The residue, when dissolved in distilled water, was stable for at least six weeks at −10°C.

The preparation was chromatographically free of fatty acids and prostaglandins but traces of phospholipid material remained. There was no prostaglandin-like activity when tested on the rat fundus strip, rat colon and chick rectum (Ferreira & Vane, 1967). High doses (5.0 U) contracted the guinea-pig ileum and rat stomach strip. However, RCS-RF was distinguished from SRS-A by its potent RCS releasing properties. Furthermore, RCS-RF activity was not destroyed by incubation with the enzyme arylsulphatase which selectively inactivates SRS-A (Orange, Murphy & Austen, 1974). Indeed, RCS-RF activity was often increased by incubation with arylsulphatase.

RCS-RF is insoluble in ether, chloroform, ethyl acetate, acetone and ethanol but is more soluble in methanol. It is very polar as judged by its behaviour in several lipid chromatography systems. It is destroyed by acid or alkali (2 h at pH 2 or pH 12) but not by mild base treatment (0.1 N NaOH for 30 minutes). It loses < 10% activity after boiling for 2 min but is substantially (> 75%) degraded after 10 min at 100°C. Passage through a dialysis membrane suggests that it is a small molecule (< 5000 m.w.).

The release of RCS-RF was not prevented by aspirin (200 μg/ml), indomethacin (2 μg/ml), dexamethasone (2 μg/ml), di-sodium cromoglycate (20 μg/ml), colchicine (5 μg/ml), diethyl carbamazine (1 mg/ml) or mepacrine (20 μg/ml) but the release of RCS by the factor was blocked by these substances (except di-sodium cromoglycate and colchicine) in the same doses. All but dexamethasone also blocked the conversion in perfused lungs of arachidonic acid to RCS, implying direct block of the cyclo oxygenase. Dexamethasone, however, blocked the release of RCS by RCS-RF without affecting release by arachidonic acid.

RCS-RF does not release RCS or prostaglandin from guinea-pig perfused kidneys (which only convert arachidonic acid to PGE_2). RCS-RF has no inflammatory action in the rat paw (8 u/rat) oedema test but does cause hyperalgesia. This is partially (80%) blocked by indomethacin (10 mg/kg). It does not aggregate human platelets (10 u/ml).

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The effects of prostaglandin endoperoxides and thromboxane A_2 on strips of rabbit coeliac artery and certain other smooth muscle preparations

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Rabbit aorta contracting substance (RCS) contracts all vascular tissue tested and is generated by lung and other tissues after several stimuli, including immunological shock and bradykinin or arachidonic acid injection (Piper & Vane, 1969; Vargaftig & Dao Hai, 1971; Palmer, Piper & Vane, 1973). RCS is unstable and is probably a mixture comprising mainly thromboxane A_2 (TxA_2; half-life 30 s) with some prostaglandin endoperoxides (PGG_2 and PGH_2; half lives approximately 5 min) (Hamberg, Svensson & Samuelsson, 1975). We have compared the effects on various smooth muscle preparations of PGG_2, PGH_2 and TxA_2 with the stable prostaglandins.

Tissues were superfused in cascade at 10 ml/min with Krebs solution at 37°C containing a mixture of antagonists (Gilmore, Vane & Wyllie, 1968) plus indomethacin (1 μg/ml). As well as rat stomach strip, rat colon and chick rectum, strips of rabbit vascular tissue (cut spirally) were tested.

Prostaglandin endoperoxides were prepared by incubating microsomes of ram seminal vesicles with arachidonic acid at 20°C without co-factors. Endoperoxides were purified by low temperature column chromatography (Ubatuba, Moncada & Vane, unpublished). Thromboxane A_2 was prepared by incubation of PGG_2 or PGH_2 with horse platelet microsomes at 0°C (Needleman, Moncada, Bunting, Hamberg, Samuelsson & Vane, unpublished). TxA_2 was then rapidly ($\simeq$ 1 min) extracted by shaking (20 s) with two parts dry ether (0°C) at neutral pH, dried under nitrogen and redissolved in buffer immediately before testing.

The rat stomach strip was contracted by prostaglandin E_2 (PGE_2) being more potent than PGG_2 and PGH_2 (2-3 x) and TxA_2 (10 x). On rat colon, TxA_2 was inactive (up to 50 ng); $PGF_{2\alpha}$ was 12–15 times more potent than the endoperoxides. On chick rectum PGE_2 was more potent than the endoperoxides (2-3 x) and TxA_2 (4 x). On rabbit aorta, TxA_2 was 30–50 times more potent than the endoperoxides; PGE_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were inactive (up to 200 ng). Some of these results confirm ratios previously described (Nugteren & Hazelhof, 1973; Willis, Vane, Kuhn, Scott & Petrin, 1974).

The most interesting effects were on coeliac and mesenteric artery. PGE_2 (1–20 ng) relaxed these preparations, as did PGG_2 and PGH_2 at about one fifth of the potency. In some preparations the PGG_2 or PGH_2 induced relaxation preceded by a short-lasting contraction. Thromboxane A_2 contracted both preparations. Coeliac artery recovered from relaxation in 5 min whereas mesenteric artery took considerably longer.

We conclude that (a) the RCS described by Piper & Vane (1969) was predominantly TxA_2 since it contracted strips of rabbit coeliac artery (Palmer *et al.*, 1973) (b) PGG_2, PGH_2 and TxA_2 are much more potent on vascular tissue than on smooth muscle from gastrointestinal tract and (c) the coeliac artery preparation distinguishes between the endoperoxides and TxA_2. It will be interesting to compare these activities with vascular effects of the same substances *in vivo*.

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Bioassay and thin-layer chromatography of prostaglandins and their pulmonary metabolites

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Piper & Vane (1969) reported that prostaglandins are released from guinea-pig lungs during anaphylaxis. Mathé & Levine (1973) and Leibig, Bernauer & Peskar (1974) have shown that prostaglandin metabolites are also released. We have therefore investigated the effects of the pulmonary metabolites of the prostaglandins on tissues routinely used to assay parent prostaglandins. Earlier results indicate that high doses of the pulmonary metabolites of prostaglandin E_2 (PGE_2) are indistinguishable from lower doses of PGE_2 on the tissues used (Crutchley & Piper, 1975). The present studies were carried out on the pulmonary metabolites of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

Prostaglandins and metabolites were assayed on the rat stomach strip, chick rectum, rat colon and oestrogen-primed rat uterus as described (Crutchley & Piper, 1975). Thin-layer chromatography was carried out using the AI, AII and AIII systems (Green & Samuelsson, 1964; Änggard & Samuelsson, 1964).

Results of bioassay on rat stomach strip, chick rectum and rat colon indicated that 13,14-dihydro-$PGF_{2\alpha}$ was the most potent metabolite, having approximately 0.3 times the activity of $PGF_{2\alpha}$. 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ had approximately 0.01 times the activity of the parent prostaglandin. However, the order of potency of metabolites was the same on all three assay tissues, making distinction on this basis impossible (Figure 1).

In the AII system, 13,14-dihydro-PGE_2 (R_f 0.80) was almost indistinguishable from PGE_1 (R_f 0.82) and 13,14-dihydro-PGF_2 (R_f 0.50) was similarly indistinguishable from PGE_2 (R_f 0.50). Thin-layer chromatography in the AI and AIII systems also failed to separate these prostaglandins. When tested on rat stomach strip, rat colon and chick rectum