(0.5–1 μg/ml) and flufenamic acid (5–10 μg/ml) in all species except the guinea-pig. The addition of $PGE_2$ and $PGF_{2\alpha}$ (10–20 ng/ml) reversed the effects of these substances causing a return of tone and spontaneous activity. Bioassay of the bath fluid of the rabbit detrusor strips and thin layer chromatography done in association with N.G. Bowery, indicated, by the $R_f$ value of the biological activity, the presence of PG-like activity of the E-series. In the presence of indomethacin no activity was detectable.

The addition of physostigmine (1 μg/ml) to the rabbit isolated detrusor strip produced an increase in tone and spontaneous activity which was prevented by the prior addition of indomethacin. Prostaglandin $E_2$ reversed this effect of indomethacin. Hyoscine (300 ng/ml), which did not affect the resting tone or spontaneous activity, abolished the response to physostigmine. These results suggest that there may be a link between acetylcholine output and PG production in the bladder preparation. Preliminary experiments with hemicholinium-3 support this possibility.

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## Effects of metyrapone on rat uterine prostaglandin release and on spontaneous smooth muscle activity *in vitro*

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Parnham & Sneddon (1975) have shown that metyrapone, an inhibitor of corticosteroid biosynthesis (Chart & Sheppard, 1959), when administered *in vivo* inhibits the subsequent release of prostaglandin F (PGF) from the isolated pregnant rat uterus. It also inhibits the conversion of $^{14}$C-arachidonic acid to prostaglandin $E_2$ ($PGE_2$) by crude homogenates of pregnant rat uteri at the same time stimulating the synthesis of labelled prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In an attempt to clarify the action of metyrapone its direct effect on the isolated pregnant rat uterus has been investigated.

Uteri were removed from pregnant rats on the morning of day twenty-two. Individual horns were mounted in 75 ml organ baths and bath fluid collected every 15 min for 1 h, as described previously (Vane & Williams, 1973), while uterine activity was recorded isotonically. Metyrapone (to give 0.5, 1, 2 or 4 mM) was injected into one bath at the start of the experiment and the corresponding volume of vehicle (0.33M (+)-tartaric acid; 0.05–0.4 ml) was injected into a second organ bath containing the contralateral uterine horn. Both solutions were left in contact with the tissue for 15 min, and then washed out. Prostaglandins were extracted from bath fluid as described by Vane & Williams (1973), subjected to column chromatography to separate PGs E and F (Parnham & Sneddon, 1975) and the relevant fractions were resuspended in 2 ml saline for bioassay either on the isolated rat colon against authentic $PGF_{2\alpha}$ or on the isolated rat stomach strip against authentic $PGE_2$. At low doses of metyrapone the release of both PGE and PGF was stimulated when compared with controls ($291 \pm 67\%$ and $182 \pm 25\%$ at 0.5 mM, respectively; 4 exp/dose); but at higher doses PGF release was inhibited ($65 \pm 8\%$ at 4 mM), whereas PGE release was unaffected.

Metyrapone rapidly inhibited spontaneous uterine contractions which reappeared within 5–10 min following washout. This inhibition was dose-dependent and involved reduction of tone at high doses. Similar dose-dependent inhibition of spontaneous contractions by metyrapone, was observed on the isolated rabbit ileum. This did not involve a reduction in tone at the doses used. Propranolol (1 μg/ml) antagonized the inhibitory effect of isoprenaline on this preparation, but had no effect on the response to metyrapone. The dose-response curve to metyrapone on the rabbit ileum was similar to that for papaverine, which was

approximately 10 times more potent than metyrapone on a molar basis.

In two experiments on the isolated uterus of the non-pregnant rat (primed with 200 μg oestradiol benzoate i.m. 18 h previously) metyrapone antagonized contractile responses to acetylcholine and to $PGE_2$ but responses to $PGF_{2\alpha}$ suggested slight stimulation.

These results suggest that metyrapone has a non-specific direct smooth muscle inhibiting action. They also support the earlier suggestion (Parnham & Sneddon, 1975) that metyrapone has a differential effect on the synthesis of PGF and PGE in the pregnant rat uterus *in vitro*, similar to that recently observed with gold salts and phenylbutazone on sheep seminal vesicles (Stone, Mather & Gibson, 1975).

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## Effects of anti-inflammatory drugs on macrophage prostaglandin biosynthesis

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Chronic inflammation in diseases such as rheumatoid arthritis is typified by a persistent mononuclear cell infiltrate in which macrophages are prominent. Macrophages, derived from inflammatory exudates, generate substantial concentrations of prostaglandin activity when cultured *in vitro* (Bray, Gordon & Morley, 1974). We have, therefore, examined the effect of established anti-inflammatory drugs on macrophage prostaglandin biosynthesis *in vitro*.

Macrophage-rich peritoneal exudate cell populations (60–80% macrophages) were collected from guinea-pigs, 2–3 days after intraperitoneal injection of 20 ml 2% starch solution. Cells ($1 \times 10^6$/ml) were cultured in Eagles MEM containing antibiotics, and 10% heat-decomplemented foetal calf serum, in 5% $CO_2$ in air at 37°C for 24 hours. Prostaglandin E-like activity in the supernatant was measured by radioimmunoassay utilizing sheep anti-$PGE_2$/BSA antiserum whose cross-reactivity was $PGE_2$ (100%), $PGE_1$ (55%), $PGF_{2\alpha}$ (1.5%), 15 keto-$PGE_2$ (1.2%), $PGA_2$ (0.6%) and $PGB_2$ (0.2%).

Macrophage PG production was $12.0 \pm 2.76$ ng $PGE_2$ equivalent/$10^6$ cells per 24 h (range 2.2–43.2). In agreement with previous studies (Vane, 1971; Flower, 1974), non-steroidal anti-inflammatory drugs (NSAIDs) caused dose-related inhibition of prostaglandin biosynthesis with rank order of potency (Table 1) approximating anti-inflammatory activity.

**Table 1** Inhibition of macrophage prostaglandin synthesis

| *Drug* | *$ID_{50}$ (ng/ml)* | *Relative Potency** |
|---|---|---|
| Arylalkanoic Acids: | | |
| Indomethacin | 1.15(8)† | 100 |
| Ketoprofen | 13.6 (2) | 2.06 |
| Naproxen | 225 (2) | 0.12 |
| Pyrazolidinediones: | | |
| Phenylbutazone | 1450 (2) | 0.15 |
| Feprazone | 400 (2) | 0.55 |
| Salicylates: | | |
| Acetyl Salicylic Acid | 280 (2) | 0.35 |
| Sodium Salicylate | 5300 (2) | 0.018 |
| Glucocorticosteroids: | | |
| Hydrocortisone | 108 (2) | 1.01 |
| Prednisolone | 26.7 (2) | 4.09 |
| Dexamethasone | 4.35(2) | 25.13 |

* Relative potency was obtained by comparison of the $ID_{50}$ with the $ID_{50}$ for indomethacin (expressed as 100) determined in the same experiment.
† Number of experiments in parenthesis.