Br. J. Pharmac. (1976), **56**, 469–477

PROSTAGLANDIN PRODUCTION BY RABBIT ISOLATED JEJUNUM AND ITS RELATIONSHIP TO THE INHERENT TONE OF THE PREPARATION

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1 Pieces of rabbit jejunum were bathed in Krebs solution at 37°C in an isolated organ bath bubbled with O_2 and 5% CO_2. The bathing fluid was collected regularly and assayed for prostaglandins.

2 The preparations maintained a continuous sub-maximal muscle contraction, referred to as inherent 'tone'. Prostaglandins E_2 and $F_{2\alpha}$ were continuously generated by the intestine and released into the bathing fluid. The amounts released first declined over 2 h and then steadily increased. The release was also greater after 48 h storage in the refrigerator and after mechanical damage.

3 There was no change in prostaglandin release when the rabbit jejunum was contracted by acetylcholine or physostigmine or relaxed by adrenaline, hyoscine, papaverine, dinitrophenol, or calcium-free Krebs solution.

4 Addition to the bathing fluid of the prostaglandin precursor, arachidonic acid, did not increase the release of prostaglandins although it contracted the tissue. Thus, output of prostaglandins from the tissue was not limited by substrate concentration but more probably by the capacity of the prostaglandin synthetase.

5 Prostaglandin output was decreased by bubbling the bathing fluid with N_2 rather than O_2; at the same time the preparation relaxed.

6 Aspirin-like drugs such as indomethacin also decreased or abolished prostaglandin formation and this, too, was accompanied by loss of tone of the isolated preparation.

7 Pieces of rabbit jejunum stored in Krebs solution containing indomethacin initially released little or no prostaglandin into the bathing fluid. However, prostaglandin release increased with repeated washing of the preparation.

8 The results suggest that intra-mural prostaglandin production contributes to the inherent tone of the rabbit jejunum, that trauma increases prostaglandin production and that the inhibitory effects of anoxia are linked with the lack of prostaglandin production and activity. The relevance of these findings to intestinal activity *in vivo* is discussed.

Introduction

Some isolated smooth muscle preparations exhibit a sustained sub-maximal contraction or inherent 'tone', demonstrated by the fact that catecholamines and spasmolytic drugs cause relaxation. Weiland (1912) found a smooth muscle stimulant in the bath fluid in which segments of rabbit intestine were suspended. Similar stimulant activity, called 'Darmstoff', was obtained from frog isolated intestine (Vogt, 1949) and its diffusable component was later identified as a mixture of prostaglandins (Suzuki & Vogt, 1965). The spontaneous release of prostaglandins into the nutrient fluid by several other isolated preparations has since been described (Bennett, Friedmann & Vane, 1967; Coceani, Pace-Asciak, Volta & Wolfe, 1967; Vane & Williams, 1973). In general, tissues do not store prostaglandins so that release indicates fresh synthesis (Piper & Vane, 1971) and both synthesis and release are inhibited by non-steroidal anti-inflammatory drugs (Vane, 1971).

We have investigated the effects of indomethacin, a potent inhibitor of prostaglandin synthetase, on the generation of prostaglandins and on the tone of the rabbit isolated jejunum. We also investigated the effects on prostaglandin release of several other

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procedures which changed the tone of the isolated tissue. Some of these results have been presented to the British Pharmacological Society (Ferreira, Herman & Vane, 1972).

Methods

Rabbit isolated jejunum preparation

Rabbits (2–4 kg) were killed by exsanguination after a blow on the head. A segment (6–7 cm) of the proximal jejunum was removed and suspended in a 15 ml organ bath containing Krebs solution at 37°C and bubbled with 95% O_2 and 5% CO_2. In some experiments, N_2 was substituted for the O_2 and in others, the rabbit jejunum was superfused (Gaddum, 1953) with Krebs solution at 10 ml/min, rather than using an organ bath. The Krebs solution had the following composition in g/l (mM): NaCl 6.9 (118), KCl 0.35 (4.7), $CaCl_2.6H_2O$ 0.55 (2.5), KH_2PO_4 0.16 (1.2), $MgSO_4.7H_2O$ 0.29 (1.17), glucose 1.0 (5.6), $NaHCO_3$ 2.1 (25.0).

As well as the intact tissue, the following preparations were also used: (1) a strip of longitudinal smooth muscle prepared according to the method of Ambache (1954); (2) preparations in which the jejunum was everted, the mucosa damaged by scraping it with the sharp edge of a scalpel blade and then everted once more; (3) jejunum stored 48 h at 4°C in 50 ml Krebs solution with or without indomethacin (10 μg/ml).

Contractions of the muscles were detected by auxotonic levers (Paton, 1957) attached to Harvard Smooth Muscle Transducers. The bath fluid was left unchanged for periods of 30 min (collection period) and then drained for assay. In some experiments arachidonic acid was added to the bath fluid and after 30 min of incubation, the prostaglandin content of the fluid was compared with that found during the periods before adding and after removing the arachidonic acid.

Biological assay of prostaglandin-like material

Samples (1 ml) of the collected fluid were assayed on a bank of tissues consisting of a rat stomach strip, a rat colon and a chick rectum superfused in series with Krebs solution at 37°C at a rate of 10 ml/min to which a mixture of antagonists was added in order to make them specifically sensitive for detection and quantitation of prostaglandin-like material (Gilmore, Vane & Wyllie, 1968). The samples were assayed using prostaglandin E_2 as a standard and the results were expressed in terms of prostaglandin E_2 equivalents.

In the experiments in which physostigmine was used, the concentration of hyoscine was increased tenfold. When indomethacin or papaverine was added to the organ bath containing the rabbit jejunum, the same drug was also added to the Krebs solution superfusing the assay tissues (1 μg/ml for indomethacin and 4 μg/ml for papaverine). The results were analysed statistically by Student's *t*-test for paired or unpaired samples. Differences were considered significant if $P < 0.05$.

Destruction of prostaglandins by the rabbit isolated jejunum

Destruction of prostaglandins in the organ bath was studied by adding a known amount of prostaglandin E_2 to the bath fluid; after 30 min of incubation, the bath fluid was assayed and the amount of added prostaglandin E_2 recovered was calculated by subtracting the basal output of prostaglandins obtained during the 30 min collection period just before the addition of prostaglandin E_2, from the total amount assayed in the presence of the added prostaglandin E_2. The amount of added prostaglandin E_2 recovered after 30 min of incubation is expressed as a percentage of the activity added at the beginning of the incubation.

Chromatography

To confirm the presence of prostaglandins, bath fluid from scraped mucosa preparations was used: samples of consecutive collection periods were pooled, acidified with HCl to pH 3, extracted twice with ethyl acetate (1 : 1 v/v) and evaporated under reduced pressure. The residue was taken up in ethanol, spotted on a silica gel plate (F 254-Merck) and developed in the AI or AII system (Gréen & Samuelsson, 1964). The bands corresponding to prostaglandin E or prostaglandin F standards respectively were scraped off and the silica gel eluted with Krebs solution for bioassay. Intermediate bands were also assayed.

Drugs

The following drugs were used: arachidonic acid (Sigma), acetylcholine hydrochloride (BDH), adrenaline bitartrate (BDH), 2,4-dinitrophenol (BDH), physostigmine (eserine) sulphate (Hopkins & Williams Ltd), hyoscine hydrobromide (BDH), indomethacin (Merck, Sharp & Dohme), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), noradrenaline bitartrate (Sigma), papaverine hydrochloride (Hopkins & Williams), phenoxybenzamine hydrochloride (SKF), propranolol hydrochloride (ICI), prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ (Upjohn). Concentrations of salts are expressed in terms of base.

Results

Basal output of prostaglandin-like material by different preparations of the rabbit isolated jejunum

Table 1 shows the basal output of prostaglandin-like material from different preparations at different time periods. The outputs from intact tissues were usually biphasic: the first sample tended to contain more activity than the third sample although these differences were not statistically significant. Thereafter, there was a gradual increase in output of activity and this continued for the rest of the experiment, so that after 4 h of incubation, the output was significantly higher than during the first 2 h ($P<0.05$). In 2 experiments the jejunum was incubated for 8 h and the output was still increasing without reaching a plateau value.

The output from the scraped mucosa preparations was significantly higher than that from intact tissue ($P<0.01$). This output was also biphasic: the 3rd or the 6th sample had significantly less activity ($P<0.05$) than the first but then there was a gradual increase in release up to 8 hours.

Greater release was also seen when the tissues were stored for 48 h at 4°C as compared with the intact tissues ($P<0.01$), and this release remained higher for the rest of the experiment. Addition of indomethacin to the Krebs solution during storage of the tissue prevented the initial increased release but the prostaglandin output rose steeply when the bath fluid was repeatedly changed and after 4–5 h reached values that were not significantly different from those of tissues stored without indomethacin.

In contrast to the progressive increase in output of prostaglandin-like material from these preparations, the output from the longitudinal muscle strip decreased with time, although the initial output was significantly greater than that from the intact tissues ($P<0.01$).

Identification of the smooth muscle stimulating activity

In each of 9 experiments, extracts of the pooled bath fluid were chromatographed in the AI system. Activity was recovered in positions corresponding to F and E standards in a proportion of $1:1.01\pm0.1$ (mean ± s.d.). With the AII solvent system activity was only detectable in the E_2 and $F_{2\alpha}$ zones (4 experiments). No smooth muscle stimulating activity was found in other areas of the plates.

To find out how much of the rat stomach stimulating activity of the bath fluid was due to prostaglandin-like material, pooled bath fluid of mucosa-scraped preparations was assayed for its prostaglandin content before and after extraction plus chromatography. All ($96.4\pm4.6\%$; mean ± s.d.) of the rat stomach stimulating activity was accounted for by prostaglandin-like material.

Influence of contraction or relaxation of the jejunum on the output of prostaglandin-like material

Contraction of the tissue induced by acetylcholine (0.2–1 µg/ml) for 30 min (6 experiments) did not consistently increase the prostaglandin production by intact preparations of the rabbit jejunum. Physostigmine (0.1–1 µg/ml; 6 experiments) induced a strong contraction of the tissue; often the bath fluid became clouded with tissue debris. In 2 experiments in which the lumen of the jejunum was open, physostigmine caused an increase in output of prostaglandin-like material (2–5 times). However,

Table 1 Total (mean ± s.e. mean) output of prostaglandin-like material (expressed as ng prostaglandin E_2 equivalents/g wet weight for the specified min collection period) from different preparations of the rabbit jejunum

Preparation (number of experiments)	*Mean weight (g)*	*Prostaglandin output (ng/g wet weight tissue)* *1st* (0–30 min)	*3rd* (60–90 min)	*6th* (150–180 min)	*9th* (240–270 min)
Intact jejunum (11)	1.7 ± 0.1	11.8 ± 1.6	7.8 ± 1.5	16.8 ± 5.0	21.5 ± 7.0
Mucosa scraped (8)	0.9 ± 0.09	259.0 ± 69.7	113.8 ± 20.4	102.0 ± 8.9	158.6 ± 31.5
Longitudinal muscle strip (6)	0.12 ± 0.02	628.2 ± 214.7	238.2 ± 84.9	63.1 ± 25.8	27.3 ± 11.1
Intact jejunum stored for 48 h at 4°C (5)	1.7 ± 0.2	65.2 ± 10.5	68.2 ± 25.1	85.2 ± 32.1	109.8 ± 32.7
Intact jejunum stored for 48 h with indomethacin (10 µg/ml) (5)	1.5 ± 0.04	9.6 ± 4.0	20.7 ± 9.8	68.5 ± 30.9	101.3 ± 44.3

For statistical analysis, see text.

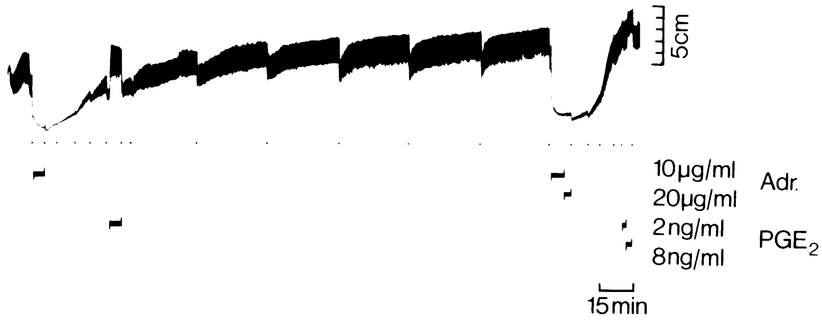

Figure 1 Rise in tone and effect of changing the bath fluid during the incubation of the rabbit jejunum in an isolated organ bath. The degree of tone at different times of the experiment was estimated by administration of adrenaline (Adr). Prostaglandin E_2 (PGE_2) contracted the tissue and the response diminished as the tone of the tissue increased. Black dots indicate change of bath fluid. Time 15 min; vertical scale 5 cm (which represents a muscle shortening of 1.25 cm).

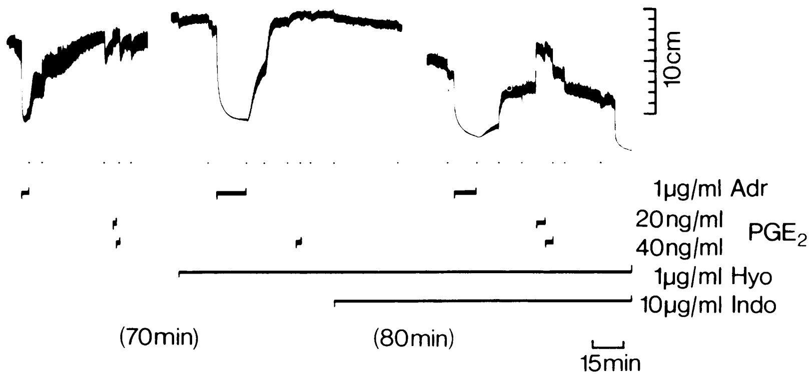

Figure 2 Effect of hyoscine (Hyo; 1 µg/ml) and indomethacin (Indo; 10 µg/ml) on the tone of the rabbit jejunum (mucosa scraped) suspended in an organ bath. The amount of tone at different times of the experiment was estimated by administration of adrenaline (Adr; 1 µg/ml). The tone of the tissue was already high in the beginning of the experiment and prostaglandin E_2 (PGE_2) produced a contraction only at doses of 20 and 40 ng/ml. Hyoscine produced a small relaxation but did not prevent the tissue from gaining further tone. Indomethacin caused a gradual fall in tone, after which the responses to prostaglandin E_2 were enhanced. The figures between brackets represent the time elapsed between different parts of the tracing. Black dots indicate change of the bath fluid. Time 15 min; vertical scale 10 cm (which represents a muscle shortening of 2.5 cm).

when the lumen of the tissue was closed to avoid expulsion of luminal contents into the bath fluid, the bath fluid from physostigmine-treated and control preparations contained similar quantities of prostaglandin-like material (2 experiments). Similarly, no increased prostaglandin output was seen in this system when physostigmine was administered after the tissue had been contracted several times by acetylcholine (1 µg/ml). No increase in the outut of prostaglandin-like material was observed during contractions of strips of longitudinal smooth muscle induced by acetylcholine (0.1–0.2 µg/ml; 2 experiments) or physostigmine (1 µg/ml; 2 experiments).

Arachidonic acid (10 µg/ml; 9 experiments) present in the organ bath for 30 min caused contraction of intact as well as of mucosa-scraped preparations. The contraction, which was quite instantaneous and which equalled a contraction induced by 15–20 ng/ml prostaglandin E_2, waned over 30 min in 6 out of 9 experiments, but was sustained in the remaining 3 experiments. There was no increase in output of prostaglandin-like material when arachidonic acid was

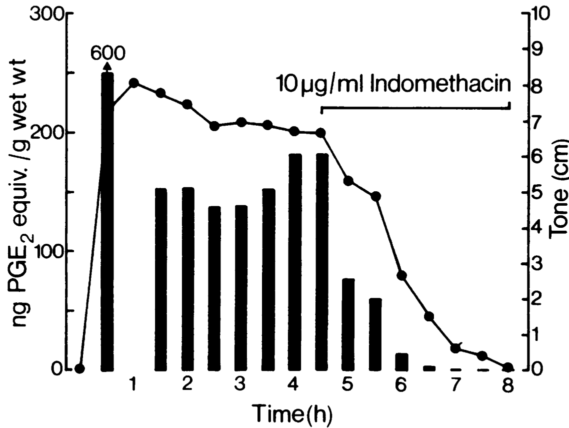

Figure 3 Effect of indomethacin (10 μg/ml) on the tone of, and production of prostaglandin-like material by, the rabbit isolated jejunum (scraped mucosa) suspended in an organ bath. The variation of the tone above the level seen after mounting the tissue in the bath was measured in cm displacement of the pen on the recorder (10 cm displacement corresponds to a muscle shortening of 2.5 cm) and is represented by the line. The total output of prostaglandin-like material during the 30 min collection periods was measured in ng prostaglandin E_2 (PGE_2) equivalents per g wet weight and is represented by the columns. Indomethacin reduced both the production of prostaglandin-like material and the tone of the tissue. After 4 h of treatment, the prostaglandin production had fallen to undetectable levels and the tone was reduced to the same level as that seen immediately after suspending the tissue in the bath. The time after suspending the tissue in the organ bath is indicated in hours.

added either at the beginning of the experiment or after the tissue had been in the organ bath for a few hours. Prostaglandin E_2 (10 ng/ml; 6 experiments) caused a sustained contraction of the preparation and after 30 min, all (105 ± 14.2; mean $\pm$ s.d.) of the added activity could be recovered from the bath fluid.

Adrenaline (2 μg/ml; 2 experiments) or noradrenaline (2 μg/ml; 2 experiments) strongly relaxed the jejunum, whereas hyoscine (0.1 μg/ml; 2 experiments) produced only a slight reduction of the tone. No consistent change in the output of prostaglandin-like material occurred during the administration of these drugs.

Relationship between tone of the rabbit isolated jejunum and prostaglandin production

The tone of the rabbit jejunum preparation tended to increase throughout the experiment, reaching a plateau after 3–5 hours. This increase in tone was partially reversed by each replacement of bath fluid (Figure 1). During the 30 min collection period the tone then gradually recovered reaching a slightly higher level than previously until a plateau was reached. The addition of a mixture of methysergide, mepyramine and hyoscine (0.1–0.2 μg/ml; 6 experiments) or hyoscine alone (0.1–1 μg/ml; 3 experiments) to the bath fluid did not prevent the tissue from gaining tone and, as shown in Figure 2, hyoscine (0.1–1 μg/ml; 7 experiments) only temporarily decreased the tone already developed.

As the tone of the tissue rose, so there was a gradual decrease in the amplitude of contractions produced by prostaglandin E_2 (Figures 1 and 2) or acetylcholine: the concentration of the agonists had therefore to be increased four to tenfold to obtain a contraction comparable with the one produced at the start of the experiment. In experiments in which the jejunum was superfused, there was also a gradual increase in tone over a similar time period.

Indomethacin (10 μg/ml) induced a gradual loss in tone (Figure 2) and a diminution in output of prostaglandin-like material. The close relationship between tone and prostaglandin production is shown for a single tissue in Figure 3. After indomethacin treatment adrenaline produced a much smaller relaxation, indicating a small degree of residual tone. This could be further reduced by addition of hyoscine but was insensitive to the administration of methysergide or mepyramine.

The longitudinal smooth muscle strip relaxed completely with indomethacin (1 μg/ml) within 30 min and no further relaxation could be obtained with adrenaline (1–10 μg/ml).

In preparations in which the tone had been substantially reduced by indomethacin, it could be restored by the addition to the bath of a prostaglandin E_2 (Figure 2). Furthermore, after indomethacin treatment, the contractions induced by prostaglandin E_2 or acetylcholine were somewhat enhanced as compared with the contractions obtained before indomethacin. This enhancement was most pronounced in the longitudinal muscle strip and in the jejunum with scraped mucosa (Figure 2). In some tissues, the contraction by arachidonic acid was blocked after 30 min of indomethacin administration, in others not until after 1 to 2 hours.

Bubbling the bath with N_2 rather than O_2 (10 experiments) was accompanied by a relaxation of the tissue and a 50–75% reduction in the production of prostaglandins (3 experiments; Figure 4). Bubbling once more with O_2 restored the prostaglandin production and was accompanied by a contraction of the tissue. Contractions induced by acetylcholine or prostaglandin E_2 were not sustained during N_2 and faded within 3 min despite the continuous presence of the drug.

Addition of 2,4-dinitrophenol (20 μg/ml:

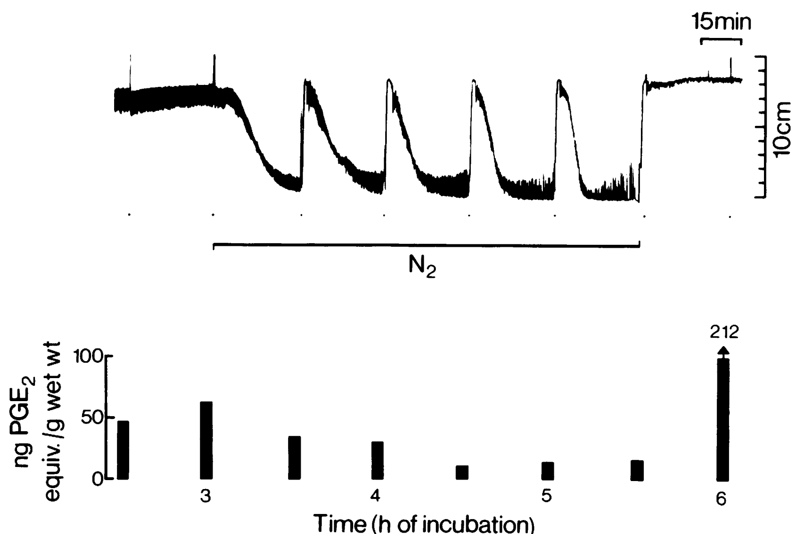

Figure 4 Effect of N_2 on the tone (upper trace) of the rabbit jejunum (mucosa scraped) suspended in an organ bath. Production of prostaglandin is shown in the lower part of the figure, expressed as ng prostaglandin E_2 (PGE_2) equivalents per gram wet weight of tissue for 30 min collection periods. The Krebs solution was bubbled with N_2 after the tissue had been incubated for 3 hours. Concomitantly with the fall in tone the output of prostaglandin-like material into the bath fluid decreased. Bubbling once more with O_2 restored the tone and the production of prostaglandin-like material. Black dots indicate the change of bath fluid (which caused a temporary muscle contraction). Time scale 15 min; the time (hours of incubation) indicates the time after suspending the tissue in the organ bath. Vertical scale 10 cm which represents a muscle shortening of 2.5 cm.

3 experiments) or changing the bath fluid to calcium-free Krebs solution (2 experiments) also relaxed the tissue. The output of prostaglandins was not affected by these treatments. After 2,4-dinitrophenol, prostaglandin E_2 no longer caused a contraction, whereas acetylcholine caused a quick non-sustained contraction which was also reduced in amplitude. After removal of the 2,4-dinitrophenol, there was an immediate restoration of the tone and a reversal of the effects on contractions induced by prostaglandin or acetylcholine.

Discussion

As found for other isolated intestinal muscle, pieces of rabbit isolated jejunum release prostaglandins into the Krebs solution in which they are bathed. All of our experiments are consistent with the concept that prostaglandin generation and consequent release is proportional to the amount of manipulation of or damage to the tissue. For example, the sample of bath fluid collected during the first half-hour contained higher amounts of prostaglandins, especially when a strip of longitudinal muscle was used. Others have noted that manipulation of tissues, such as handling of the dog spleen *in vivo* (Ferreira & Vane, 1967) or stroking the external surface of the guinea-pig lungs *in vitro* (Piper & Vane, 1971) release prostaglandins or prostaglandin-like substances. The prostaglandin output from pieces of rabbit jejunum also increased as the experiment progressed and this again could be an expression of a gradual deterioration of the integrity of the preparation: loss of structural integrity was already observable 15 min after isolation of the rat intestine in an organ bath (Levine, McNary, Kornguth & LeBlanc, 1970). Increased release of prostaglandins with time has also been observed during incubation of the bovine isolated iris (Posner, 1970) and during perfusion of the cat isolated spleen (Ferreira, Moncada & Vane, 1973) and the dog kidney (Itskovitz, Stemper, Pacholczyk & McGiff, 1973). Certainly, in our hands any procedure which damaged the tissues was followed by an increase in prostaglandin output. The increase was seen both from damaged muscle and damaged mucosa; it was interesting in this respect that the strips of longitudinal muscle released declining amounts of prostaglandins. This may have been because, after the initial trauma of dissection, the tissue did not suffer progressive damage through lack of nutrients. However, it is also possible that the bulk of prostaglandins is derived from the mucosal layer:

four times more prostaglandins can be extracted from the mucosa of the human stomach than from the submucosa, whereas only traces of prostaglandin activity could be found in the muscle layer (Bennett, Murray & Wyllie, 1968).

Contraction or relaxation of the rabbit jejunum did not affect prostaglandin output. Thus, any prostaglandin production associated with increased or decreased muscle activity is small compared with the continuous output measured here. The increase in output seen with physostigmine was accompanied by and perhaps explained by the expulsion of tissue debris from the lumen of the gut. There is now substantial evidence that contractions of isolated smooth muscle induced by arachidonic acid are due to prostaglandins generated within the tissue (see Ferreira & Vane, 1974). In these experiments also, addition of arachidonic acid to the bath fluid caused contraction of the tissue, but did not lead to an increase in prostaglandin production. A similar observation was made by Posner (1971) in bovine iris. Adequate amounts of substrate must already be present and the increased production which leads to further contraction must be small compared with the continuous output. The increase in prostaglandin output observed during the course of the experiment is unlikely to be the result of an increased liberation of precursors by phospholipase activity, as proposed by Bartels, Kunze, Vogt and Wille (1970) working with perfused frog intestine.

Since output of prostaglandins from a tissue probably represents a balance between production and metabolism within the tissue, an increased output could also be due to a decreased inactivation. In this context, Blackwell, Flower & Vane (1975) have shown that prostaglandin 15-OH dehydrogenase is a short-lived enzyme, so that continued activity depends upon continued enzyme production. Thus, isolated tissues may well lose dehydrogenase activity within an hour or so. However, addition of prostaglandin E_2 to the bath fluid showed that even from the very beginning of the experiment, there was little or no measurable metabolism by the rabbit isolated jejunum of the exogenously added prostaglandins. Thus, the gradual increase in the prostaglandin content of the bath fluid after 3 h is probably due to an increased synthesis rather than a gradual decline in the metabolism of the prostaglandins.

The increased production may be due to an increase in enzyme level or enzyme availability or to an increase in the availability of O_2 to the synthesizing system. Molecular oxygen is certainly necessary for prostaglandin production (Samuelsson, Granström & Hamberg, 1967; Nugteren, Beerthuis & Van Dorp, 1967) and we have shown that bubbling the organ bath with N_2 reduced prostaglandin production by the rabbit jejunum. At the same time, the tone of the tissue fell, probably because O_2 is not only required for the production of prostaglandins but also for their activity (Coceani & Wolfe, 1966; Eckenfels & Vane, 1972).

The involvement of prostaglandin production in the maintenance of the tone of this isolated tissue was further shown by the actions of indomethacin, which reduced not only the prostaglandin output but also the tone of the preparation. These effects of indomethacin are probably due to specific inhibition of prostaglandin biosynthesis, for much higher concentrations are needed to inhibit agonist-induced smooth muscle contractions (Northover, 1971; Sorrentino, Capasso & Di Rosa, 1972) or to uncouple oxidative phosphorylation (Whitehouse, 1964). Dinitrophenol, a much more powerful uncoupler of oxidative phosphorylation, also relaxes smooth muscle but we have shown that output of prostaglandins is not affected. However, dinitrophenol inhibits the response of the tissue to prostaglandins and this action could explain its effect on the inherent tone of the preparation, rather than an action on the energy supply to the tissue (Born & Bülbring, 1955).

Our experiments with hyoscine and indomethacin show that the tone of the rabbit isolated jejunum is maintained by at least three different mechanisms: one mechanism, which accounts for about 5–10% of the tone, is hyoscine-sensitive, and is presumably due to the continuous generation of acetylcholine; a second one, which accounts for about 70–80% of the tone, is indomethacin-sensitive and therefore related to the production of prostaglandins. The remainder of the tone after treatment of the tissue with hyoscine and indomethacin is insensitive to methysergide and mepyramine, but could be abolished by bubbling the organ bath with N_2.

The relationship between tone and prostaglandin production is further supported by work with indomethacin on other isolated smooth muscle preparations (Farmer, Farrar & Wilson, 1972; Botting & Salzmann, 1974), with other prostaglandin synthetase inhibitors (Eckenfels & Vane, 1972; Davison, Ramwell & Willis, 1972) and with prostaglandin antagonists (Bennett & Posner, 1971; Ganesan & Karim, 1973).

Our results do not preclude the possibility that prostaglandin production in the rabbit jejunum *in vivo* also contributes to muscle tone and movement. However, the fact that manipulation and trauma increase prostaglandin production in the isolated preparation suggests that the prostaglandin production which we measure and which maintains the tone of the isolated preparation could be linked with removal of the tissue from the body and its incubation in an artificial medium. Such a conclusion stresses the importance of prostaglandins in pathophysiological conditions; indeed the involvement of prostaglandins in the inflammatory process (Willis, 1969) is another indication of involvement of prostaglandins in pathological conditions. Thus pro-

staglandin production by intestine may be induced, not only by incubation in an isolated organ bath, but also by inflammation of the intestine *in vivo* as seen in some intestinal infections, mechanical obstructions, ischaemic necrosis, irradiation, etc. All of these conditions may lead to increased prostaglandin production which would increase motility of the gut and could also cause diarrhoea.

We thank Miss V. Pontieri and Mr M.J. Parnham for technical assistance; Dr J. Pike of Upjohn, Kalamazoo, U.S.A. for the gift of prostaglandins; Merck, Sharp & Dohme for the gift of indomethacin and The Wellcome Trust for a grant supporting this work.

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(Received October 16, 1975.
Revised November 14, 1975.)