 sigmoid) were studied. Prostaglandin-like activity was measured in extracts of sections (see Methods section) homogenized in Krebs solution. A typical result, illustrated in Figure 1, shows relatively little prostaglandin throughout the mucosa with more in the muscle, tending to form two peaks. In another sigmoid colon the taeniae (longitudinal muscle), circular muscle and mucosa were separated by dissection. The respective total quantities were 320, 550 and 420 ng prostaglandin E_2 equivalents/gram.

Prostaglandin release

Clamping gastric mucosal tissue for 2 to 4 min released 2 to 24 ng prostaglandin E_2 equivalents,

Table 1 Prostaglandin-like material (total, basal and synthesized) extracted from mucosa and muscle of the human gastrointestinal tract

	Mucosa			*Muscle*		
Region	*Total*	*Basal*	*Synthesized*	*Total*	*Basal*	*Synthesized*
Stomach corpus	520 ± 170 (5)	180 ± 61 (5)	340 ± 120 (5)	220 ± 52 (14)	47 ± 10 (13)	160 ± 130 (13)
Stomach antrum	1100 ± 310 (5)	360 ± 97 (5)	790 ± 240 (5)	300 ± 69 (13)	73 ± 35 (7)	340 ± 260 (7)
Duodenum	120 (1)	–	–	150 (1)	–	–
Jejunum	–	–	–	60 (1)	–	–
Terminal ileum	540 ± 160 (7)	29 ± 11 (7)	510 ± 170 (7)	200 ± 44 (13)	45 ± 11 (9)	200 ± 50 (9)
Colon ascending	240 ± 50 (10)	29 ± 9 (8)	180 ± 270 (8)	670 ± 230 (9)	67 ± 25 (7)	720 ± 220 (7)
Colon descending	180 ± 43 (5)	8, 25 (2)	180, 140 (2)	730 ± 250 (5)	110–140 (3)	670–1300 (3)
Colon sigmoid	260 ± 63 (15)	23 ± 6 (9)	240 ± 150 (9)	710 ± 110 (15)	130 ± 41 (9)	670 ± 110 (9)
Rectum	75 (1)	26 (1)	49 (1)	210 (1)	26 (1)	180 (1)

Mean levels are expressed as ng prostaglandin E_2 equivalents/g tissue ± s.e. mean corrected to 2 significant figures. The numbers of specimens are given in parentheses.

Table 2 Tentative identification of prostaglandins E and F in gastrointestinal extracts by alkaline hydrolysis (prostaglandin E inactivated) or chromatography

	Alkaline hydrolysis				*Chromatography (XI, AI)*			
	Mucosa		*Muscle*		*Mucosa*		*Muscle*	
	%E	%F	%E	%F	%E	%F	%E	%F
Stomach body			80(20–100)(7)	20(0–80)(7)	100(5)	0(5)	100(3)	0(3)
Stomach antrum			50(0–100)(8)	50(0–100)(8)	100(2)	0(2)	100(1)	0(1)
Stomach pylorus					100(2)	0(2)	100(2)	0(2)
Duodenum					60(1)	40(1)	50(1)	50(1)
Terminal Ileum	40–95(3)	5–60(3)	50(5–100)(7)	50(0–95)(7)	20–95(3)	5–80(3)	50–100(3)	0–50(3)
Ascending colon	100(45–100)(5)	0(0–55)(5)			92(1)	8(1)	80(1)	20(1)
Descending colon	70,100(2)	0,30(2)	65(1)	35(1)	20,100(2)	0,80(2)	90,98(2)	2,10(2)
Sigmoid colon	80(0–90)(7)	20(10–100)(7)	75–90(4)	10–20(4)	80(0–100)(9)	20(0–100)(9)	93(65–98)(8)	8(2–35)(8)
Rectum	100(1)	0(1)	100(1)	0(1)				

No samples were studied by both methods. Prostaglandin levels are expressed as ng prostaglandin E_2 equivalents/g (medians and ranges, corrected to 2 significant figures). The number of samples is given in parentheses.

Table 3 Prostaglandins extracted from human gastric and colonic mucosa and muscle separated by AII chromatography

Specimen	n	$\%E_1$	$\%E_2$	$\%E_3$	$\%F_{1\alpha}$	$\%F_{2\alpha}$	$\%F_{3\alpha}$
Mucosa							
Gastric body	8	15 (5–20)	85 (80–95)	0	0	0	0
Ascending colon	1	0	82	0	6	0	12
Descending colon	3	0	27–100	0	0–50	0–23	0–14
Sigmoid colon	11	0 (0–12)	80 (0–100)	0 (0–100)	0 (0–90)	0 (0–20)	0–(0–70)
Muscle							
Ascending colon	1	0	95	0	5	0	0
Descending colon	4	0–30	50–95	0	2–20	0	0
Sigmoid colon	8	0 (0–90)	94 (0–100)	0 (0–28)	2 (0–22)	0	0

Amounts are expressed as ng prostaglandin E_2 equivalents/g (medians and ranges corrected to 2 significant figures); n=number of specimens chromatographed.

Table 4 Amounts of prostaglandin-like material extracted from tissues incubated and homogenized in media containing combinations of substances which modify prostaglandin synthesis (+, upper part of table)

					Krebs solution			*Acid-ethanol*	
	Cofactors				+		+		+
	Arachidonic acid					+	+		+
Tissue	*ng PGE_2/g in Krebs soln.*	(n)	*Incubation (min)*						
Antral muscle	590	(4)	0	100	200		490	20	
Antral mucosa	1300	(4)	0	100	110		400	10	
Ileal muscle	95	(1)	0	100			260	10	40
Ileal mucosa	200	(1)	30	100		150	280	12	32
Colonic muscle	910	(4)	30	100	170	150		37	

Cofactors (μg/ml) were: reduced glutathione (100 stomach; 400 ileum; 200 colon) and adrenaline (100 stomach; 400 ileum and colon). Arachidonic acid concentrations were (μg/ml) 100 stomach; 20 ileum and colon. Results are expressed as % biological activity assayed against prostaglandin E_2 (PGE_2) corrected to 2 significant figures.

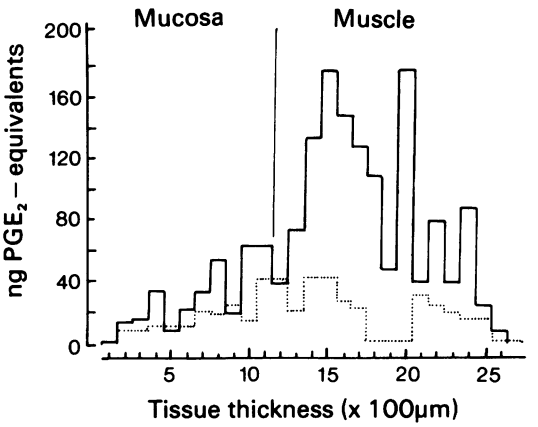

Figure 1 Prostaglandin-like (PG-like) material extracted from a sigmoid colon (female aged 76 years): —— homogenates in Krebs solution; . . . . . homogenates in acid-ethanol. Numbers on the horizontal axis represent 100 μm sections homogenized, and numbers on the vertical axis refer to amounts from 100 μm sections.

presumably from the clamped area (50 mm^2) (5 experiments). Aspirin (100 or 200 μg/ml) inhibited this release by 80 to 100% ($n=4$). Similarly, loads of 2 to 50 g applied to gastric mucosa released 0 to 15 ng (0 to 4 ng/g) prostaglandin E_2 equivalents ($n=5$). Aspirin (100 μg/ml, $n=1$) inhibited output by 80%. Enough material was obtained from one clamped gastric mucosa to allow characterization: alkaline hydrolysis destroyed the biological activity, and AII chromatography indicated prostaglandin E_2.

Factors affecting prostaglandin formation

Various specimens of gut tissue were homogenized or incubated and then homogenized, in media alone or containing substrate and/or cofactors (see Methods section). The experiments are summarized in Table 4 and indicate that more prostaglandin-like activity was produced with added substrate and/or cofactors and less with acid-ethanol. In a further experiment homogenized gastric antral mucosa and muscle were incubated for 15 min at 37°C in Krebs solution; subsequent addition of a very high concentration of indomethacin (200 μg/ml) did not decrease the amount of prostaglandins of (muscle $110 \pm 5\%$ of control; mucosa $100 \pm 10\%$; $n=4$ each). Thus virtually all the prostaglandin seemed likely to be formed before extraction. Homogenization of gastric antral muscle and mucosa with arachidonic acid yielded mainly prostaglandin E-like material on AI chromatography.

Prostaglandin synthetase

The amount of prostaglandin formed by synthetase prepared from 2 specimens of colon mucosa (1 sigmoid, 1 ascending colon) varied with substrate concentration (0.5, 1, 1.25, 1.5, 1.75, 2, 2.25 and 3 mM arachidonic acid). Peaks of product formation occurred with 1.25 and 2.25 mM arachidonic acid, with a trough at 1.5 mM substrate. XI chromatography of the products formed by mucosal synthetase from the ascending colon showed that the two peaks contained prostaglandin E- and F-like material and a substance with an intermediate R_F value. Products formed at the trough (1.5 mM arachidonic acid) were similar except that no prostaglandin F was found.

Discussion

The differences between various gut regions in formation of prostaglandin-like material seem unlikely to be artefacts, but they may be affected by several factors. The material was obtained from operation specimens and it is not known to what extent medication, anaesthesia, gastrointestinal disease, or delay in obtaining the samples may have affected the results. Some patients may have taken aspirin-like drugs several hours before surgery, but prostaglandin synthetase was never completely inhibited since homogenates in Krebs solution contained more activity than those in acid-ethanol. The nutritional status of the patients might also be important: rabbits fed on an essential-fatty-acid-free diet had low kidney levels of prostaglandin E_2 (Van Dorp, 1971), and vitamin C can affect prostaglandin production (Pugh, Sharma & Wilson, 1975).

The small amounts of prostaglandins in homogenates with acid-ethanol indicate that basal quantities are low, even though tissue levels of arachidonic acid rise quickly after death (e.g. in rat brain; Wolfe, Pappius & Marion, 1976). It is generally considered that tissues do not store prostaglandins, though they presumably contain prostaglandins released during activity or when cells die. We therefore do not know to what extent basal measurements represent amounts actually in the tissue, or material formed during homogenization before enzyme activity has been inhibited. Nor do we know the amounts and types of prostaglandin precursor in human gut, but arachidonic acid (which forms prostaglandin D_2, E_2, etc) is widely distributed in tissues, and the same types of prostaglandin seemed to be formed from endogenous precursor and added arachidonic acid. However, small amounts of 20:3 and 20:5 acids (which form prostaglandins E_1 and E_3 respectively) occur, for example, in shark intestine (Ogata & Nomura, 1975).

Samples were assayed on rat fundic strips in terms

of prostaglandin E_2 which is about equipotent with E_1 but more potent than F and other primary prostaglandins. The total prostaglandin content in a mixture of different prostaglandin classes is therefore underestimated, and correction cannot be calculated since the relative amounts and potencies of all the prostaglandin types are not known.

On AII chromatography with silica-gel-impregnated paper, prostaglandin D_1 is indistinguishable from E_1 but the R_F of D_2 (0.45) differs from those of the other primary prostaglandins tested. The 13,14-dihydro-15-oxo metabolite of prostaglandin $F_{2\alpha}$ runs with $F_{1\alpha}$, but 15-oxo-prostaglandin $F_{2\alpha}$ runs a fraction slower (Bennett, Charlier, McDonald, Simpson & Stamford, 1976; Bennett, Raja & Stamford, unpublished). These metabolites are only weakly active on the rat stomach strip (Crutchley & Piper, 1976; Bennett *et al.*, unpublished), but 13,14-dihydro-prostaglandin $F_{2\alpha}$, which would also be likely to run with prostaglandin $F_{1\alpha}$ has about one third the activity of $F_{2\alpha}$. 13,14-Dihydro-15-oxo-prostaglandin E_2 runs slightly faster than prostaglandin E_1, and 15-oxo-prostaglandin E_2 runs between prostaglandins E_1 and $F_{1\alpha}$. Thus metabolites of prostaglandin $F_{2\alpha}$ might contribute to the activity we have described tentatively as 'prostaglandin $F_{1\alpha}$-like'. Similarly, the rest of the chromatographic data must be interpreted cautiously. Although it is unlikely that the unstable endoperoxides prostaglandins G_2 and H_2, thromboxane A_2 or prostaglandin I_2 (prostacyclin, Moncada, Gryglewski, Bunting & Vane, 1976) would be present in the extracts, the contribution of other substances (e.g. thromboxane B_2 or 6-oxo-prostaglandin $F_{1\alpha}$, formed from thromboxane A_2 or prostaglandin I_2 respectively), to the biological activity is not known.

Only prostaglandin E was found in gastric mucosa and the substantial amounts were similar to those reported previously (Bennett *et al.*, 1968; Bennett *et al.*, 1973). Smaller quantities of 'prostaglandin E' were extracted from intestinal mucosa, but 'prostaglandin F' was also present. The analogue 15(R)-15-methyl-prostaglandin E_2 methyl ester stimulates human gastric mucus production (Fung, Lee & Karim, 1974), and drugs which inhibit prostaglandin synthesis (aspirin; corticosteroids, Gryglewski, Panczenko, Korbut, Grodzinska & Ocetkiewicz, 1975) decrease mucus secretion in rats and dogs (Menguy & Masters, 1963, 1965). Perhaps increased mucus secretion in gut inflammation involves prostaglandins. It is interesting that gastric and intestinal mucosa differ both in prostaglandin content and type of mucus secreted.

The responsiveness of gastrointestinal muscle to prostaglandin E and F compounds is well known (Bennett & Fleshler, 1970). More recently, experiments with prostaglandin antagonists and inhibitors of prostaglandin synthesis suggest that prostaglandins contribute to the regulation of gastrointestinal tone, activity and nerve mediated responses (see Bennett 1976a, for references). The importance of gut prostaglandins *in vivo* is not clear because we do not know the extent of their release during normal activity or in disease, or their contribution compared with other products of fatty acid metabolism. However, the experiments by Dilawari, Newman, Poleo & Misiewicz (1975) suggest that prostaglandin E_2 may be involved in both the physiology and pathophysiology of the human lower oesophageal sphincter.

We thank Mrs E.M. Charlier and Mrs B. Raja for technical assistance, the Wellcome Trust for support (H.L.S., E.M.C.), Dr J.E. Pike, Upjohn Co. Ltd. for prostaglandins and Merck, Sharp & Dohme Ltd. for indomethacin.

Note added in proof: Recent mass spectrometric data on two extracts of human gastric mucosa homogenized in Krebs solution show mostly 6-oxo-prostaglandin $F_{1\alpha}$ with small amounts of prostaglandins D_2, E_2, $F_{2\alpha}$ and thromboxane B_2 (A. Bennett, J.R. Boot, W. Dawson, D.N. Mallen, D.J. Osborne & I.F. Stamford, unpublished).

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(Received February 21, 1977.
Revised June 15, 1977)