Br. J. Pharmac. (1978), **63**, 635–642

PROSTAGLANDIN PRODUCTION BY MACROPHAGES AND THE EFFECT OF ANTI-INFLAMMATORY DRUGS

M.A. BRAY & D. GORDON[1]

Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, London SW3 6HP

1 Macrophages derived from peritoneal cavity inflammatory exudates of guinea-pigs produced substantial amounts of prostaglandin E_2-like activity during *in vitro* culture, so providing the basis for an experimental model of prostaglandin production during inflammatory reactions.

2 Dose-related inhibition of prostaglandin biosynthesis was demonstrated by 16 acidic non-steroidal anti-inflammatory drugs.

3 Seven anti-inflammatory glucocorticosteroid preparations inhibited prostaglandin production in a dose-related manner. The relative potencies of dexamethasone, prednisolone and hydrocortisone were consistent with clinical anti-inflammatory ranking. Cortisone, however, failed to inhibit macrophage prostaglandin production.

4 Three other agents used in the treatment of inflammatory joint diseases were examined. Sodium aurothiomalate inhibited prostaglandin production, although higher concentrations were toxic to macrophages. D-Penicillamine did not affect macrophage prostaglandin production. Colchicine, in contrast, enhanced prostaglandin production at some concentrations.

5 The probable significance of macrophages as a source of prostaglandins, during inflammatory responses, is discussed.

Introduction

There is considerable evidence that prostaglandins are involved as mediators of inflammation (Vane, 1972; Ferreira & Vane, 1974). However, the cellular origin of increased prostaglandin production in inflammatory reactions has remained uncertain and this aspect has received less attention than other criteria of mediator identification. The similar time course of appearance of prostaglandins, lysosomal enzymes and infiltrating leucocytes in carrageenin-induced exudates has been taken to suggest that the cellular infiltrate is the source of prostaglandin production (Anderson, Brocklehurst & Willis, 1971). The polymorphonuclear (PMN) leucocyte has been suggested as a possible source of inflammatory prostaglandins on the basis of observed *in vitro* prostaglandin E (PGE) production by rabbit PMN leucocytes (Higgs, McCall & Youlten, 1975). However, human peripheral blood PMN leucocytes showed little capacity for PGE production (Zurier & Sayadoff, 1975) and certainly no correlation exists between prostaglandin concentration and PMN leucocyte count in several types of inflammatory response (Levine, 1973; Blackham, Farmer, Radziwonik & Westwick, 1974; Glatt, Peskar & Brune, 1974).

The macrophage, another prominent infiltrating haematogenous cell, is known to contain a high proportion of arachidonic acid in membrane phospholipids (Mason, Stossel & Vaughan, 1972), to possess phospholipases A_1 and A_2, that can be activated by phagocytosis (Munder, Ferber, Modolell & Fischer, 1969; Franson & Waite, 1973) and to undergo lipid peroxidation following phagocytosis (Stossel, Mason & Smith, 1974). These observations would suggest that this cell type is a likely source of prostaglandins and we have previously reported that macrophages derived from inflammatory exudates produce substantial amounts of PGE-like activity during short-term *in vitro* culture (Bray, Gordon & Morley, 1974). The present experiments have examined the effects of a range of steroidal and non-steroidal anti-inflammatory drugs with established therapeutic value, on prostaglandin biosynthesis by macrophages, with the view to evaluating such an *in vitro* system for the screening of potential anti-inflammatory agents acting upon this component of the inflammatory response. A preliminary account of some of this work has been published (Bray & Gordon, 1976).

[1] Present address: Department of Pharmacology, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN

Methods

Cell harvesting and culture

Peritoneal exudate cells were obtained from male, outbred Hartley guinea-pigs, weighing 300 to 500 g, following intraperitoneal injection of 20 ml 2% sterile starch solution (Sigma). The animals were killed 2 to 3 days later and the macrophage-rich cell populations were removed, through a ventral midline incision, by washing out the peritoneal cavity with 50 to 100 ml cold (4°C) Hanks balanced salt solution (Oxoid) containing penicillin (200 iu/ml) and streptomycin (100 iu/ml) (Glaxo). The cell pellet obtained after centrifugation at 400 *g* for 10 min was resuspended in 10 ml cold Hanks solution and the number of viable cells determined by vital dye exclusion (Eosin-Y: Oxoid) using a modified Neubauer haemocytometer (Hawksley). The cells were recentrifuged at 400 *g* for 10 min and suspended to a concentration of 1×10^6 viable cells/ml in cold Eagle's Minimal Essential Medium (MEM) (Flow Labs) containing penicillin (200 iu/ml) and streptomycin (100 iu/ml) supplemented with 10% heat-decomplemented foetal calf serum (Flow Labs).

Aliquots (0.5 ml) of the cell suspensions were dispensed into sterile, disposable, 12 × 75 mm, plastic culture tubes (Falcon) and incubated in an atmosphere of 5% CO_2 in air for up to 24 h at 37°C. Drugs in Eagle's MEM were added immediately before culture (20 µl volumes). Cell culture was terminated by centrifugation at 600 *g* for 5 min at 4°C, and the supernatants removed and stored at −20°C before assay. Sterile techniques were employed throughout and no infections were noted during the culture period.

Prostaglandin radioimmunoassay

Prostaglandin-like activity in culture supernatants was measured by radioimmunoassay (Jaffe, Smith, Newton & Parker, 1971) using sheep anti-PGE_2/bovine serum albumin and anti-$PGF_{2\alpha}$/bovine serum albumin antisera. Tritium-labelled prostaglandins (20,000 d/min per 0.1 ml) were equilibrated with the appropriate antiserum (0.1 ml) and unknown or standard (15 to 400 pg/0.1 ml) solutions of prostaglandins in 0.1 M phosphate buffer pH 7.2 at 4°C overnight. Bovine gamma globulin (2.5 mg/0.1 ml) was then added and antibody-bound [^{3}H]-prostaglandin precipitated by the addition of saturated ammonium sulphate solution (0.4 ml). The precipitate was washed once with 50% saturated ammonium sulphate solution (0.8 ml), transferred, with two 0.6 ml aliquots of distilled water, into vials containing 10 ml scintillation fluid (Fisons Toluene Cocktail P: Fisons Emulsifier mixture No. 1, at a ratio of 1:0.5 v/v), and counted in a Packard Tri-Carb liquid scintillation counter. Anti-PGE antiserum cross reacted 100% with PGE_2; 55% with PGE_1; 11% with 13,14-dihydro-PGE_2; 1.5% with $PGF_{2\alpha}$; 1.2% with 15-keto PGE_2; 0.8% with 13,14-dihydro-15-keto-PGE_2; 0.6% with PGA_2; 0.3% with thromboxane B_2; and 0.2% with PGB_2. Anti-PGF antiserum cross-reacted 100% with $PGF_{2\alpha}$; 27% with $PGF_{1\alpha}$; 0.21% with 15-keto-$PGF_{2\alpha}$; 0.16% with 13,14-dihydro-15-keto-$PGF_{2\alpha}$; 0.13% with PGE_2; 0.013% with PGA_2; 0.017% with thromboxane B_2; and 0.016% with PGB_2. Since these antisera are not mono-specific, immunoreactive prostaglandin-like activity was expressed in terms of PGE_2 or $PGF_{2\alpha}$ weight equivalents.

Drugs

The following drugs were used: indomethacin (Merck, Sharp & Dohme); ketoprofen, sodium salt (SPECIA, France); sodium flurbiprofen (Boots); fenoprofen, calcium salt (Eli Lilly); naproxen (Syntex); alclofenac (Berk); azapropazone (E.H. Robins); oxyphenbutazone (Geigy); feprazone, sodium salt (Di Angeli, Italy); phenylbutazone, sodium salt (Di Angeli, Italy); meclofenamic acid (Parke Davis); niflumic acid (Squibb); mefenamic acid (Parke Davis); flufenamic acid (Parke Davis); acetylsalicylic acid (BDH); sodium salicylate (BDH); D-penicillamine (Dista); colchicine (Sigma); dexamethasone sodium phosphate (Decadron; Merck, Sharp & Dohme); prednisolone sodium phosphate (Codelsol; Merck, Sharp & Dohme); prednisolone acetate (Precortisyl; Roussel); methylprednisolone acetate (Depo-Medrone; Upjohn); triamcinolone acetonide (Adcortyl; Squibb); hydrocortisone sodium phosphate (Efcortesol; Glaxo); hydrocortisone sodium succinate (Organon); cortisone acetate (Cortisyl; Roussel) and sodium aurothiomalate (Myochrysin; May & Baker).

[5,6,8,11,12,14,15(n)-^{3}H]-prostaglandin E_2, 160 Ci/mmol, and [9-^{3}H]-prostaglandin $F_{2\alpha}$, 15 Ci/mmol, were purchased from the Radiochemical Centre, Amersham.

Results

Prostaglandin production by macrophage populations

Peritoneal exudate cells obtained from guinea-pigs, 2 to 3 days following intraperitoneal starch injection, consisted of 60 to 80% macrophages, 10 to 30% PMN leucocytes and up to 10% lymphocytes. These macrophage-rich cell populations produced substantial PGE_2-like activity (mean 12.6 ± 2.1 (s.e. mean) ng PGE_2 equivalent/10^6 cells) during 24 h culture. The presence of PGE_2 tentatively identified by bioassay and thin layer chromatography (Gordon, Bray &

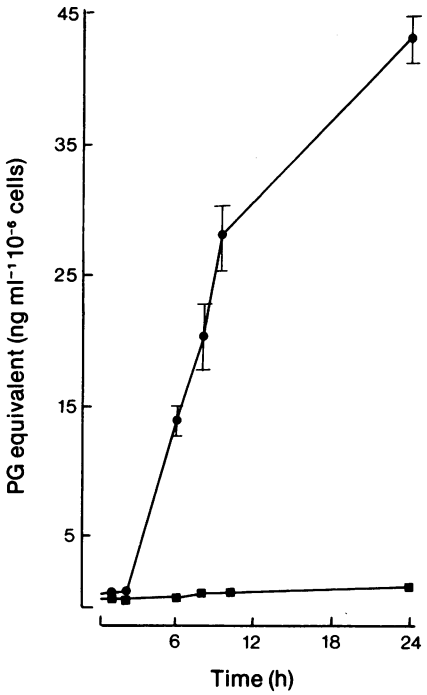

Figure 1 Time course of prostaglandin production by cultured guinea-pig peritoneal exudate macrophages. Macrophages were incubated for the times indicated, and prostaglandin (PG) activities in the culture supernatants determined by radioimmunoassay as described. Each point represents the mean of 6 replicate samples; vertical lines show s.e. means. (●) PGE_2 equivalent; (■) $PGF_{2\alpha}$ equivalent.

Morley, 1976) has been confirmed by combined gas chromatography/mass spectrometry (Nugteren & Van Dorp, Unilever—personal communication). The time course of prostaglandin production by macrophage-rich cell populations is shown in Figure 1. Prostaglandin-like activity was undetectable in supernatant fluids collected up to 2 h; PGE synthesis then proceeded at a fairly constant rate up to 24 h. PGF synthesis was much lower than PGE synthesis. Post-culture viability of cells was >90% viable as estimated by vital dye exclusion. A similar time course and ratio of PGE:PGF production has been reported for cultured human rheumatoid synovial fragments (Kantrowitz, Levine & Robinson, 1975a; Kantrowitz, Robinson, McGuire & Levine, 1975b).

Inhibition of prostaglandin production by indomethacin and other nonsteroidal anti-inflammatory agents

Indomethacin (0.1 ng to 0.1 μg/ml) produced dose-related inhibition of prostaglandin biosynthesis (Figure 2), with 70% inhibition at a concentration of 3.7 ng/ml (range 1.9 to 4.8 ng/ml in 8 experiments). Indomethacin was arbitrarily assigned a potency of 100 and used as a reference compound for comparison with other drugs (Table 1).

Fifteen other nonsteroidal anti-inflammatory agents were examined representing four chemical classes: salicylates (acetylsalicylic acid and sodium salicylate); pyrazolidinediones (phenylbutazone, azapropazone, oxyphenbutazone and feprazone); arylalkanoic acids (ketoprofen, flurbiprofen, fenoprofen, and naproxen) and fenamates (meclofenamic acid, niflumic acid, mefenamic acid and flufenamic acid).

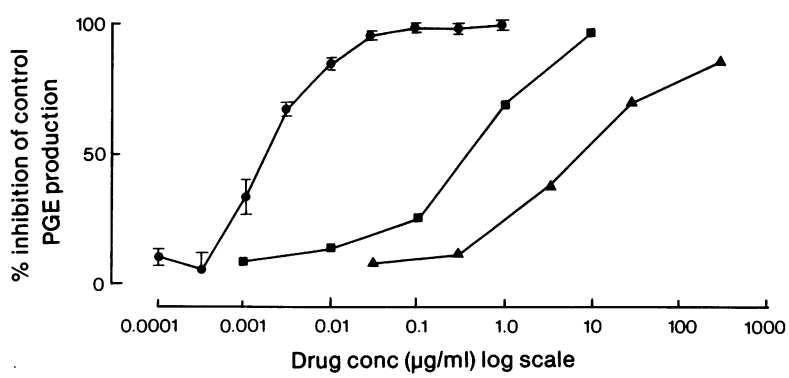

Figure 2 Effect of indomethacin and salicylates on prostaglandin E (PGE) production by cultured macrophages. Macrophage cultures were incubated for 24 h in the presence of indomethacin (●), acetylsalicylic acid (■) and sodium salicylate (▲) at the concentrations shown. For indomethacin each point represents the mean of 12–24 replicate samples from 4 to 8 separate experiments; vertical lines show s.e. mean. For the salicylates each point represents the mean of 6 replicate samples from 2 separate experiments.

Each compound produced dose-related inhibition of macrophage prostaglandin production (Figure 2; Table 1).

Inhibition of prostaglandin production by steroidal anti-inflammatory agents

Dexamethasone (0.1 to 100 ng/ml), prednisolone (1.0 ng to 1.0 μg/ml) and hydrocortisone (10 ng to 1.0 μg/ml), tested as their soluble phosphate esters, exhibited dose-related inhibition of macrophage prostaglandin production. Dexamethasone and prednisolone were, respectively, approximately 8 and 2 times as potent as hydrocortisone, judged on the basis of ID_{70} concentrations of base (Table 1).

In other experiments, dose-related inhibition of prostaglandin production has been observed with triamcinolone acetonide, prednisolone acetate, methylprednisolone acetate and hydrocortisone sodium succinate (Figure 3; Table 1). In contrast, cortisone acetate had no significant effect on prostaglandin production at concentrations up to 1 μg/ml, and stimulated PGE production at concentrations above 10 μg/ml.

Inhibition of prostaglandin production by dexamethasone was unaffected by concurrent addition of insulin (0.1 to 100 mu/ml) suggesting that the action of the anti-inflammatory steroid was not simply a result of depressed glucose utilisation by the cells.

Effects of other anti-inflammatory agents on prostaglandin production

Sodium aurothiomalate (1 μg to 1 mg/ml) produced dose-related inhibition of prostaglandin production, with 70% inhibition being observed at a concentration of 42 μg/ml (Figure 4). In contrast to non-steroidal anti-inflammatory drugs, at concentrations above 10 μg/ml there was some loss of cell viability as measured by vital dye exclusion.

D-Penicillamine, at concentrations up to 100 μg/ml, had no significant effect on macrophage prostaglandin production; however, at 1 mg/ml 20% inhibition was observed (Figure 4). Colchicine increased prostaglandin production at some concentrations with maximal effect at 0.1 μg/ml (no drug control = 6.2 ± 0.7: colchicine 0.1 μg/ml = 11.1 ± 0.4 ng PGE_2 equivalent per 10^6 cells in 24 h) though less

Table 1 Inhibition by anti-inflammatory drugs of macrophage prostaglandin E (PGE) production

Compound	*ID_{70} (ng/ml)*	*Relative potency*
Group 1		
Indomethacin	3.7	100*
Acetylsalicylic acid	1120	0.34
Sodium salicylate	51000	0.006
Azapropazone	530	0.56
Oxyphenbutazone	860	0.35
Feprazone	1400	0.20
Phenylbutazone	3050	0.09
Meclofenamic acid	2.65	183
Niflumic acid	8.6	59.9
Mefenamic acid	20.0	22.7
Flufenamic acid	48.5	9.3
Ketoprofen	28.0	7.0
Flurbiprofen	88.0	2.2
Fenoprofen	405	0.47
Naproxen	750	0.25
Alclofenac	3800	0.05
Group 2		
Dexamethasone (phosphate)	44.0	8.1
Prednisolone (phosphate)	158	2.3
Hydrocortisone (phosphate)	358	1.0†
Triamcinolone (acetonide)	48.1	12.9
Methyl prednisolone (acetate)	57.5	10.7
Prednisolone (acetate)	80.6	7.7
Hydrocortisone (succinate)	1534	0.4

Group 1: relative potency obtained by comparison of the ID_{70} with the ID_{70} for indomethacin* (expressed as 100) determined in the same experiment.
Group 2: relative potency obtained by comparison of the ID_{70} of base with the ID_{70} of hydrocortisone phosphate† (as base) expressed as 1.0 determined in the same experiment.

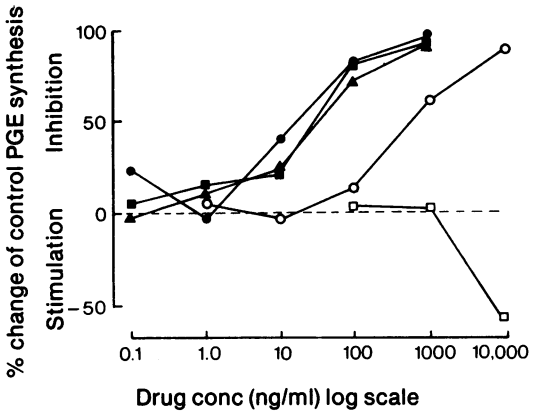

Figure 3 Effect of glucocorticosteroids on prostaglandin E (PGE) production by cultured macrophages. Macrophage cultures were incubated for 24 h in the presence of triamcinolone acetonide (●), methylprednisolone acetate (■), prednisolone acetate (▲), hydrocortisone succinate (○) and cortisone acetate (□) at the concentrations indicated. Each point represents the mean of 6 replicate samples from 2 separate experiments.

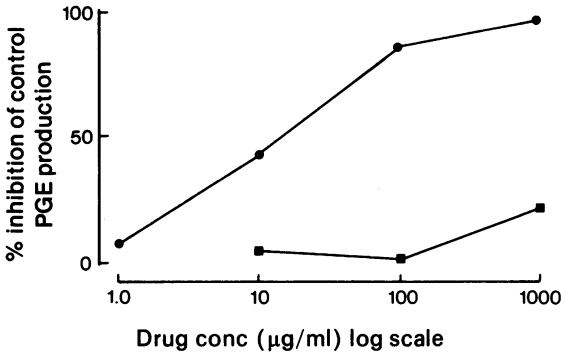

Figure 4 Effect of anti-rheumatic drugs on prostaglandin E (PGE) production by cultured macrophages. Macrophage cultures were incubated for 24 h in the presence of triamcinolone acetonide (●), and D-penicillamine (■) at the concentrations indicated. Each point represents the mean of 6 replicate samples from 2 separate experiments.

stimulation was seen at higher concentrations (colchicine 1.0 μg/ml = 8.3 ± 0.3 ng PGE_2 equivalent per 10^6 cells in 24 h).

Discussion

The observation that macrophages derived from inflammatory exudates produce substantial amounts of prostaglandin E_2-like activity during *in vitro* culture, indicates that this cell type should be considered as a major source of the elevated prostaglandin levels found in inflammation, particularly during chronic stages but also contributing to some earlier manifestations of the inflammatory response. For example, in carrageenin-induced inflammation, although the onset of prostaglandin production apparently coincides with the migration of PMN leucocytes and mononuclear cells into the inflamed site (Anderson *et al.*, 1971), elevated prostaglandin levels persist for longer than PMN leucocyte infiltration and remain elevated during the period of mononuclear cell predominance (Willis, 1969; DiRosa & Willoughby, 1971; DiRosa, Giroud & Willoughby, 1971). In contrast, in urate crystal-induced synovitis, the onset of prostaglandin production and several symptoms of inflammation precede PMN leucocyte accumulation (Glatt *et al.*, 1974); moreover ingestion of urate crystals by synovial-lining macrophages also precedes the onset of vasodilatation, oedema and the subsequent PMN leucocyte accumulation (Schumacher, Phelps & Agudelo, 1974).

Inhibition of prostaglandin synthesis by nonsteroidal anti-inflammatory drugs has been demonstrated in many preparations (Flower, 1974). Dose-related inhibition of guinea-pig peritoneal macrophage prostaglandin synthesis has been demonstrated by 16 acidic non-steroidal anti-inflammatory drugs (Figure 2, Table 1) with at least 70% inhibition at concentrations known to be achieved during anti-inflammatory therapy in man. Hamberg (1972) showed that therapeutic doses of indomethacin, aspirin and sodium salicylate inhibited whole body prostaglandin production in man to a similar degree (greater than 70%).

The sensitivity of macrophage prostaglandin biosynthesis to inhibition by indomethacin is higher than that of homogenates or sub-cellular fractions derived from several tissues (see Flower, 1974) but is comparable to that of intact cells in culture, including human rheumatoid synovium (Kantrowitz *et al.*, 1975a).

An approximate correlation between inhibition of dog spleen prostaglandin synthetase and inhibition of carrageenin-induced rat paw oedema for several nonsteroidal anti-inflammatory agents has been reported, and ID_{50} concentrations were generally less than peak drug levels in man (Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972). Our results on macrophages are consistent with this viewpoint, as based upon a comparison of the ID_{50} values obtained from dose-response data. For example, ketoprofen was approximately 17 times more active than naproxen which is in accordance with reports that effective blood levels of these two drugs are in the ranges 2

to 7 μg/ml and 35 to 70 μg/ml respectively (Runkel, Forchielli, Serelius, Chaplin & Segre, 1974; Mitchell, Scott, Kennedy, Brooks, Templeton & Jeffries, 1975). Similarly feprazone was approximately 3.7 times more active than phenylbutazone on macrophage PGE production *in vitro* whilst effective blood levels are respectively 20 to 60 μg/ml and 80 to 140 μg/ml (Burns, Rose, Chenkin, Goldman, Schulert & Brodie, 1953; Chériè-Lingnière, Colombo, Carraba, Ferrari & Gallazi, 1974). Aspirin was approximately 30 times more active than sodium salicylate whilst effective blood levels are 10 to 14 μg/ml and 200 to 300 μg/ml respectively (Rosenthal, Bayles & Fremont-Smith, 1964; Sholkoff, Eyring, Rowland & Riegelman, 1967).

Although there is an observed correlation between inhibition of *in vitro* macrophage prostaglandin production and clinically effective blood levels within each chemical class of non-steroidal anti-inflammatory agent tested, no account has been taken of factors such as plasma protein binding, which is important *in vivo* because only free drug can reach the sites of action and exert a pharmacological effect (Koch-Weser & Sellers, 1976). However, pharmacokinetic studies with indomethacin have shown that symptomatic relief (analgesia) of inflammatory joint disease in man is more closely associated with drug levels in synovial fluid than in blood (Emori, Champion, Bluestone & Paulus, 1973; Brooks, Bell, Lee, Rooney & Dick, 1974). Nonetheless the level of indomethacin attained in synovial fluid (approximately 0.7 μg/ml) following the oral administration of therapeutic dosage in man (Emori *et al.*, 1973) is sufficient to inhibit by more than 90%, prostaglandin production by guinea-pig macrophages (Figure 2) even allowing for a high degree (95%) of protein binding. Similarly, synovial free drug concentrations achieved following clinically active doses of three other drugs which have been studied, ketoprofen (Mitchell *et al.*, 1975), feprazone (Chériè-Lignière *et al.*, 1974) and aspirin (Rosenthal *et al.*, 1964; Sholkoff *et al.*, 1967) are sufficient to inhibit macrophage prostaglandin synthesis to a comparable degree. The ratio of acetylsalicylate to salicylate in synovial fluid after analgesic doses is approximately 1:20–30, and the potency ratio inhibiting macrophage prostaglandin production (30:1) is not inconsistent with this.

Corticosteroids have been reported to produce no inhibition of prostaglandin biosynthesis (Vane, 1971; Smith & Willis, 1971; Ferreira, Moncada & Vane, 1971), small degrees of inhibition at high concentrations (Greaves & McDonald-Gibson, 1972a & b) or marked inhibition at therapeutic concentrations (Lewis & Piper, 1975; Gryglewski, 1975; Kantrowitz *et al.*, 1975b; Tashjian, Voelkel, McDonough & Levine, 1975) depending on the prostaglandin synthesizing preparation used. Prostaglandin production by cultured macrophages has been shown in this study to be inhibited by seven anti-inflammatory steroid preparations, with complete inhibition again being achieved at concentrations attained during anti-inflammatory therapy in man. The potency ratios of dexamethasone, prednisolone and hydrocortisone (Table 1) are in reasonable agreement with their clinical anti-inflammatory activities (Goodman & Gilman, 1975). The sensitivity of macrophage prostaglandin production to inhibition by these three drugs closely resembles that of human rheumatoid synovium *in vitro* (Kantrowitz *et al.*, 1975b).

It is also noteworthy that inhibition by hydrocortisone of macrophage prostaglandin production was evident at concentrations within the physiological range (Goodman & Gilman, 1975). The lack of inhibition of macrophage prostaglandin production by cortisone acetate suggests that these cell populations are unable to convert cortisone to its biologically active derivative hydrocortisone.

Of the two drugs tested with anti-rheumatic activity, D-penicillamine was essentially inactive at concentrations achieved during anti-rheumatic treatment (100 μg/ml plasma with a daily dose of 1 g of drug) (Figure 4). Thus inhibition of prostaglandin biosynthesis seems unlikely to be important in the mechanism of action of this drug. Aurothiomalate caused a dose-related inhibition of PGE production at concentrations known to be attained in serum and synovial tissue of patients on maintenance gold therapy (Lorber, Atkins, Chang, Lee, Starrs & Bovy, 1973; Rubenstein & Dietz, 1973; Grahame, Billings, Laurence, Marks & Wood, 1974). However, the observed dose-related increase in cell death at concentrations above 20 μg/ml seen in some experiments may contribute to the observed inhibition of prostaglandin synthesis, since prostaglandin synthesis was a feature of viable macrophages. Colchicine, which has a selective anti-inflammatory effect in acute attacks of gouty arthritis, but which is not therapeutically useful in other types of arthritis, enhanced macrophage prostaglandin biosynthesis with maximal effect at a concentration of 0.1 μg/ml. Colchicine (0.1 μg/ml) has also been reported to increase the production of prostaglandins by human rheumatoid synovium (Robinson, Smith & Levine, 1973). This effect of colchicine occurs at concentrations greater than those achieved during normal therapy (Wallace, Omojuku & Ertel, 1970). Prostaglandin production during urate crystal-induced synovitis was also enhanced by colchicine, although PMN leucocyte infiltration and the oedema of inflammation were simultaneously depressed (Glatt *et al.*, 1974).

In these experiments the effects of established anti-inflammatory drugs have been examined on the spontaneous *in vitro* prostaglandin production by macrophages which had been activated *in vivo*. We have previously demonstrated that such cell populations

increase prostaglandin production in response to lymphocyte activation products (Gordon *et al.*, 1976). More recently, other workers have shown that macrophage prostaglandin production may also be enhanced by agents that activate the complement system via the alternative pathway (Humes, Bonney, Pelus, Dahlgren, Sadowski, Kuehl & Davies, 1977; Glatt, Wagner & Brune, 1977; Wahl, Olsen, Sandberg & Mergenhagen, 1977).

The striking similarities in prostaglandin production by cultured guinea-pig macrophages and human rheumatoid synovia, and their qualitative and quantitative responses to pharmacological agents, support the recent suggestion that the macrophage is the cell type responsible for prostaglandin production by synovial fragments (Dayer, Krane, Russell & Robinson, 1976; Bray & Gordon, 1976). Therefore, it seems likely that guinea-pig macrophages may provide a relevant, simple and inexpensive *in vitro* system for the evaluation of analgesic-anti-inflammatory drugs on the prostaglandin component of the inflammatory response. Furthermore, the potential exists for simultaneous investigation of drug effects on two other important inflammatory mediators released by these mixed cell populations viz. lymphocyte activation products (lymphokines) and macrophage lysosomal enzymes.

This study was supported by a grant to Dr J. Morley from the Arthritis and Rheumatism Council.

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(Received January 16, 1978.
Revised March 10, 1978.)