Br. J. Pharmac. (1968), **34**, 630–638.

Effects of prostaglandins E_1 and E_2 on human, guinea-pig and rat isolated small intestine

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1. Prostaglandins E_1 and E_2 contracted the longitudinal muscle of human, guinea-pig and rat isolated ileum.

2. The site of action varied with the species. In the rat and in some strips of human tissue prostaglandin appeared to have only a direct action on or in the muscle cells. In the other strips of human tissue and in guinea-pig ileum the prostaglandins seemed to stimulate both the intrinsic cholinergic nerves and the muscle cells.

3. In contrast to the longitudinal muscle, the circular muscle of human, guinea-pig and rat isolated ileum was usually inhibited by prostaglandin, apparently by an action directly on the muscle cells.

4. Prostaglandins may play a part in the control of intestinal motility.

Prostaglandins have been found in the gastrointestinal wall of man and other animals (Ambache, Brummer, Rose & Whiting, 1966; Vogt, Suzuki & Babilli, 1966; Coceani, Pace-Asciak, Volta & Wolfe, 1967; Bennett, Friedmann & Vane, 1967; Bennett, Murray & Wyllie, 1968), and are released from the serosal and mucosal surface of the rat isolated stomach during peristalsis elicited by electrical stimulation (Coceani *et al.*, 1967; Bennett, Friedmann & Vane, 1967). The longitudinal gastrointestinal muscle of many species responds with contraction to low concentrations of prostaglandins, but in contrast the circular muscle of the human stomach is inhibited (Bergström, Eliasson, Euler & Sjövall, 1959; Horton & Main, 1963; Bennett, Murray & Wyllie, 1968). The experiments described here were made to determine the effects and modes of action of prostaglandins E_1 and E_2 on the longitudinal and circular muscle layers of the human, guinea-pig and rat small intestine.

Methods

Human small intestine was obtained from specimens removed at operation, at least 6 cm from any pathological lesion, and strips 2–3 cm long and 2–3 mm wide were cut parallel to either the longitudinal or circular muscle layers. The mucosa was usually removed because its presence made no difference to the response, and

the preparations were suspended in Krebs solution at 37° C bubbled with 5% carbon dioxide in oxygen. The movements of the muscle were recorded on a kymograph by an isotonic frontal writing lever (magnification × 8). The load on the tissue was 0.5–1.5 g. Whole segments of guinea-pig and rat ileum 2–3 cm long were used to study the longitudinal muscle. Strips of circular muscle were prepared either by cutting the tissue into a spiral, or by zig-zag transverse cuts in the way described by Harry (1963). The rest of the procedure was as described for human tissue except that the load on the muscle was approximately 0.5 g.

The drugs used were acetylcholine perchlorate, adrenaline hydrogen tartrate, dimethyphenylpiperazinium iodide (DMPP), hexamethonium bromide, histamine hydrogen phosphate, " Hydergine," Sandoz Ltd. (containing equal parts of dehydroergocornine, dehydroergocristine and dehydroergokryptine), 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, mepyramine maleate, methysergide bimaleate, neostigmine methylsulphate, nicotine hydrogen tartrate, physostigmine sulphate, potassium chloride, pronethalol hydrochloride, prostaglandins E_1 and E_2, and tetrodotoxin. The concentrations of the salts are expressed in terms of base.

Results

Effects of prostaglandins E_1 and E_2 on the longitudinal intestinal muscle

Both prostaglandins caused similar effects in each preparation and their potencies were approximately the same. Slight tachyphylaxis occurred in one strip of guinea-pig ileum, but not in any other experiment.

Human. Four strips of jejunum and seven strips of ileum were obtained from eleven patients and were studied the same day. Prostaglandins E_1 and E_2 (0.2–1 μg/ml., five and nine experiments respectively) were added to the bath fluid for 1–4 min and caused a contraction of the longitudinal muscle which took 1–3 min to reach its peak. Relaxation after the drug was washed out of the bath was also slow (Figs. 1 and 2).

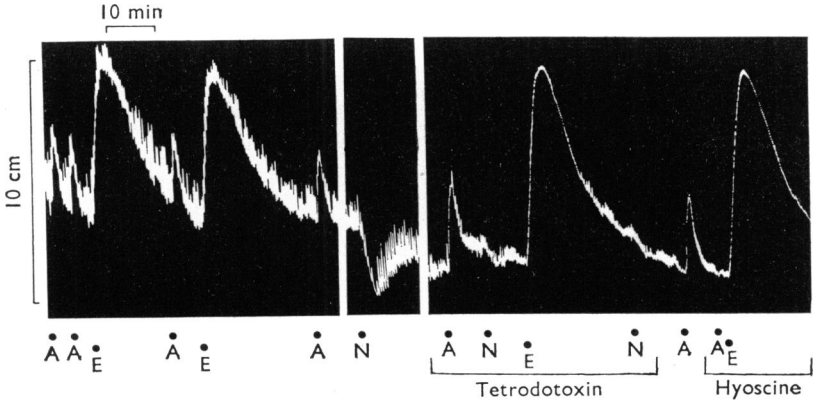

FIG. 1. Longitudinal muscle strip of human ileum showing responses to acetylcholine (A, 0.4 μg/ml.), prostaglandin E_2 in contact with tissue for 2 min (E, 0.4 μg/ml.) and nicotine (N, 20 μg/ml.). Tetrodotoxin (0.2 μg/ml.) prevented the effect of nicotine but not of prostaglandin. Hyoscine (0.2 μg/ml.) prevented the effect of acetylcholine but not of prostaglandin.

Guinea-pig. On eight tissues prostaglandin E_1 (six experiments) and E_2 (three experiments) caused contraction of the longitudinal ileal muscle in doses ranging from 1 to 200 ng/ml. Guinea-pig ileum contracted faster than human muscle strips on addition of prostaglandin and relaxed more quickly on changing the bath fluid (Fig. 3).

Rat. Prostaglandins E_1 and E_2 (three experiments each) caused the longitudinal muscle of rat ileum to contract in doses of 0.05–1 μg/ml. (Fig. 4). In one instance the contraction was preceded by a small relaxation.

Site of action of prostaglandins on the longitudinal muscle of human, guinea-pig and rat small intestine

Consistent submaximal contractions of the longitudinal intestinal muscle were produced by prostaglandins E_1 and E_2, and drugs were then added to determine whether the contractions were due to activation of intrinsic nerves, to the release of other substances, or to a direct action on the muscle cells.

Hexamethonium (20–40 μg/ml.) greatly reduced or abolished the response to nicotine or DMPP, but had no effect on prostaglandin-induced contractions of human or rat small intestine (six and three experiments, respectively, Figs. 2 and 4). The response of guinea-pig ileum to prostaglandin was either unaltered or reduced by approximately 10% (two experiments each).

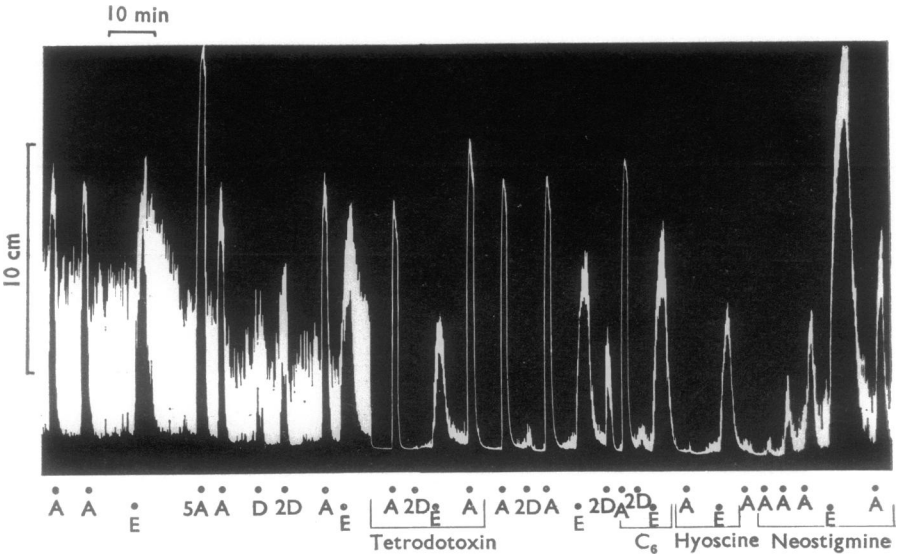

FIG. 2. Longitudinal muscle strip of human jejunum showing contractions to acetylcholine (A, 0.05 μg/ml.), prostaglandin E_1 in contact with the tissue for 2 min (E, 0.5 μg/ml.), and DMPP (D, 2 μg/ml). Tetrodotoxin (0.5 μg/ml.) prevented the effect of DMPP and reduced the response to prostaglandin. Contractions to both drugs returned in parallel as the effect of tetrodotoxin wore off. Hexamethonium (C_6, 25 μg/ml.) blocked the effect of DMPP but not of prostaglandin. Hyoscine (0.2 μg/ml.) blocked the response to acetylcholine and, like tetrodotoxin, reduced the response to prostaglandin. Neostigmine (0.1 μg/ml.) potentiated the effect of prostaglandin.

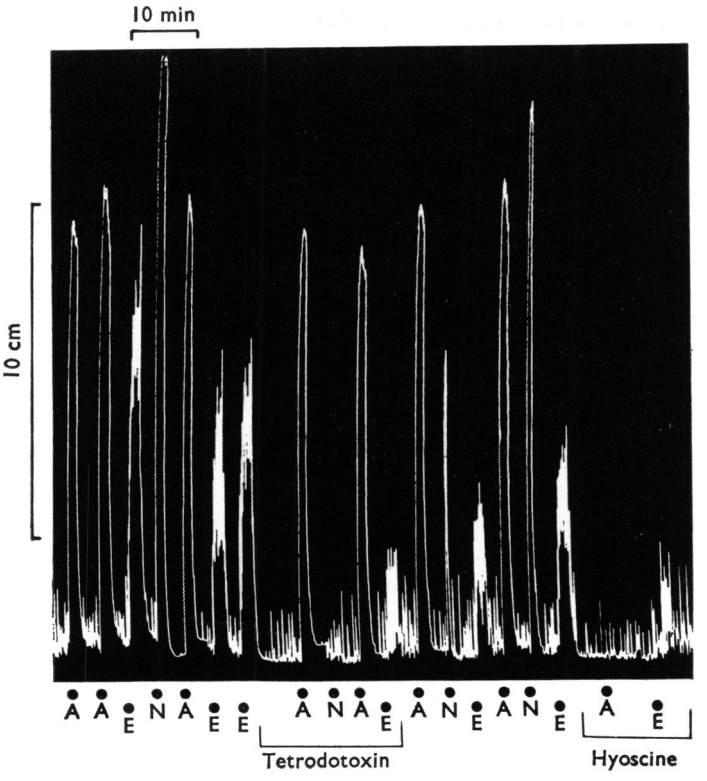

FIG. 3. Longitudinal muscle of guinea-pig ileum, showing contractions to acetylcholine (A, 10 ng/ml.), prostaglandin E_1 (E, 25 ng/ml.) and nicotine (N, 1.25 μg/ml.). Tetrodotoxin (0.1 μg/ml.) blocked the response to nicotine and reduced the response to prostaglandin. Both effects returned in parallel as the effect of tetrodotoxin wore off. Hyoscine (0.2 μg/ml.) blocked the effect of acetylcholine and, like tetrodotoxin, reduced the response to prostaglandin.

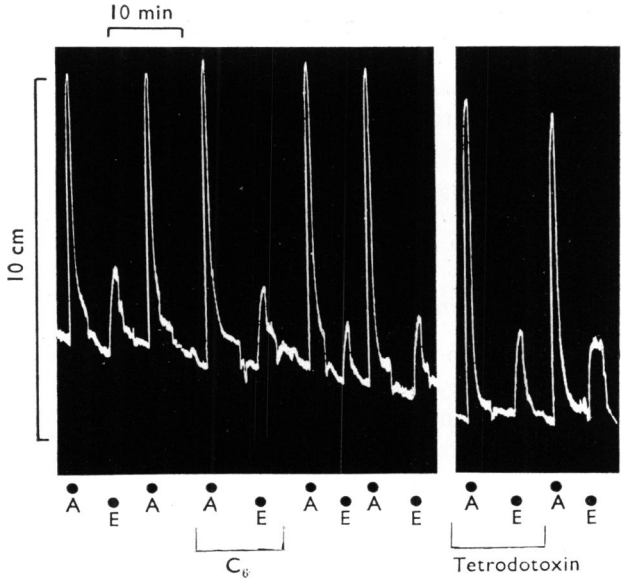

FIG. 4. Longitudinal muscle of rat ileum showing contractions to acetylcholine (A, 0.2 μg/ml.) and prostaglandin E_2 (E, 0.5 μg/ml.). Hexamethonium (C_6, 40 μg/ml.) and tetrodotoxin (0.5 μg/ml.) had no effect on the response to prostaglandin.

Tetrodotoxin (0.3–0.5 μg/ml.) was used to block conduction in the intrinsic nerves (Gershon, 1967). It greatly reduced or abolished the responses to nicotine or DMPP (Figs. 1–4), and it often lowered the tone of the tissue and reduced spontaneous contractions (Fig. 2). The effect of tetrodotoxin on the response of the longitudinal muscle to prostaglandin varied in the different species. In the guinea-pig it inhibited the contractions by 48–75% (four experiments, Fig. 3) but in the rat ileum the contractions were unaffected (three experiments, Fig. 4). In four out of seven experiments on human small intestine tetrodotoxin reduced the response by 10–80% (Fig. 2), but in the other three experiments the contractions were unaltered (Fig. 1).

Hyoscine (0.1–0.4 μg/ml.) abolished the contractions of the tissues to acetylcholine, but its effects on responses to prostaglandin were similar to those of tetrodotoxin. Hyoscine always reduced the response of guinea-pig ileum to prostaglandin (five experiments, 45–90% reduction, Fig. 3), but had no further effect when the response was already reduced by tetrodotoxin (two experiments). Hyoscine had no effect on contractions of rat ileum (four experiments), or on the three strips of human intestine in which the response to prostaglandin was unaltered by tetrodotoxin (Fig. 1). It reduced the response to prostaglandin, however, in three of the human strips which gave smaller contractions in the presence of tetrodotoxin (Fig. 2), and in another experiment in which the toxin was not used (20–70% reduction). This variation between strips of human intestine is unlikely to have been caused by block of the nerves by premedication or anaesthetic drugs, because the preparations responded to nicotine.

Physostigmine or neostigmine (10–100 ng/ml.) potentiated the response to acetylcholine, and its effects on prostaglandin-induced contractions were opposite to those of tetrodotoxin and hyoscine. The anticholinesterases enhanced the contractor effect of prostaglandins on both the guinea-pig ileum (three experiments) and on the four strips of human intestine in which hyoscine and tetrodotoxin inhibited the responses to prostaglandin (Fig. 2).

Mepyramine (0.2 μg/ml.) abolished the response of human and guinea-pig intestine to histamine, but it had no effect on the response to prostaglandin (human four, guinea-pig two, and rat two experiments).

Methysergide (0.2 μg/ml.), like mepyramine, had no effect on prostaglandin-induced contractions of human, guinea-pig or rat intestine (four, two and two experiments respectively).

Effects of prostaglandins E_1 and E_2 on the circular intestinal muscle

Human. Six strips of ileum and one of jejunum were obtained from six patients. They were studied on the day of operation except for one piece of ileum which was stored at 4° C overnight in Krebs solution. Prostaglandin E_1 (two experiments) and E_2 (five experiments) were left in contact with the tissue for 1–6 min. Their effects in doses of 0.15–0.6 μg/ml. varied in different tissues. In one experiment a relaxation occurred and the response to acetylcholine, added in the presence of prostaglandin, was reduced. In two experiments the only effect was a reduction in the response to acetylcholine, whereas in the other four experiments there was a small initial contraction (unaffected by hyoscine) followed by a long-lasting relaxation during which the response to added acetylcholine was depressed (Fig.

5). Thus the only common effect of prostaglandin in these experiments was inhibition (25 to 60%) of the response to acetylcholine.

Guinea-pig. Prostaglandin consistently inhibited the circular muscle of the guinea-pig isolated ileum (E_1, nine experiments ; E_2, three experiments). This effect was shown as a reduction in the response to potassium because the muscle was very insensitive to acetylcholine (Fig. 6). Prostaglandin (0.01–1 μg/ml.) reduced submaximal contractions to potassium (0.5–2 mg/ml.) by 10–75% in different experiments.

Rat. Prostaglandins E_1 and E_2 (three experiments, 0.2–1 μg/ml.) inhibited responses of spirally cut intestinal muscle to potassium by 25–40%. When the circular muscle strip was prepared by zig-zag cuts, however, prostaglandin had no effect in two experiments and caused very small contractions in two others. This may have been the result of inclusion of longitudinal muscle fibres in the strip which was contracted by prostaglandin and obscured an inhibitory effect on the circular muscle fibres.

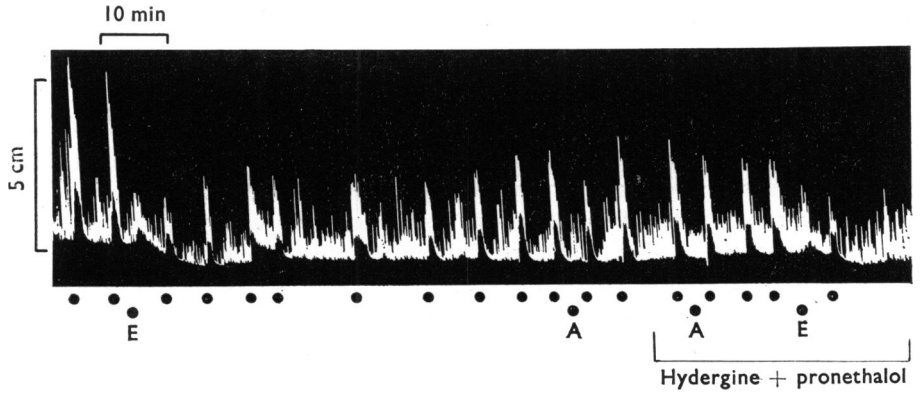

FIG. 5. Circular muscle strip of human terminal ileum showing contractions to acetylcholine (unlabelled dots, 0.4 μg/ml.). Prostaglandin E_2 (E, 0.5 μg/ml.) in contact with the tissue for 4 min caused a small contraction followed by a small relaxation. The subsequent dose of acetylcholine was added while prostaglandin was still in the bath. "Hydergine" and pronethalol (1 μg/ml. and 10 μg/ml. respectively) prevented the inhibitory effect of adrenaline (A, 0.2 μg/ml.) but not of prostaglandin.

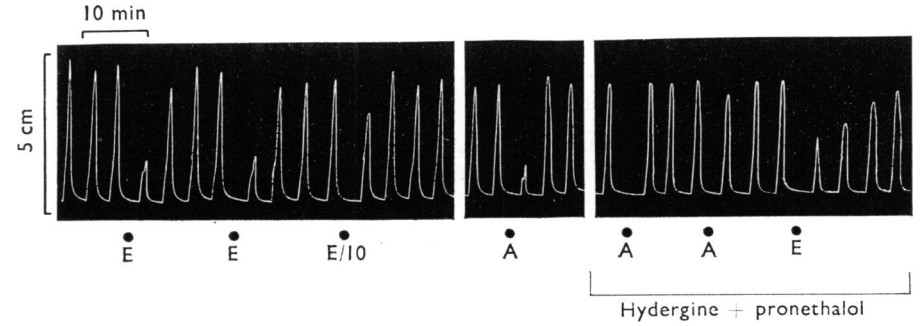

FIG. 6. Circular muscle strip of guinea-pig ileum showing contractions to potassium (unlabelled responses, 1 mg/ml.). Prostaglandin E_1 (E, 0.1 μg/ml.) and adrenaline (A, 0.2 μg/ml.) inhibited the response to potassium added to the bath 1 min after the prostaglandin or adrenaline. "Hydergine" and pronethalol (1 μg/ml. and 10 μg/ml. respectively) blocked the action of adrenaline but not of prostaglandin.

Site of action of prostaglandins on the circular muscle of the human, guinea-pig and rat small intestine

Tetrodotoxin (0.1–0.5 μg/ml.) prevented the relaxant response of human ileum to nicotine but did not alter the inhibitory action of prostaglandin on contractions to acetylcholine (three experiments), or to 5-hydroxytryptamine in the presence or absence of hyoscine (0.1 μg/ml.). Tetrodotoxin was also without effect on the inhibition of responses to potassium in rat spiral strips and in guinea-pig intestine (two and four experiments, respectively). In three of the experiments on guinea-pig ileum, hyoscine (0.2 μg/ml.) was added to the bath fluid: the contractions caused by potassium and the inhibitory action of prostaglandin were unaltered, so excluding the possibility that prostaglandins have an atropine-like effect.

"Hydergine" and pronethalol (1 μg/ml. and 10 μg/ml., respectively) prevented the inhibitory effect of adrenaline but not of prostaglandin (human two, guinea-pig four, and rat two experiments, Figs. 5 and 6).

Discussion

The effects of prostaglandins E_1 and E_2 on the human small intestine and on the guinea-pig and rat ileum were similar. In general, the longitudinal muscle was stimulated to contract whereas the circular muscle was inhibited, thus following the pattern first seen in the body of the human stomach (Bennett, Murray & Wyllie, 1968). The longitudinal muscle from different regions of the rat small intestine, however, appears to vary in its response to E-type prostaglandin: the duodenum relaxes (Khairallah, Page & Türker, 1967), the jejunum contracts but sometimes first relaxes (Berkström *et al.*, 1959), whereas in the present experiments the contraction of the ileum was preceded by a relaxation in only one experiment. The tendency of the rat small intestine to relax in response to E-type prostaglandins therefore seems to diminish from the duodenum to the ileum.

The way in which prostaglandins E_1 and E_2 stimulated the longitudinal muscle of the human, guinea-pig and rat ileum to contract was different in each species, as shown by the experiments with tetrodotoxin and hyoscine. In the rat ileum, and in some strips of human jejunum and ileum, these blocking drugs did not alter the contraction caused by prostaglandin, so that stimulation of intrinsic nerves or of muscarinic receptors was not involved. In contrast, the contractions of guinea-pig ileum and of the remaining strips of human small intestine were reduced, but not abolished, by tetrodotoxin and by hyoscine. This suggests that prostaglandin stimulates at two sites: one site appears to be the intrinsic cholinergic nerves (supported by the finding that anticholinesterases potentiate the contraction) and the other appears to be non-neuronal. Our results agree with the findings of Harry (1968) who used procaine to anaesthetize the intramural nerves in the guinea-pig small intestine, and who suggested that prostaglandin potentiates the response of intrinsically released acetylcholine. All the neuronal action of prostaglandin in human small intestine, and most or all in the guinea-pig ileum was unaffected by hexamethonium, indicating that prostaglandin stimulates the postganglionic cholinergic nerves. We do not know the reason why stimulation of nerves occurred in only some strips of human small intestine, but block of the nerves or of cholinergic receptors with drugs administered to the patients did not seem to be the cause.

Mepyramine and methysergide had no effect on the response of any tissue studied, so that release of histamine or 5-hydroxytryptamine, or stimulation of their receptors by prostaglandin was unlikely. However, a possible release of 5-hydroxytryptamine by prostaglandin in guinea-pig ileum cannot be excluded because methysergide does not block the neuronal action of the amine (Day & Vane, 1963).

In contrast to the longitudinal muscle, the prostaglandins inhibited the circular muscle by a mechanism which appeared to be the same in the human, guinea-pig and rat intestine. Neither tetrodotoxin nor blockade of α- and β-adrenoceptive receptors altered the inhibition, so that the effect was probably due to an action directly on or in the muscle cells, and not to stimulation of inhibitory nerves or to the release of catecholamine.

Our results show a difference in the sensitivity to prostaglandin between species and regional differences within the same species. Both the circular and the longitudinal muscle layers of the human and the rat small intestine are less sensitive than those of the guinea-pig, and they are also less sensitive than the human and rat isolated stomach (Bennett, Murray & Wyllie, 1968; Bennett, Friedmann & Vane, 1967). These regional differences may occur because the stomach and ileum arise from embryologically different parts of the gut; both quantitative and qualitative variations in the response of the two regions are found with other naturally occurring substances such as acetylcholine, histamine, 5-hydroxytryptamine and gastrin (Bennett, 1965; Bennett & Whitney, 1966a, b; Bennett, Misiewicz & Waller, 1967).

The actions of prostaglandin on the isolated muscle, and the presence of prostaglandins E_2 and $F_{2\alpha}$ in guinea-pig intestine (Ambache *et al.*, 1966) and of prostaglandin-like material in the human and rat ileum (unpublished observations) raise the possibility that the substances play a part in controlling intestinal motor activity. This seems most likely in the guinea-pig because of its high sensitivity. The results in our following paper (Bennett, Eley & Scholes, 1968) suggest that prostaglandin released in the gut wall is more likely to affect motility than prostaglandin in the blood or in the lumen of the bowel.

We thank Dr. J. E. Pike, The Upjohn Company, Kalamazoo, U.S.A. for kindly supplying the prostaglandins.

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(*Received August* 16, 1968)