cyclic AMP levels decreased until day 18 but rose again between days 18 and 22, when paw volume was still increasing (Bonta *et al.*, this volume). PGE_1 (0.5 mg kg^{-1} day^{-1}) did not significantly alter the late increase in cyclic AMP, though paw volume was increased.

These results suggest that, in adjuvant arthritis, gross changes in cyclic AMP levels at the inflammatory site cannot be correlated with the inflammatory response. They may reflect different changes in individual cell populations, as observed with lymphocyte subpopulations *in vitro* (Bach, 1975).

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Actions of phospholipase-A on mast-cell histamine release and paw oedema in the rat

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Local administration of E-prostaglandins potentiates carrageenin-induced oedema formation in the rat paw (Moncada, Ferreira & Vane, 1973). A prostaglandin precursor, arachidonic acid, also potentiates carrageenin paw oedema and this action is abolished by administration of a non-steroid anti-inflammatory drug, indomethacin, known to inhibit prostaglandin synthesis (Lewis, Nelson & Sugrue, 1975). In the present work, we have investigated the effects on paw oedema of phospholipase-A (PL-A), an enzyme involved with the liberation of endogenous prostaglandin precursors, and have studied the actions of anti-inflammatory agents. However, since crude PL-A is known to release inflammatory mediators from rat mast cells (Thomas & Whittle, 1976) which therefore could lead to oedema formation, we have first compared the ability of PL-A obtained from various sources to liberate histamine from rat mast cells with their effects on rat paw oedema.

Mast cells were obtained by lavage of the rat peritoneal cavity with a modified buffer solution (pH7) and the histamine release following incubation (20 min at 37°C) was determined by fluorometric assay. Crude phospholipase-A from *vipera russellii* venom caused a dose-dependent release of histamine; a concentration of 20 μg/ml (0.1 unit/ml enzyme activity) gave a $75 \pm 7\%$ (mean ± s.e. mean, $n=5$) release of the total histamine content of the mast cells. However, the PL-As from other sources gave only a low histamine release; in five experiments, PL-A from *crotalus terrificus* venom (20 μg/ml; 6 units/ml) gave $9 \pm 2\%$ release, that from bee venom (20 μg/ml; 31 units/ml) gave $8 \pm 2\%$ release and the PL-A_2 from pig pancreas (20 μg/ml; 16 units/ml) $13 \pm 1\%$ release. Thus, the histamine-releasing activity was not correlated with PL-A enzyme activity, and may be due to presence of a lytic factor in the crude PL-A.

Changes in rat paw volume following subplantar injections were determined with a mercury-displacement plethysmograph (Van Arman, Begany, Miller & Pless, 1965). PL-A from *vipera russellii* (0.5–5 μg in 0.1 ml) gave a rapid marked rise in paw volume reaching a maximum after 30 min, as was found with the histamine liberator, compound 48/80 (1–10 μg in 0.1 ml). The PL-A from *crotalus* and bee venoms (5–20 μg) also caused an increase in paw volume following local injection, but were less effective. In contrast, pig pancreas PL-A_2 (5–20 μg) had no consistent effect on paw volume. However, this PL-A_2 (10 μg) significantly potentiated the increase in paw volume (by 212 ± 28 μl, $n=20$; after 1.5 h, $P<0.001$) following simultaneous subplantar administration of carrageenin (0.1 ml, 2% suspension). Pretreatment with indomethacin (15 mg/kg, s.c.; 1 h prior to carrageenin) in a dose causing 75% inhibition of the carrageenin-induced paw oedema, reduced this potentiation with PL-A_2 (to 92 ± 10 μl, $n=14$; $P<0.01$), whereas pretreatment with an equi-active anti-inflammatory dose of dexamethasone (100 μg/kg, s.c.) did not significantly alter this response (177 ± 33 μl, $n=14$).

These results show that local administration of PL-A_2, like exogenous prostaglandins, can potentiate carrageenin-induced rat paw oedema. The ability of a prostaglandin synthetase inhibitor to reduce this response may suggest that endogenous prostaglandin formation is involved in the potentiation. The failure of dexamethasone to inhibit this PL-A_2 response could indicate that the anti-inflammatory steroids exert their effects at a stage prior to, or independent of, the involvement of PL-A_2 (see Gryglewski, 1976), although the present findings do not preclude the possibility of actions on the activation or release of endogenous PL-A_2.

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Phospholipase A_2 activity of guinea-pig perfused lungs: stimulation and inhibition by anti-inflammatory steroids

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Guinea-pig isolated lungs release prostaglandin (PG) endoperoxides and thromboxane A_2 (TXA_2) into the perfusion fluid in response to stimuli including antigen challenge (Piper & Vane, 1969), mechanical trauma (Palmer, Piper & Vane, 1973), bradykinin or arachidonic acid (Vargaftig & Dao Hai, 1972) and rabbit aorta contracting substance – releasing factor (RCS-RF; Piper & Vane, 1969; Nijkamp, Flower, Moncada & Vane, 1976). Nijkamp *et al.* (1976) demonstrated that in relation to their anti-inflammatory potency, corticosteroids inhibited the generation of TXA_2 induced by RCS-RF, but not that due to the precursor arachidonic acid. Thus, inhibition of the release of arachidonic acid could be related to the therapeutic action of these steroids. Flower & Blackwell (1976) demonstrated that arachidonic acid was released from cellular phosphatides in response to similar stimuli, and this led us to speculate that agents which release TXA_2 from lungs do so by "activating" phospholipase A_2.

For these experiments, the guinea-pig perfused lungs and cascade superfusion apparatus were prepared and TXA_2 generation was measured as previously described (Nijkamp *et al.*, 1976). For assay of phospholipase A_2 activity, a mixture of 18.0 nmoles 2-acyl ([^{3}H]-oleoyl) phosphatidylcholine and 1.8 nmoles [^{14}C] oleic acid was injected into the pulmonary artery. The perfusate was collected for 7 min and the labelled fatty acids selectively extracted at pH 8.0 with 10 ml *n*-hexane. The solvent was evaporated to dryness and the $^3H/^{14}C$ ratio estimated by conventional liquid scintillation counting techniques.

When injected into the pulmonary artery, histamine (2–5 μg), RCS-RF (5–10 u), bradykinin (1–5 μg) and arachidonic acid (1–5 μg) caused a release of PG endoperoxides and TXA_2. Release of TXA_2 was blocked by indomethacin (1 μg/ml). The release induced by histamine or RCS-RF was also blocked by dexamethasone (ID_{50} 1.5 μg/ml) and hydrocortisone (ID_{50} 33 μg/ml).

There was a small (1–3%) basal hydrolysis of the labelled phosphatide by the perfused lung, which increased gradually with time. This hydrolysis was inhibited by mepacrine (20 μg/ml), procaine (40 μg/ml), betamethasone (2 μg/ml), dexamethasone (2 μg/ml) and hydrocortisone (50 μg/ml). The steroids exhibited a time-dependent inhibition, the maximum effect occurring after 30 min infusion. Histamine (2 μg), RCS-RF (5 u) and bradykinin (1 μg) stimulated phospholipid hydrolysis by 150–300%. Steroids and mepacrine blocked (60–90%) the stimulation due to histamine and RCS-RF but had only a small effect (10–20%) on the bradykinin stimulation.

In homogenates of guinea-pig lung, phospholipase A_2 activity was inhibited by mepacrine and procaine. Steroids were without effect, indicating that these agents require intact cells to function effectively.

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