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# OCCURRENCE OF PROSTAGLANDIN $E_2$ IN THE HUMAN STOMACH, AND A STUDY OF ITS EFFECTS ON HUMAN ISOLATED GASTRIC MUSCLE

BY

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We have previously shown the presence of an unidentified smooth-muscle stimulating factor in human stomach mucosa (Murray & Wyllie, 1964, 1965). The work described in the present paper has shown that the material is prostaglandin $E_2$ ($PGE_2$) and that it is present in moderately high concentration. We have also found that $PGE_2$ is remarkably active on human stomach muscle, a tissue generally unreactive to pharmacological agents (Bennett & Whitney, 1966).

## METHODS

### *Material*

Excised portions of human stomach, taken at least 6 cm from any macroscopic pathological lesion, were obtained fresh in the operating theatre. They were taken in cold Krebs solution to the laboratory where the mucosa and submucosa were dissected from the muscle. The mucosa was either extracted immediately or was stored at −20° C, usually for less than a week. The muscle was studied the same day or was stored overnight in Krebs solution at 4° C.

### *Extraction procedure*

Extracts were made initially with 80% acetone by the method of Parratt & West (1957) and subsequently by the method of Samuelsson (1963). The latter procedure involves ethanol extraction followed by partition firstly between diethyl ether and an aqueous phase acidified to pH 3, and secondly between petrol and aqueous ethanol. Parallel experiments showed that the initial stages of the two methods yielded approximately equal amounts of prostaglandins from the same sample of mucosa. In a few experiments tissues were extracted with Krebs solution by homogenization in a Potter-Elvehjem homogenizer or by grinding with sand.

### *Chromatography*

Chromatography of acetone extracts on Whatman No. 1 paper with butanol:acetic acid:water (60:15:25 vol.) was used to separate prostaglandins from 5-hydroxytryptamine. The E and F series of prostaglandins were separated by the method of Samuelsson (1963), using a 1 g column of silicic acid, and were also distinguished by thin layer chromatography using the solvent AI of Gréen & Samuelsson (1964). Plates impregnated with silver nitrate (0.5 g $AgNO_3$/15 g silica gel G, Mallinkrodt) were used with solvent system AII of Gréen & Samuelsson (1964) to distinguish prostaglandins with different numbers of double bonds.

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Paper chromatograms were prepared for bioassay by evaporating the organic solvents *in vacuo* or under a stream of nitrogen. Rectangles were cut from the paper and eluted either into Krebs solution or directly into the isolated organ bath. Ehrlich's aldehyde reagent was used to locate 5-hydroxytryptamine on some paper chromatograms (Smith, 1960). Thin layer plates were dried *in vacuo* over potassium hydroxide for 1 hr. Measured areas on the plate were scraped into test tubes and eluted with chloroform : methanol (1 : 1 vol.) which was decanted and evaporated *in vacuo* with centrifugation to prevent splashing. The material in the various tubes was then dissolved in 1 ml. of Krebs solution for bioassay. Where practicable, manipulations were carried out under nitrogen at 3° C. Storage was under nitrogen at −25° C in the dark.

*Bioassay*

The small quantities of prostaglandin in the extracts were detected on the rat stomach strip preparation (Vane, 1957). In some experiments rabbit duodenum was used because of its sensitivity to compounds of the F series, especially $F_{2\alpha}$. Extracts were also tested on rat duodenum to find whether bradykinin could be detected. These tissues were suspended in a 6 ml. bath of Krebs solution at 37° C gassed with oxygen : carbon dioxide (95 : 5%).

Strips of human stomach muscle were cut parallel to either the circular or the longitudinal fibres of body or antrum. They were studied in the way described by Bennett & Whitney (1966).

Chemicals used in the preparation of extracts for bioassay were all of analytical grade and were not further purified. The composition of Krebs solution was (g/l.): NaCl, 6.9; KCl, 0.35; $CaCl_2.6H_2O$, 0.55; $KH_2PO_4$, 0.16; $MgSO_4.7H_2O$, 0.29; glucose, 1.0; $NaHCO_3$, 2.1.

The drugs used were: acetylcholine perchlorate, (−)-adrenaline bitartrate, bromolysergic acid diethylamide (BOL), chymotrypsin, cocaine hydrochloride, hexamethonium bromide, histamine acid phosphate, " Hydergine " (a mixture of equal parts of dihydroergocornine, dihydroergokryptine and dihydroergocrystine as methanesulphonates), 5-hydroxytryptamine creatinine sulphate (5-HT), (−)-hyoscine hydrobromide, mepyramine maleate, methysergide bimaleate, pronethalol hydrochloride and $PGE_1$, $PGE_2$, $PGF_{1\alpha}$ and $PGF_{2\alpha}$. Except for " Hydergine " and the prostaglandins, drug concentrations refer to the base.

## RESULTS

Extracts of mucosa made with Krebs solution, aqueous acetone or ethanol contracted the rat fundus strip in the presence of methysergide, hyoscine and mepyramine (all $2 \times 10^{-7}$ g/ml.); these drugs abolished the effects of large doses of 5-hydroxytryptamine, acetylcholine and histamine, respectively. The material in the extracts was also distinguishable from these substances by the somewhat slower time course of the contraction and relaxation of the strip (Fig. 1).

### *Identification of $PGE_2$ in extracts*

Paper chromatography separated the total activity in acetone extracts into two components (Fig. 2). One component detected by the untreated rat stomach strip was 5-hydroxytryptamine because it ran with an $R_F$ of 0.5 identical with authentic 5-hydroxytryptamine and was blocked by methysergide or by BOL. The other component was resistant to these antagonists of 5-hydroxytryptamine and ran near the solvent front. This high $R_F$ readily distinguished it from adenosine mono- di- or triphosphate, substance P, gastrin and bradykinin which remained near the origin. Also, the unknown contracted the rat duodenum, a tissue relaxed by bradykinin. Incubation with chymotrypsin (100 $\mu$g/ml., pH 8, 30 min, 37° C) had no effect on the substance.

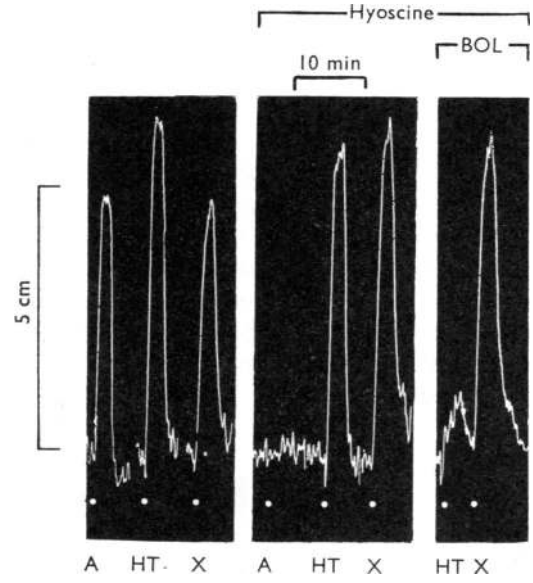


Fig. 1. Rat stomach strip. Responses to 50 ng of acetylcholine (A) and 5 ng of 5-hydroxytryptamine (HT) were abolished, respectively by hyoscine $10^{-7}$ g/ml. and BOL $10^{-6}$ g/ml. Responses to acid lipid material extracted from 80 mg of gastric mucosa (X) were unaffected.

The position of the unknown on paper chromatograms suggested that it might be one of the pharmacologically active acidic lipids. This was confirmed by its distribution between an organic phase (diethyl ether or chloroform) and an aqueous phase of differing pH values. Figure 3 shows the result of a typical experiment in which the dry residue from an extract of 350 mg mucosa, previously purified from 5-hydroxytryptamine by paper chromatography, was re-suspended in either 1 ml. 0.025 M Tris-(hydroxy methyl)-amino-methane buffer of the stated pH or 1 ml. 0.1 N-HCl, and was partitioned by shaking with 1 ml. of ether. After centrifuging, the phases were separated and the ether was removed in a stream of nitrogen. The dry material from the ether phase was shaken with 1 ml. of Krebs solution and assayed. The ether-free aqueous phase was adjusted to pH 7 with sodium hydroxide before assay. The change in partition coefficient in favour of the ether phase when the aqueous phase was acidified indicated that the active material was an acidic lipid. In similar experiments neither benzene nor petrol extracted the material from an acid aqueous phase. Unlike 5-hydroxytryptamine, the unidentified material was destroyed by exposure of paper chromatograms to bromine vapour, suggesting that olefinic double bonds were essential for activity and that the material might be a prostaglandin.

The $R_F$ of the substance on thin layer chromatography using the solvent system of Horton and Thompson (1963), was 0.37 and was indistinguishable from that of $PGE_1$ ($R_F = 0.36$).

Extracts made by the method of Samuelsson (1963) were applied to 1 g silicic acid columns to separate dihydroxy (E-type) from trihydroxy (F-type) prostaglandins. They were eluted successively with 50 ml. lots of ethyl acetate: benzene in proportions by

volume 30:70; 60:40; 80:20, followed by ethyl acetate:methanol (95:5). Most of the activity was found in the 60:40 fraction, and traces in the 80:20 fraction. Control columns were run with $PGE_1$ (2 $\mu$g), $PGE_2$ (2 $\mu$g) and $PGF_{1\alpha}$ (15 $\mu$g). Most of the E compounds were eluted, as stated by Samuelsson (1963), in the 60:40 fraction. The F compound was eluted mainly in the 80:20 fraction together with a little of the E compounds. Confirmation that the substance in the tissue extracts was an E-type prostaglandin was therefore sought by thin layer chromatography of the column eluates using the AI system of Gréen & Samuelsson (1964), which distinguishes the E and F compounds. All the activity was found at the same $R_F$ as authentic $PGE_1$ ($R_F=0.33$) and none at the $R_F$ of $PGF_1$ ($R_F=0.26$). This confirmed that the activity eluted in ethyl acetate:benzene (60:40) was the result of an E-type prostaglandin, and showed also that the trace of activity found in the 80:20 fraction was the result of an E compound not completely eluted by the less polar mixture.

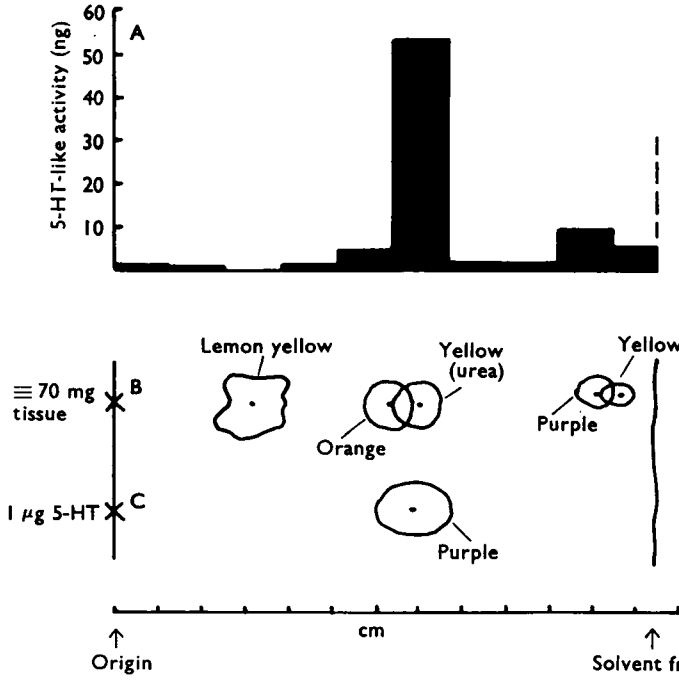


Fig. 2. Parallel paper chromatograms of 1 $\mu$g of 5-hydroxytryptamine (C) and evaporated acetone extract of 70 mg of mucosa (A, B) run in butanol:acetic acid:water (60:15:25 vol.). B and C were developed with Ehrlich's aldehyde reagent to show the position of 5-hydroxytryptamine and the presence of several unidentified materials in the extract. In A bioassay on the rat stomach strip of ≡70 mg of the same tissue extract run in parallel showed 5-hydroxytryptamine and another component near the solvent front.

The solvent system AI of Gréen & Samuelsson (1964) and that of Horton & Thompson (1963) both produce wide separation of prostaglandins from other gut-stimulating substances such as unsaturated carboxylic acids and phospholipids, which run faster and slower respectively. It therefore seemed certain that our material was an E-type prostaglandin.

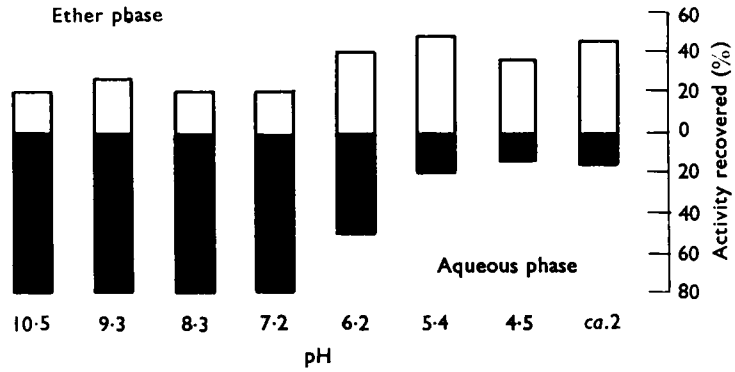


Fig. 3. Partition of active material in gastric mucosal extracts between equal volumes of diethyl ether (clear areas) and an aqueous phase (black areas) of differing pH values.

The prostaglandin in the extracts was characterized further using the solvent system AII of Gréen & Samuelsson (1964) and thin layer plates impregnated with silver nitrate. The material in the extracts had an $R_F$ (0.68) clearly different from that of $PGE_1$ (0.82) and identical with that of $PGE_2$ (0.65). Since $PGE_3$ runs very much more slowly than $PGE_2$, this identifies the material in the extract as $PGE_2$.

Two other types of experiment showed that all the activity in crude extracts could be attributed to $PGE_2$. A crude extract was made by homogenizing mucosa with 4 volumes of ethanol, evaporating the supernatant to dryness at room temperature on a rotary film evaporator and dissolving the dry material in water (1 ml./g of wet mucosa). Fifteen tubes were arranged to contain either extract from 0.5 g of tissue or prostaglandin (2 $\mu$g $PGE_1$, 2 $\mu$g $PGE_2$, 15 $\mu$g $PGF_{1\alpha}$, or 3 $\mu$g $PGF_{2\alpha}$) in a final volume of 1.0 ml. of each of the following: 0.1 N-NaOH, 0.1 N-HCl and 0.1 N-NaCl. After incubating at 38° C for 30 min, the acid and alkali were neutralized and the contents of the tubes were assayed on a rat stomach strip treated with methysergide and hyoscine. Table 1 shows the height of contractions caused by 1/25 of the materal in each tube. The activity of the extract, and that of the E compounds, was totally destroyed by alkali in each of two experiments whereas that of the F compounds was unaffected. This seems to exclude the presence in the extract of concentrations of F-compounds high enough to affect the rat stomach strip.

TABLE 1

DESTRUCTION OF E-TYPE PROSTAGLANDINS AND OF ACTIVITY IN EXTRACTS BY MILD ALKALINE HYDROLYSIS (30 MIN AT 38° C)

| | Height of contraction of rat stomach strip (cm) after hydrolysing extracts and prostaglandins in: | | |
|---|---|---|---|
| | 0·1 N NaOH | 0·1 N HCl | 0·1 N NaCl |
| $PGE_1$ | 0 | 15 | 14 |
| $PGE_2$ | 0 | 11 | 11 |
| $PGF_{1\alpha}$ | 5 | 4 | 5 |
| $PGF_{2\alpha}$ | 5 | 4 | 5 |
| Extract | 0 | 6 | 6 |

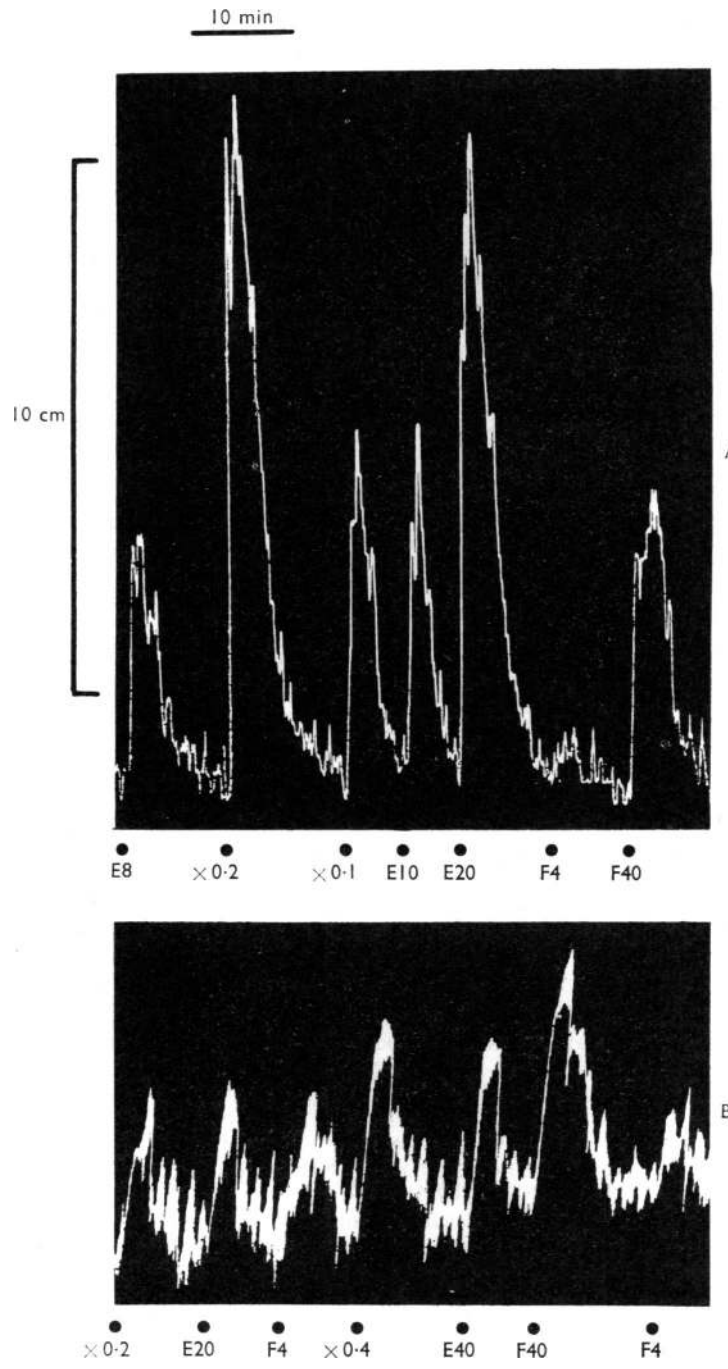


Fig. 4. Comparative assay of mucosal extract (X, dose in ml. added to bath) on rat stomach strip (A) and rabbit duodenum (B). $PGE_2$ (E) 20 ng/ml. and extract 0.2 ml. were equiactive on both preparations. The rabbit duodenum was less sensitive to $PGE_2$, but more sensitive to $PGF_{2\alpha}$ (F) than was the rat stomach strip. Doses of prostaglandins in ng/ml.

We found, however, that rat stomach strips were not equally sensitive to all four prostaglandins. Equiactive doses producing a moderate contraction were on average: $PGE_2$, 2 ; $PGE_1$, 3 ; $PGF_{2\alpha}$, 15 ; $PGF_{1\alpha}$, 80 or more ng/ml. The corresponding values in rabbit duodenum were $PGF_{2\alpha}$, 10 ; $PGE_1$, 20 ; $PGE_2$, 20 ; $PGF_{1\alpha}$, 40 ng/ml. Thus an extract containing a mixture, for example, of $PGE_2$ and $PGF_{2\alpha}$ should give different values when assayed against $PGE_2$ on the two preparations. There was no evidence for the presence of F compounds in any of three experiments (Fig. 4). Thus, by chromatography, hydrolysis, and parallel assay, we were unable to find prostaglandins other than $PGE_2$ in our extracts. Nor did we find activity at unexpected places on chromatograms to show the presence of other unidentified components.

*Concentration of $PGE_2$ in the stomach wall*

The amounts of $PGE_2$ per g of wet tissue varied from 0.01 to 2.4 $\mu$g (Table 2). These values are uncorrected for losses which were estimated to be of the order of 50%. In general, the concentrations in a given stomach were similar in the body and the pyloric mucosa. It is interesting that the values in two out of three cases of gastric cancer were lower than normal.

TABLE 2

QUANTITIES OF $PGE_2$ IN EXTRACTS OF MUCOSA FROM DIFFERENT STOMACHS

| Patient | Diagnosis | $PGE_2$ content ($\mu$g/g wet weight) Body | Pylorus |
|---|---|---|---|
| 1 | Duodenal ulcer | 0·12 | — |
| 2 | Duodenal ulcer | 1·1 | |
| 3 | Duodenal ulcer | 0·44 | |
| 4 | Duodenal ulcer | 1·5 | 1·7 |
| 5 | Duodenal and gastric ulcers | 2·4 | 2·1 |
| 6 | Gastric ulcer | 1·1 | — |
| 7 | Gastric ulcer | 2·4 | 1·7 |
| 8 | Gastric carcinoma | 1·5 | 1·6 |
| 9 | Gastric carcinoma | 0·19 | 0·01 |
| 10 | Gastric carcinoma | 0·14 | 0·01 |

The concentration of extractable prostaglandin was about four times higher in mucosa than in submucosa from the same area of the stomach. Only traces of activity were found in muscle. The material in submucosa and muscle was not identified, but it behaved similarly to that in mucosa on paper chromatography, solvent extraction, and bioassay.

*Effect of prostaglandins on human stomach muscle* in vitro

$PGE_2$ was mainly used, because it occurs naturally in the human stomach. Twenty-nine strips of circular and longitudinal muscle were obtained from the gastric body and antrum from eighteen patients.

There was a striking difference between the response of circular and longitudinal muscle, especially in preparations from the body of the stomach. Prostaglandins had an inhibitory effect on all strips of circular muscle from the body and the antrum. They reduced any spontaneous activity, and they depressed the response to acetylcholine added to the bath (Body: $PGE_1$, three experiments ; $PGE_2$, seven experiments. Antrum: $PGE_1$, two experiments ; $PGE_2$, four experiments).

This inhibition was dose dependent, and was detected with concentrations as low as 4–10 ng/ml. of either $PGE_1$ or $PGE_2$. On a molar basis, $PGE_1$ and $PGE_2$ were several times more potent than adrenaline. Larger doses (200 ng/ml.) reduced the effect of acetylcholine by as much as 87% in one experiment. This effect was unaltered by cocaine ($4 \times 10^{-5}$ g/ml.; two experiments) or by adrenergic receptor blockade with a combination of "Hydergine" ($10^{-6}$ g/ml.) and pronethalol ($10^{-5}$ g/ml.; four experiments; Fig. 5). Responses to potassium chloride (3 mg/ml.) were also reduced by 0.08–0.6 $\mu$g/ml. $PGE_2$ (20–34%; three experiments, Fig. 6) thus showing that the inhibition of responses to acetylcholine was not the result of an atropine-like action.

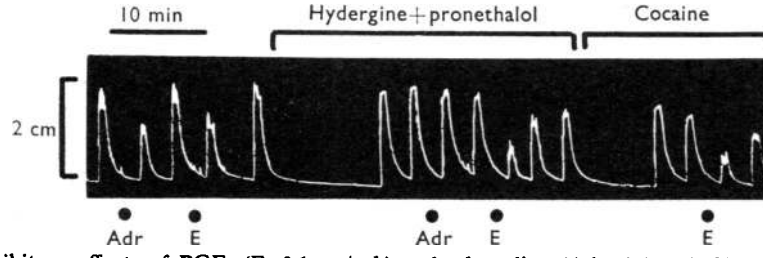


Fig. 5. Inhibitory effects of $PGE_1$ (E, 0.1 $\mu$g/ml.) and adrenaline (Adr. 0.4 $\mu$g/ml.) on the response of a circular muscle strip from human gastric antrum. Each contraction (unlabelled) is caused by acetylcholine 0.6 $\mu$g/ml. The effects of $PGE_1$ and of adrenaline were demonstrated as a reduced response to acetylcholine which was added to the bath without changing the fluid 1 min after the dose of inhibitor. Neither "Hydergine" ($10^{-6}$ g/ml.) in combination with pronethalol ($10^{-5}$ g/ml.), which blocked the effect of adrenaline, nor cocaine ($4 \times 10^{-5}$ g/ml.) affected the response to $PGE_1$.

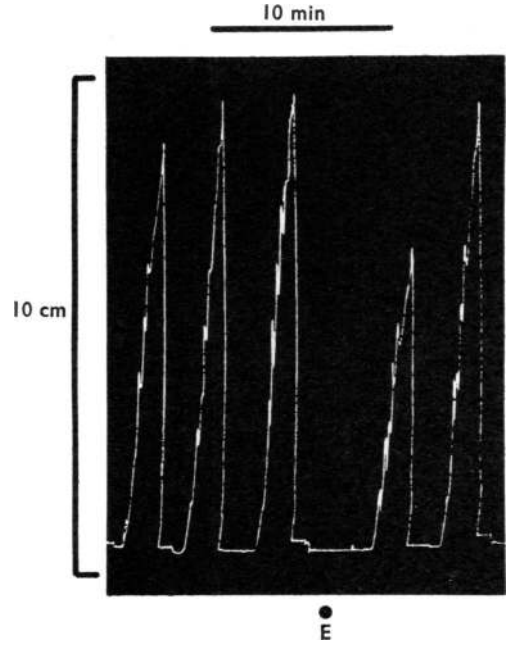


Fig. 6. Inhibitory effect of $PGE_2$ (E) on the response of a circular muscle strip from human gastric body to potassium chloride (3 mg/ml.; unlabelled contractions). $PGE_2$ (0.1 $\mu$g/ml.) was added 1 min before the potassium chloride. Drum off during relaxation of the strip.

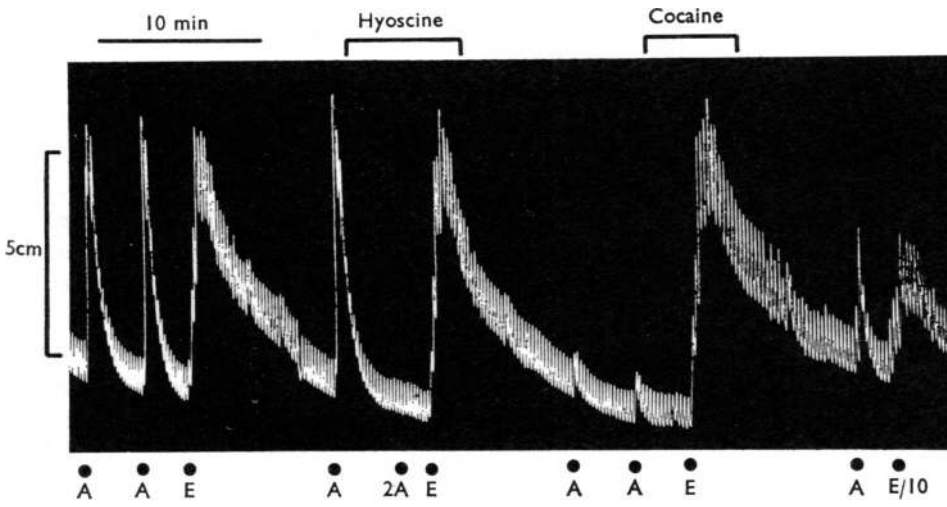


Fig. 7. Contractions of a longitudinal muscle strip from body of human stomach in response to acetylcholine 150 and 300 ng/ml. (A and 2A) and $PGE_2$ 20 and 2 ng/ml. (E and E/10). Relaxation of the strip was much slower after $PGE_2$ than after acetylcholine. Neither hyoscine ($4 \times 10^{-7}$ g/ml.), which abolished the effect of acetylcholine, nor cocaine ($5 \times 10^{-5}$ g/ml.) altered the response to $PGE_2$.

In contrast, the longitudinal muscle from the body of the stomach responded to prostaglandins by contraction in each of seven experiments. In some cases (Fig. 7) the threshold dose was as low as 2 ng/ml. of $PGE_2$. The longitudinal muscle of the antrum, however, responded less constantly. In one experiment, $PGE_2$ 200 ng/ml. caused a contraction. In two others the response to acetylcholine was potentiated by low doses (20 and 200 ng/ml., respectively) but was reduced by higher doses (200–800 ng/ml.) in these and in three other experiments. The contraction of longitudinal muscle from both the body and the antrum was unaffected by hexamethonium ($4 \times 10^{-5}$ g/ml.; two experiments) or by hyoscine ($4 \times 10^{-7}$ g/ml.; two experiments). Cocaine $5 \times 10^{-5}$ g/ml. had no effect on the response of a strip from the body of the stomach to $PGE_2$, and methysergide ($2 \times 10^{-7}$ g/ml.) or mepyramine ($10^{-7}$ g/ml.) had no effect on the response of an antral strip to $PGE_2$. In the one experiment where high doses ($10^{-5}$ g/ml.) of mepyramine were used, the contraction to $PGE_2$ was reduced, but it returned to normal when the bath fluid was changed.

## DISCUSSION

The activity in our extracts was caused by an acidic lipid, which behaved like a prostaglandin on thin layer chromatography in the solvent system of Horton & Thompson (1963). It had the properties of an E-type prostaglandin in the system AI of Gréen & Samuelsson (1964), and in the column elution system of Samuelsson (1963). It was proved to be $PGE_2$ with the system AII of Gréen & Samuelsson (1964).

No F-type compounds were detected using thin layer chromatography to separate E and F compounds, or mild alkaline hydrolysis to destroy E-compounds, or parallel assays on two tissues having different relative sensitivities to E and F prostaglandins.

The occurrence of prostaglandin in the intestine has been mentioned by Bergström *et al.* (1962), and by Vogt, Suzuki & Babilli (1966), who found what was thought to be a mixture of $E_1$ and $F_{1\alpha}$ in a dialysate of frog intestine (" Darmstoff "). Ambache, Brummer, Rose & Whiting (1966) showed that longitudinal intestinal muscle incorporating Auerbach's plexus contained both $PGE_2$ and $PGF_{2\alpha}$.

E-type prostaglandin occurs in the rat stomach (Wolfe, Coceani & Pace-Asciak, 1967 ; Bennett, Friedmann & Vane, 1967), and in the rat intestine, which possesses high enzymic activity for synthesizing E-type but not F-type prostaglandins (Nugteren, Beerthuis & Van Dorp, 1966). So it seemed likely that the gastro-intestinal tract is an important site for production of E-type prostaglandins.

The physiological and pathological significance of the prostaglandins and the importance of differences in their distribution are not yet known. It seems unlikely that prostaglandins play a physiological part as circulating hormones, because they are almost cleared from blood in one passage through the lungs (Ferreira & Vane, 1967). But massive release of prostaglandin might contribute to the hypotension which occurs, for example, in gut strangulation.

It is interesting that $PGE_2$ is active on strips of isolated human stomach muscle in doses lower than those of other substances (Bennett & Whitney, 1966). As shown in Fig. 7 2 ng/ml. was sometimes sufficient to contract the longitudinal muscle. The remarkable opposing actions on circular and longitudinal muscle are also shown by prostaglandins acting on isolated guinea-pig ileum (Bennett & Eley, unpublished). This may reflect a role of these compounds in peristalsis.

The experiments with pharmacological blocking agents indicate that both excitatory and inhibitory actions of E-type prostaglandins are exerted directly on the muscle. The large stores of prostaglandin in the mucosa would have to reach the muscle in some way if they were to affect gastric motility. We have, however, been unable to find statements in the literature to support or refute the possibility of direct vascular links between mucosa and muscle. On the other hand, the mucosal prostaglandin may have a different, and so far unsuspected, role.

## SUMMARY

1. Human stomach mucosa contains a pharmacologically active acidic lipid which is indistinguishable from $PGE_2$ by chromatography in four different systems, and by other tests.

2. The concentrations present in mucosa are of the order of 1 $\mu$g/g of wet tissue. Concentrations are similar in mucosa of the body and the pyloric part of the stomach ; much smaller amounts are present in submucosa and muscle.

3. Prostaglandin $E_2$ acts directly on both the longitudinal and the circular muscle layers of the human isolated stomach.

4. The longitudinal muscle of the body of the stomach is contracted by doses of $PGE_2$ which may be as low as $2 \times 10^{-9}$ g/ml.

5. In contrast, $PGE_2$ inhibits the contractions of circular muscle caused by acetylcholine or potassium.

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