permeability is maintained by kinin release up to 2·5 h and then by prostaglandin release up to 6 hours. Mononuclear leucocyte migration into the inflamed site occurs during this latter phase.

Whilst testing different strains of rat for their reactivity to carrageenan, we found that Wistar rats, genetically resistant to the anaphylactoid reaction produced by dextran, failed to react to carrageenan (0·1 ml of 1%), injected into the subplantar aspect of one hind paw, until 1 h after injection after which the oedema increased to reach, by 6 h, almost the value obtained using Wistar rats sensitive to dextran. On the other hand, these sensitive rats always showed a marked reaction to 1 mg of carrageenan by 30 minutes. The resistant animals contain comparable amounts of histamine and 5-HT but release only relatively small quantities when injected with dextran (Harris & West, 1963), and form and release kinins only with difficulty when subjected to different forms of shock (Starr & West, 1970). Hence, their prostaglandin release by carrageenan appears to be unimpaired and they yield a result which closely follows that of Di Rosa & Willoughby (1971) who used rats sensitive to dextran but depleted of their stores of histamine, 5-HT and kinin before injecting carrageenan.

The intradermal injection of complete Freund's adjuvant into one hind paw of a rat results in disseminated inflammatory lesions of joints and skin after a latent period of about 14 days, a condition which resembles human rheumatoid arthritis in many respects. In 1969 Pelczarska reported that this adjuvant-induced arthritis was inhibited by treating the animals with hypostamine, an inhibitor of histidine decarboxylase. However, daily doses of 50 mg/kg intraperitoneally of NSD-1034 (N-(3-hydroxybenzyl)-N-methylhydrazine dihydrogen phosphate), another potent inhibitor of histidine decarboxylase, failed to modify the course of the reaction which therefore appears not to involve histamine. In contrast, Wistar rats resistant to dextran did not develop the arthritis, even without inhibitor; the secondary symptoms of the cell-mediated inflammation did not show at any time.

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Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum

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Prostaglandin (PG) synthesis and release *in vitro* or *in vivo* is blocked by non-steroidal anti-inflammatory drugs (Vane, 1971; Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Aiken & Vane, 1971). We have now studied the action of a

potent PG synthesis inhibitor, indomethacin, on the rabbit isolated jejunum preparation.

A segment of jejunum (4–6 cm) or a strip of longitudinal muscle (Ambache, 1954) was suspended in Krebs solution in an organ bath (15 ml) at 37° C. Contractions of the muscle were detected by an auxotonic lever attached to a transducer and displayed on a pen recorder. The bath fluid was left unchanged for 30 min ('collection period') and then drained and assayed for prostaglandin-like activity on a rat stomach strip, rat colon and chick rectum (Ferreira & Vane, 1967), superfused in series with Krebs solution containing antagonists (Gilmore, Vane & Wyllie, 1968) to increase the specificity of the assay.

During the collection period, the tone of the jejunum gradually increased, but fell again when the bath was washed with fresh Krebs solution. The mean PG-like activity (assayed as PGE_2) released during the first 30 min was 15 ng/g tissue (six experiments: range 6–30 ng/ml). The amount of activity which was released increased with time so that 4–6 h later, the release in 30 min was about 45 ng/g tissue. Inhibition of resting tone by adrenaline or papaverine did not affect the output of prostaglandin-like activity.

Jejunum which had been stored at 4° C for 48 h generated even more activity (78 ng/g tissue). Strips of longitudinal smooth muscle also generated PG-like activity [(> 150 ng/g tissue)/30 min; two experiments]. Acid–lipid extraction of the collected bath fluid and thin layer chromatography in the AI system (Green & Samuelsson, 1964) showed the presence of PGs of the E and F series.

Indomethacin (1–10 μg/ml) progressively diminished prostaglandin release so that in 1–3 h, none was detectable. At the same time, the resting tone of the jejunum gradually fell. Furthermore, in contrast to the untreated preparations, the tone of the tissue did not rise during the collection period.

After inhibition of PG synthesis, the preparations were more sensitive to PGE_2, which caused a sustained contraction and restored the pendulum movements. The effects of acetylcholine were also augmented. Because of the low resting tone, adrenaline-induced relaxation could no longer be demonstrated.

These results suggest that the tone of the rabbit isolated jejunum is maintained by a continuous generation of prostaglandins, a conclusion supported by work with prostaglandin antagonists on other smooth muscle preparations (Bennett & Posner, 1971). The PG release may have a physiological function; however, it is also possible that it is due to the trauma (Piper & Vane, 1969) caused by isolating the tissue in an organ bath.

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Prostaglandin production by rabbit peritoneal polymorphonuclear leukocytes *in vitro*

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It has been suggested (Kaley & Weiner, 1971) that since prostaglandin E_1 (PGE_1) has chemotactic activity *in vitro* on rabbit polymorphonuclear (PMN) leukocytes it may be an important mediator of the leukocyte emigration from blood vessels seen in acute inflammation. If PGE_1 were released by PMN leukocytes during phagocytosis, this could constitute a control system for local leukocyte emigration which would continue as long as phagocytosis was occurring. PMN leukocytes *in vitro* were therefore investigated for their capacity to produce PG.

Rabbit PMN leukocytes were obtained by washing out the peritoneal cavities of animals with Hanks solution 4 h after the intraperitoneal injection of 200 ml 0·1% rabbit liver glycogen in 0·9% sterile saline as described by Hirsch & Church (1960). Each animal was used at intervals of 7–10 days for this procedure. The cell suspensions (over 90% PMN leukocytes) were centrifuged gently and resuspended in Hanks solution enriched with glucose (0·56 mM) and 0·01% bovine serum albumin, to give cell suspensions in the range $3{\cdot}5\text{–}8{\cdot}0\times10^6$/ml. These suspensions (volume 40–50 ml) were incubated at 37°, and aliquots of 4 ml taken at intervals, adjusted to pH 3·0 with HCl and extracted twice with ethyl acetate. The pooled extract was evaporated to dryness in a rotary evaporator, redissolved in 1·0 ml Krebs solution and the resulting solution assayed for PG-like activity on rat stomach strips superfused with Krebs bicarbonate solution containing methysergide 0·2 mg/l, phenoxybenzamine 0·1/mg/l, propranolol 3 mg/l, hyoscine hydrobromide 0·1 mg/l and mepyramine maleate 0·1 mg/litre.

In these circumstances only very small (less than 1 ng/ml original suspension) amounts of prostaglandins were detected, but if killed bacteria (Pertussis vaccine, Burroughs Wellcome, containing 4×10^{10} bacteria/ml) were added in doses of 100–200 bacteria/leukocyte, PG-like activity in amounts up to 10 ng/10^6 PMN leukocytes PGE_2 equivalent were found after incubation for 2 hours. The bacterial suspensions alone had no PG-like activity. Thin layer chromatography of the extract was performed after purification from solution in ethanol by extracting 4 times with petroleum ether, using Dioxan/Benzene/acetic acid (20/20/1) as solvent. Fifty-six per cent of the PG-like activity found moved with PGE_2, 28% with $PGF_{2\alpha}$ and 16% as an unidentified spot between the origin and the $F_{2\alpha}$.

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