Br. J. Pharmac. (1970), **39**, 556–563.

Antagonism of some smooth muscle actions of prostaglandins by polyphloretin phosphate

K. E. EAKINS, S. M. M. KARIM AND J. D. MILLER

Departments of Ophthalmology Research and Anesthesiology, Columbia University, New York, U.S.A., and Department of Pharmacology, Makerere University Medical School, Kampala, Uganda

Summary

1. The antagonism of the smooth muscle stimulating actions of $PGF_{2\alpha}$ and PGE_2 by polyphloretin phosphate (PPP) was studied on several isolated smooth muscle preparations and on the blood pressure of the anaesthetized rabbit.

2. PPP (2·5–20 μg/ml) reversibly inhibited contractions of the jird colon produced by PGE_2 or $PGF_{2\alpha}$; $PGF_{2\alpha}$ was more readily antagonized than PGE_2.

3. PPP (2·5–30 μg/ml) reversibly antagonized contractions produced by PGE_2 and $PGF_{2\alpha}$ on the isolated rabbit jejunum and uterus. In these preparations PPP antagonized PGE_2 as readily as $PGF_{2\alpha}$.

4. It is concluded that PPP is a selective antagonist to the prostaglandins on these tissues, for contractions produced by other agonists, such as acetylcholine, angiotensin, 5-hydroxytryptamine and bradykinin were not reduced by concentrations of PPP which markedly antagonized responses to the prostaglandins.

5. Intravenous injections of PPP (25–200 mg/kg) resulted in a variable antagonism to the fall in blood pressure produced by intravenous injections of $PGF_{2\alpha}$ in the anaesthetized rabbit; vasodepressor responses produced by PGE_2 and acetylcholine were not antagonized.

6. The mechanism of this antagonism by PPP is not clear and must await further investigation.

Introduction

The availability of specific prostaglandin antagonists would be of importance in the study of the physiological roles of the prostaglandins. This problem has been approached in the classical manner by Fried and his co-workers, by the synthesis of a series of substances structurally related to the prostaglandins. Some of these prostaglandin analogues were found to antagonize selectively the smooth muscle stimulating actions of prostaglandins E_1 and $F_{1\alpha}$ (Fried, Santhanakrishnan, Himizu, Lin, Ford, Rubin & Grigas, 1969). Using a general screening approach, Sanner (1969) found 1-acetyl-2-(8-chloro-10,11-dihydrodibenz[b,f][1,4]oxazepine-10-carbonyl) hydrazine (SC 19220) to be a specific inhibitor of contractions produced by prostaglandin E_2 on the guinea-pig ileum.

Polyphloretin phosphate (PPP), a polymeric phosphorylated polyanionic derivative of phloridzin, is a potent inhibitor of alkaline phosphatase, hyaluronidase and

urease (Diczfalusy, Ferno, Fex, Hogberg, Linderot & Rosenberg, 1953). In addition, PPP has been found to reduce the permeability of serous membranes, to diminish the formation of serous exudates (Fries, 1956, 1960) and to antagonize the rise in intraocular pressure in the rabbit produced by ocular irritation (Cole, 1961). Since irins, that is, prostaglandins, were shown to be involved in the response of the rabbit eye to irritation (Ambache, Kavanagh & Whiting, 1965 ; Ambache, Brummer, Rose & Whiting, 1966), the interaction of PPP with prostaglandin E_2 (PGE_2) was studied on rabbit intraocular pressure. PPP was found to antagonize the rise in intraocular pressure produced by intracameral injections of PGE_2 (Beitch & Eakins, 1969). Following this observation Eakins & Karim (1970), in a preliminary study, found that PPP selectively antagonized the smooth muscle stimulating actions of prostaglandins $F_{1\alpha}$ ($PGF_{1\alpha}$) and $F_{2\alpha}$ ($PGF_{2\alpha}$) on the jird colon. The structure of the non-phosphorylated phloretin is shown in Fig. 1.

In the present experiments we have investigated further the prostaglandin-blocking activity of PPP on the jird colon and also on the isolated rabbit jejunum and uterus and the blood pressure of the anaesthetized rabbit.

A preliminary report of part of this work has been given to the Federation of American Societies for Experimental Biology (Eakins, Miller & Karim, 1970).

Methods

Smooth muscle preparations

All contractions were measured isotonically with a Brush isotonic muscle transducer and a Brush 220 recorder. A 5 ml organ bath was used for all the isolated preparations. Doses of agonist yielding suitable submaximal responses from the tissues were chosen before exposure of the tissue to PPP. In most experiments on the rabbit jejunum and uterus, gassing of the bathing fluid with oxygen alone yielded more reproducible contractions with the prostaglandins. Thus, although 95% oxygen and 5% carbon dioxide were used in some early experiments, for most of the experiments oxygen was used alone.

Jird colon

The use of the sensitive colon preparations from *Meriones libycus* for irin, and later prostaglandin, assays was introduced by Ambache *et al.* (1965). The species is now known as *Meriones shawi* (Ambache *et al.*, 1966 ; Ambache & Brummer, 1968). For the present experiments animals weighing 50–150 g were killed by a blow on the head. The middle part of the ascending colon was removed and set up in an organ bath containing de Jalon's rat colon solution at 28° C gassed with oxygen.

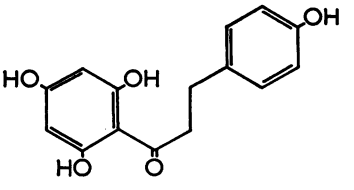

FIG. 1. Structure of phloretin.

A dose cycle of 3–4 min and a contact time of 1–2 min was used for the agonists studied. PPP was added to the bath and left in contact with the tissue for 2 min before the addition of the agonist.

Rabbit jejunum

New Zealand white rabbits (1–3 kg) were killed by a blow on the head. The jejunum was removed and a suitable segment was suspended in Tyrode solution at 37° C gassed with 95% oxygen and 5% carbon dioxide in some experiments, and with oxygen alone in others. A dose cycle of 3–4 min and a contact time of 60–90 s was used for the agonists studied. PPP was added to the bath and left in contact for 2 min before the addition of the agonist.

Rabbit uterus

A suitable portion of a uterine horn from a freshly killed New Zealand white rabbit was suspended in Tyrode solution at 37° C gassed with 95% oxygen and 5% carbon dioxide in some experiments, and with oxygen alone in others. A dose cycle of 10–15 min was used for the agonists. PPP was added to the bath and left in contact for 5 min before the addition of the agonist.

Rabbit blood pressure

Rabbits weighing 2·5–3·5 kg were anaesthetized with urethane (1–2 g/kg) injected as a 25% solution in 0·9% NaCl solution into a marginal ear vein. The trachea was cannulated. The femoral vein was cannulated for intravenous injections. Blood pressure was recorded in mmHg (1 mmHg≡1·333 mbar) from a femoral artery with a Statham P23Db pressure transducer in conjunction with a Beckman R Dynograph. Body temperature was maintained at 37° C. Each animal received heparin, 250–500 i.u./kg, intravenously.

Prostaglandins E_2 and $F_{2\alpha}$ were supplied by Dr. J. E. Pike, the Upjohn Company, Kalamazoo. PPP was supplied by Dr. B. Hogberg, A. B. Leo, Halsingborg, Sweden.

Results

PPP in concentrations of 2·5–20 μg/ml either antagonized or totally abolished contractions of the jird colon produced by prostaglandins E_2 or $F_{2\alpha}$; $PGF_{2\alpha}$ was blocked more readily than PGE_2. Contractions of the jird colon produced by other smooth muscle stimulating agents were unaltered by concentrations of PPP which markedly antagonized the prostaglandins. A typical result is seen in Fig. 2. PPP in a concentration of 20 μg/ml totally abolished the response to $PGF_{2\alpha}$ and markedly antagonized the response to PGE_2, but had little or no effect on responses elicited by angiotensin, bradykinin, 5-hydroxytryptamine (5-HT) and acetylcholine. Partial recovery of the tissue from prostaglandin-block is seen some 30 min after the last dose of PPP (Fig. 2E). In general, blockade of the responses of the jird colon to prostaglandins by PPP developed slowly and was of long duration, once established. In all jird preparations studied, it was found that the selective blockade of prostaglandins by PPP was reversible and was readily overcome by increasing the concentration of the agonist.

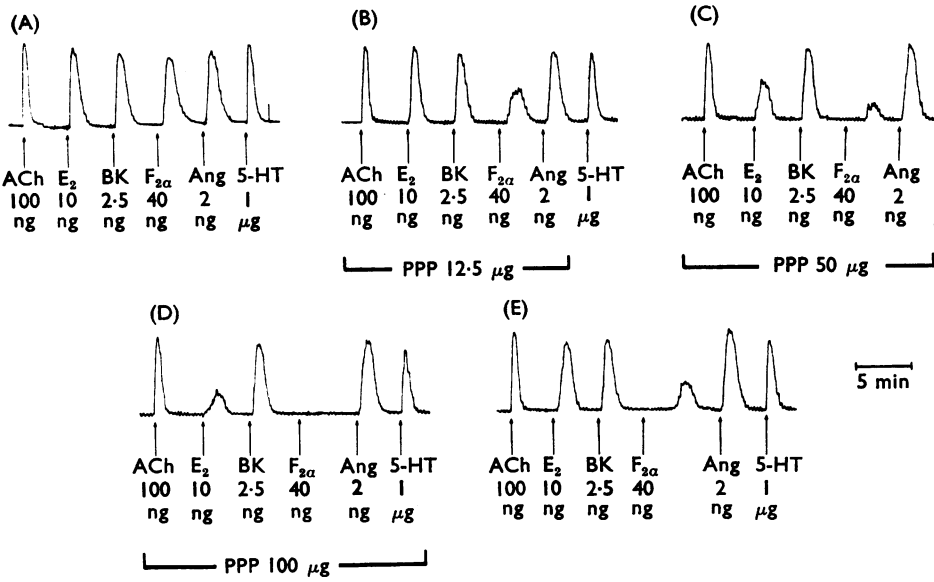

FIG. 2. Effect of polyphloretin phosphate (PPP) on contractions of jird colon produced by acetylcholine (ACh), prostaglandin E_2 (E_2), bradykinin (BK), prostaglandin $F_{2\alpha}$ ($F_{2\alpha}$), angiotensin (Ang) and 5-hydroxytryptamine (5-HT); 5 ml bath. PPP was injected 2 min before the agonist. A, Control responses; B, C and D, responses in the presence of 2·5, 10 and 20 μg/ml PPP, respectively; E, partial recovery of response to prostaglandins 30 min after last dose of PPP.

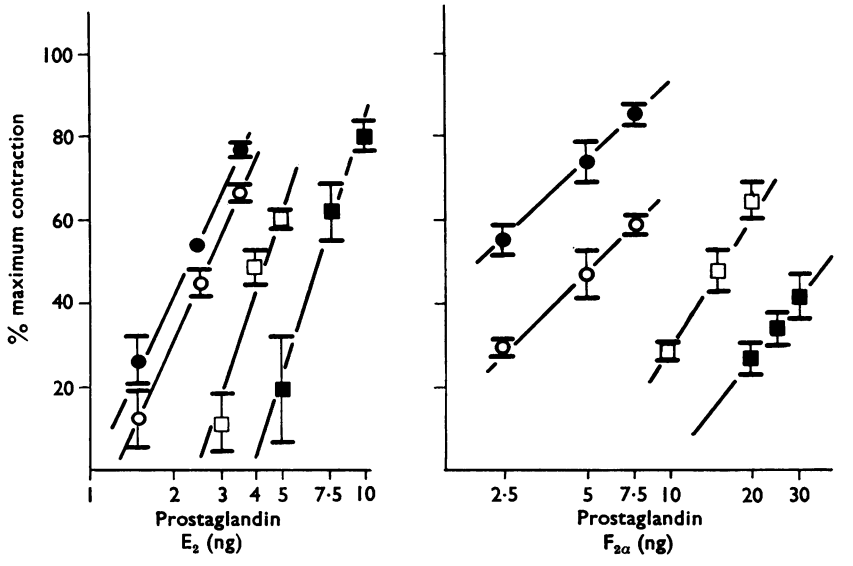

FIG. 3. Effect of polyphloretin phosphate (PPP) on dose-response curves to prostaglandins E_2 and $F_{2\alpha}$ on the isolated jird colon. ●——●, Control responses to prostaglandin; responses to prostaglandin in the presence of PPP: 2·5 μg/ml (○——○); 5 μg/ml (□——□) and 7·5 μg/ml (■——■). Each point is the mean of three observations; limits indicate standard error of the mean.

Fig. 3 (a and b) shows the effect of PPP on the log dose-response curves to prostaglandins E_2 and $F_{2\alpha}$ obtained on the jird colon. The addition of increasing doses of PPP to the bath fluid resulted in progressive shifts of the dose-response curves of both prostaglandins to the right.

PPP (2·5–30 μg/ml) was also found to antagonize selectively the contractions produced by prostaglandins E_2 and $F_{2\alpha}$ on the isolated rabbit jejunum and uterus. The antagonism was reversible and could be overcome by increasing the dose of prostaglandin. In contrast to the results obtained on the jird colon, PPP antagonized

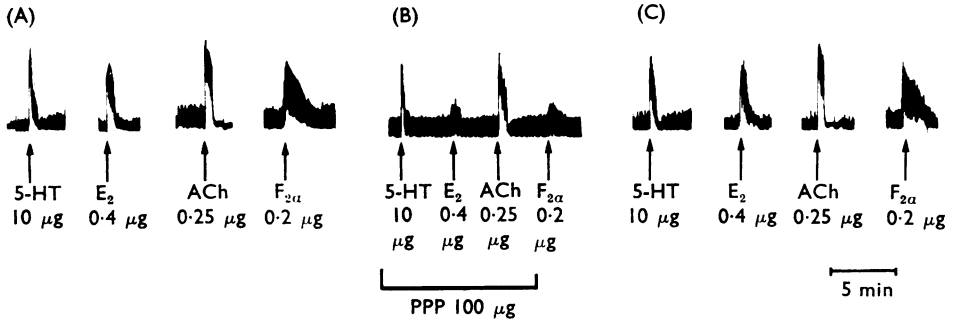

FIG. 4. Effect of polyphloretin phosphate (PPP) on the contractions of isolated rabbit jejunum elicited by 5-hydroxytryptamine (5-HT), prostaglandin E_2 (E_2), acetylcholine (ACh) and prostaglandin $F_{2\alpha}$ ($F_{2\alpha}$); 5 ml bath. A, Control responses; B, responses in the presence of PPP (20 μg/ml); C, partial recovery of response to prostaglandins 120 min after last dose of PPP.

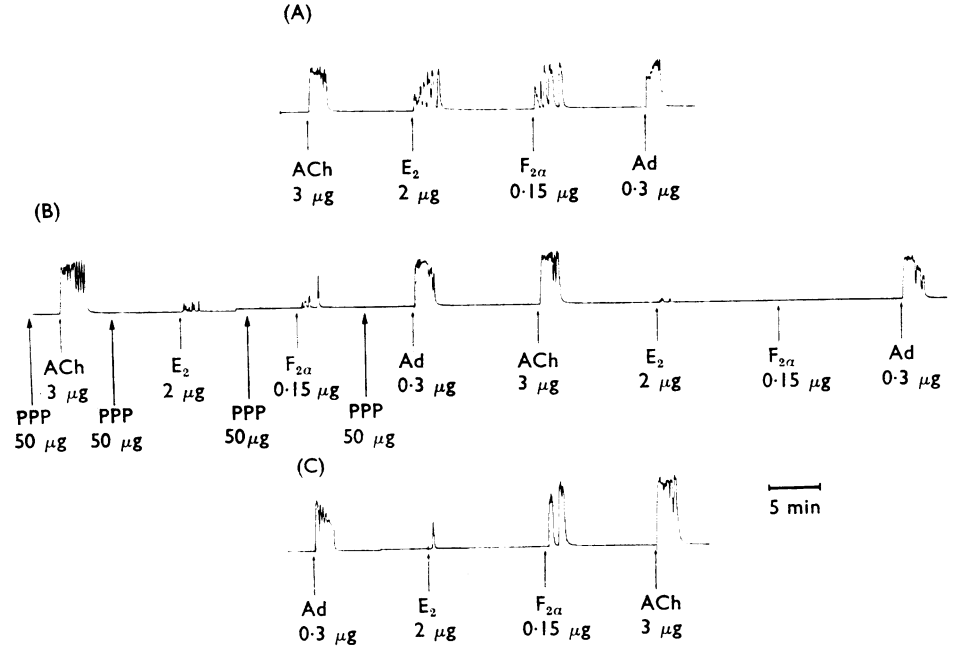

FIG. 5. Effect of polyphloretin phosphate (PPP) 10 μg/ml on the contractions of isolated rabbit uterus elicited by acetylcholine (ACh), adrenaline (Ad), prostaglandin E_2 (E_2), and prostaglandin $F_{2\alpha}$ ($F_{2\alpha}$); 5 ml bath. A, Control responses; B, selective blockade of prostaglandins by PPP; C, partial recovery of prostaglandin contractions 160 min after the last exposure to PPP.

PGE_2 as readily as $PGF_{2\alpha}$ on these isolated rabbit tissues. Figure 4 shows the selective antagonism of prostaglandins E_2 and $F_{2\alpha}$ by PPP on the isolated rabbit jejunum. In most experiments, total blockade of the prostaglandin responses was difficult to obtain on the rabbit jejunum and the antagonism once established was of long duration. In some experiments, high doses of PPP (100–200 μg/ml) produced either a small transient contraction or an increase in the amplitude of the pendular movement of the rabbit jejunum.

Figure 5 shows the selective antagonism of prostaglandins E_2 and $F_{2\alpha}$ by PPP on the isolated rabbit uterus. PPP in a concentration of 10 μg/ml abolished the contractions produced by the prostaglandins; in contrast, contractions produced by acetylcholine and adrenaline were not antagonized. PPP produced a long-lasting blockade of the responses to prostaglandins on this tissue; only partial recovery of the prostaglandin responses is seen in Fig. 5C some 160 min after the last exposure of the tissue to the antagonist.

In vivo, intravenous injections of PPP (25–200 mg/kg) resulted in a variable antagonism of the vasodepressor effect of intravenous $PGF_{2\alpha}$ but not of PGE_2 or of acetylcholine. Such a result is seen in Fig. 6. PPP was injected slowly over a 5–10 min period, because more rapid administration resulted in severe alterations in blood pressure. The duration of the antagonism varied from animal to animal, usually lasting 30–60 min. It was difficult to determine whether large doses of PPP would eventually antagonize the vasodepressor responses produced by PGE_2, because these doses of PPP produced long-lasting alterations in the blood pressure.

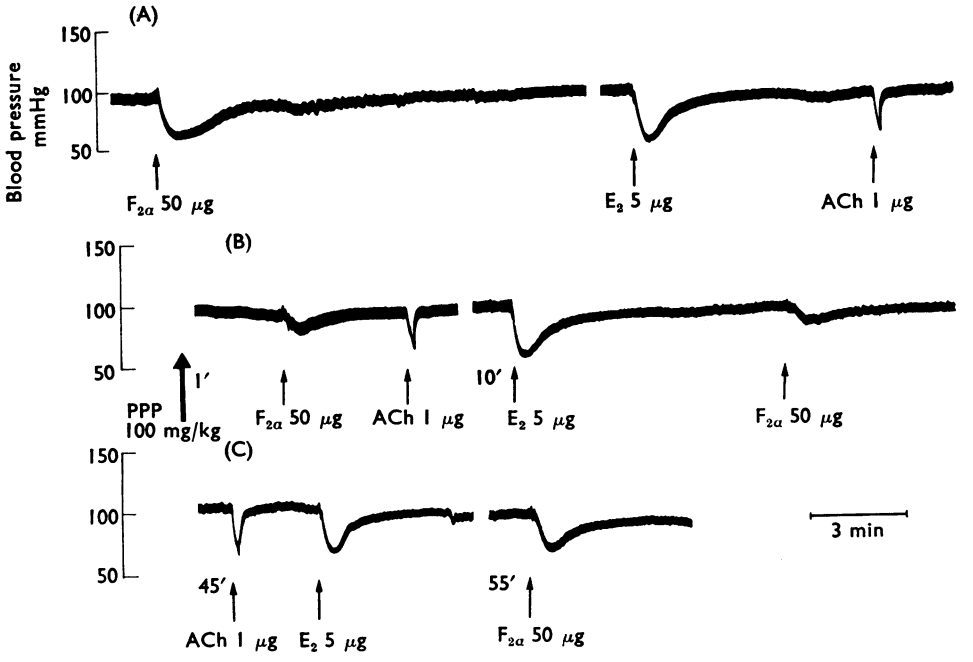

FIG. 6. Rabbit, 3 kg, urethane anaesthesia. Effect of polyphloretin phosphate (PPP) on the vasodepressor effects of prostaglandins $F_{2\alpha}$ ($F_{2\alpha}$) or E_2 (E_2) and of acetylcholine (ACh). A, Control responses; B, responses after PPP (100 mg/kg); C, partial recovery of the response to prostaglandin $F_{2\alpha}$ 55 min after injection of PPP.

Discussion

The present results demonstrate clearly that polyphloretin phosphate antagonizes the smooth muscle stimulating actions of both $PGF_{2\alpha}$ and PGE_2 on several isolated smooth muscle preparations. In a preliminary report (Eakins & Karim, 1970), it was stated that PPP antagonized only the stimulating action of F-prostaglandins on the jird colon. This apparent contradiction may be explained by the fact that in the present experiments, the contractions of the jird colon elicited by $PGF_{2\alpha}$ were more readily antagonized by PPP than those elicited by PGE_2; however, with suitable doses of PPP, the contractions produced by both prostaglandins could be inhibited. The lack of effect of PPP on the hypotensive responses to intravenous PGE_2 found in the present study most probably result from quantitative rather than from qualitative differences between the E and F prostaglandins, particularly in view of the observed variations in sensitivity of the E and F prostaglandins to blockade in the different isolated preparations. The present results also indicate that PPP is a selective antagonist to the prostaglandins on the tissues studied, since the actions of other smooth muscle stimulating agents, including acetylcholine, angiotensin, 5-HT and bradykinin, were not reduced by doses of PPP which antagonized responses to the prostaglandins.

The mechanism of this antagonism is not clear. However, the shifts to the right, apparently in parallel, of the dose-response curves for PGE_2 and $PGF_{2\alpha}$ on the jird colon in the presence of PPP, taken together with the reversible and surmountable nature of the blockade, suggest that the antagonism is competitive in nature. However, the magnitude of the shifts in the dose-response curves obtained in the present experiments are somewhat incompatible with this suggestion. A more detailed quantitative analysis would be necessary to resolve this discrepancy. Fried *et al.* (1969) have reported that the mechanism of the antagonism, on the jird colon, between one of their compounds and both E and F prostaglandins, most probably involved competition for the same receptor site. Sanner (1969) showed that SC 19220 competitively antagonized PGE_2 on the guinea-pig ileum in low doses, the antagonism becoming non-competitive at higher doses. It is of interest to note that non-steroidal anti-inflammatory drugs such as meclofenamate have been found to antagonize the contractions produced by $PGF_{2\alpha}$ on human isolated bronchial muscle (Collier & Sweatman, 1968). Whether or not the prostaglandin-blocking activity of PPP is related to its ability to inhibit a wide variety of enzymes (Diczfalusy *et al.*, 1953) must await further investigation.

This work was supported in part by U.S. Public Health Service Research Grant EY 00457 and in part by the Wellcome Trust. One of us (J.D.M.) was a Postdoctoral Research Trainee supported by U.S. Public Health Service Training Grant 5 TI-GM-56-12. We thank Mrs. Barbara Napolitano for her skilled technical assistance.

REFERENCES

AMBACHE, N. & BRUMMER, H. C. (1968). A simple chemical procedure for distinguishing E from F prostaglandins, with application to tissue extracts. *Br. J. Pharmac. Chemother.*, **33**, 162–170.

AMBACHE, N., BRUMMER, H. C., ROSE, J. G. & WHITING, J. (1966). Thin-layer chromatography of spasmogenic unsaturated hydroxy acids from various tissues. *J. Physiol., Lond.*, **185**, 77–78*P*.

AMBACHE, N., KAVANAGH, L. & WHITING, J. (1965). Effect of mechanical stimulation on rabbits' eyes; release of active substances in anterior chamber perfusates. *J. Physiol., Lond.*, **176**, 378–408.

BEITCH, BARBARA R. & EAKINS, K. E. (1969). The effects of prostaglandins on the intraocular pressure of the rabbit. *Br. J. Pharmac.*, **37**, 158–167.

COLE, D. F. (1961). Prevention of experimental ocular hypertension with polyphloretin phosphate. *Br. J. Ophthal.*, **45**, 482–489.

COLLIER, H. O. J. & SWEATMAN, W. J. F. (1968). Antagonism by fenamates of prostaglandin $F_{2\alpha}$ and of slow reacting substances on human bronchial muscle. *Nature, Lond.*, **219**, 864–865.

DICZFALUSY, E., FERNO, O., FEX, H., HOGBERG, B., LINDEROT, T. & ROSENBERG, Th. (1953). Synthetic high molecular weight enzyme inhibitors. 1. Polymeric phosphates of phloretin and related compounds. *Acta chem. scand.*, **7**, 913–920.

EAKINS, K. E. & KARIM, S. M. M. (1970). Polyphloretin phosphate—a selective antagonist for prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. *Life Sci., Oxford*, **9**, 1–5.

EAKINS, K. E., MILLER, J. D. & KARIM, S. M. M. (1970). Polyphloretin phosphate, a selective prostaglandin antagonist. *Fedn Proc.*, **29**, 745.

FRIED, J., SANTHANAKRISHNAN, T. S., HIMIZU, J., LIN, C. H., FORD, S. H., RUBIN, B. & GRIGAS, E. O. (1969). Prostaglandin antagonists: Synthesis and smooth muscle activity. *Nature, Lond.*, **223**, 208–210.

FRIES, B. (1956). Polyphloretin phosphate—a hyaluronidase inhibitor—and hyaluronidase in prevention of intra-peritoneal adhesions. An experimental study in the rabbit. *Acta chir. scand., Suppl.*, 217.

FRIES, B. (1960). The edema-inhibiting action of polyphloretin phosphate (P.P.P.) in some types of capillary damage. An experimental investigation. *Acta chir. scand.*, **119**, 1–7.

SANNER, U. H. (1969). Antagonism of prostaglandin E_2 by 1-acetyl-2-(8-chloro-10,11-dihydrodibenz[b,f] [1,4] oxazepine-10-carbonyl) hydrazine (SC 19220). *Archs int. Pharmacodyn. Thér.*, **180**, 46–56.

(*Received February* 12, 1970)