Cyclic AMP and prostaglandin E in perfusates of rat hind paws during the development of adjuvant arthritis

M. J. PARNHAM, I. L. BONTA, AND M. J. P. ADOLFS

From the Department of Pharmacology, Medical Faculty, Erasmus University Rotterdam, PO Box 1738, Rotterdam, The Netherlands

SUMMARY The contralateral uninjected hind paws of rats which had been injected with Freund's complete (FCA) or incomplete (FIA) adjuvant 10, 14, 18, or 22 days previously were perfused under urethane anaesthesia using a stainless steel coaxial catheter. In one series of experiments cyclic AMP (cAMP) levels were determined after a 2-hour perfusion. cAMP levels were also determined in rats treated on days 16-22 with either prostaglandin E_1 (PGE_1) (500 μ g/kg per day subcutaneously) or saline. When compared with control rats injected with FIA, after 22 days, cAMP levels in FCA-injected rats fell between days 10 and 18 and then rose between days 18 and 22. After PGE_1 treatment, paw volume on day 22 increased in rats injected with FCA, but cAMP levels were not significantly altered when compared with day 22 FCA-injected controls treated with saline.

In a second series of experiments, using a slightly modified perfusion method, PG levels in extractions of 30-minute perfusates were determined after chromatography and bioassay. Protein levels and paw volume were also measured. No PGF was detectable in any perfusate. Changes in PGE levels in FCA-injected animals paralleled changes in paw volume, increasing from day 14 and reaching a maximum on day 22. Both parameters remained unchanged in FIA-injected rats. Protein levels in perfusates from FCA-injected animals were significantly greater than FIA-injected controls only on day 22.

We suggest that the early changes in cAMP reflect the infiltration of activated leucocytes into the inflamed joint and that the increase in PGE is attributable to lysosomal enzyme activity. Later increases in cAMP are attributable both to the increasing PGE levels and to other changes in cellular activation. We suggest that high levels of PGE contribute to tissue damage which is reflected in raised protein levels.

Prostaglandins (PGs), particularly those of the E series, are released locally during various inflammatory responses, both in man and in laboratory animals, and exert both pro- and anti-inflammatory actions (see Zurier, 1974; Vane, 1976). PGs have thus been ascribed a dual role as modulators of inflammation (Willoughby and Dieppe, 1976).

The anti-inflammatory property of pharmacological doses of PGE was first demonstrated in adjuvant arthritis in rats (Aspinall and Cammarata, 1969; Zurier and Quagliata, 1971). The inhibitory effects of PGE on leucocyte function *in vitro* are related to increases in intracellular cyclic AMP

(cAMP) (Bourne *et al.*, 1974), and theophylline, an inhibitor of cAMP phosphodiesterase, was found *in vivo* to potentiate the PGE_1 -induced suppression of the arthritic syndrome (Bonta *et al.*, 1977b, 1978).

Adjuvant arthritis in the rat is similar in several respects to rheumatoid arthritis (RA) (Pearson, 1968) and we have suggested that the interactions between PGE and cAMP in the two diseases may also be similar (Parnham *et al.*, 1977). However, although PGE production has been shown to increase in synovial joints of RA patients, with associated changes in cAMP (Higgs *et al.*, 1974; Robinson and Levine, 1974; Robinson *et al.*, 1975a), no such changes have been reported in the inflamed joints of rats with adjuvant arthritis. We describe here the changes which occur in the levels of cAMP and PGE

in perfusates of arthritic rat joints obtained at different stages during the development of chronic inflammation. Preliminary reports of some of these data have been published (Adolfs *et al.*, 1977; Parnham *et al.*, 1977).

Materials and methods

ANIMALS AND TREATMENT

Male rats of an inbred Lewis strain (Central Animal Breeding Institute, Hanover, W. Germany), weighing 180–250 g, were maintained on standard laboratory diet and drinking water *ad libitum*. Arthritis was induced by injecting 0.1 ml Freund's complete adjuvant (FCA) (5 mg/ml *Mycobacterium butyricum* in liquid paraffin; Difco, Detroit) into the left hind paw. Control animals received 0.1 ml incomplete adjuvant (FIA) (liquid paraffin only). FCA injection was accompanied by an acute inflammatory response, and subsequent swelling in the uninjected right hind paw about 10 days after FCA injection was taken to indicate the chronic arthritic response.

In the first series of experiments, for cAMP determination, arthritic animals were anaesthetised with urethane (1.65 g/kg) 10, 14, 18, or 22 days after FCA injection. Because of the small number of

animals available, control animals were anaesthetised only on day 22 after FIA injection. A group of FCA-injected rats were also treated with either saline (1 ml/kg per day subcutaneously, 5 animals) or PGE₁ (Unilever, Vlaardingen) (0.5 mg/kg per day subcutaneously, 5 animals) on days 16–22 inclusive, before being anaesthetised on day 22. Uninjected hind paw volumes were also determined in this group on day 22, using a differential volume meter (Ugo Basile, Milan). All animals were weighed before anaesthesia.

In the second series of experiments, for PG determination, both FCA- and FIA-injected animals were anaesthetised with urethane 10, 14, 18, or 22 days after injection. Uninjected hind paw volumes and body weights of all rats were measured before adjuvant injection and before anaesthesia.

PERFUSION

Two modifications of the technique of Rocha e Silva and Antonio (1960) were used, the method of determining PG levels being an improvement of the technique initially used in determining cAMP levels. Both modifications are shown in Fig. 1 A and B.

For measurement of cAMP levels in paw perfusates, an incision was made, after anaesthesia, in the skin covering the thigh of the uninjected hind leg of

Fig. 1 Stainless-steel, coaxial catheters used to perfuse uninjected hind paws of rats. (A) Method used in determining cAMP levels. The outer efferent tube (inner diameter 2 mm) was straight, and the inner afferent tube (inner diameter 0.8 mm) was bent and exited through the side of the outer tube. The needle-shaped tip covered the tibiotarsal joint. (B) Method used in determining PGE and protein levels. The inner tube was straightened to receive a thin wire before the perfusion. The needle-shaped tip was pushed into the joint space between tibia and calcaneus.

the rat and a stainless steel coaxial catheter inserted (Fig. 1A). The catheter was pushed subdermally down the outer side of the leg until the tip covered the tibiotarsal joint. The catheter was then secured by a thread which also closed the incision. Sterile 6% dextran/saline (Dextran Poviet 70, Poviet Products, Amsterdam) was pumped for 2 hours, at 0.2 ml/min (using a Braun-Melsungen Unita II pump), down the small inner tube and the perfusate was collected on ice from the larger outer tube. To test for possible interference of the dextran, 0.1 ml of the 6% dextran/saline solution was injected into the hind paws of 4 unanaesthetised normal rats and 4 normal rats anaesthetised with urethane (1.65 g/kg subcutaneously). Urethane inhibited paw swelling by 83% after 1 hour (maximum response) and this inhibition was maintained after 2 hours confirming the results of Bertè and Crema (1962). Thus, perfusion with dextran under urethane anaesthesia was unlikely to have interfered noticeably with the arthritic response.

Initial assays of PGs from perfusates gave very low values. In an attempt to overcome the apparently inadequate perfusion of the synovial space, another coaxial catheter was used with a straight innertube, extending 0.5 mm beyond the edge of the outer tube, and with different tube outlets (Fig. 1B). A fine wire could thus be inserted down the inner tube before perfusion so that any tissue preventing access of the perfusate to the synovial space might be removed. Furthermore, the catheter was pushed subdermally down the back (instead of the side) of the uninjected leg so that the tip lay in the space between the tibia and the calcaneus. As in the earlier perfusion experiments, the catheter was secured round the incision with thread. Although the perfusion rate was maintained at 0.2 ml/min, the perfusion time was limited to 30 minutes, and since the perfusate was collected over ice, breakdown of the unstable PGs was reduced. Initial experiments, using this modified technique, were carried out with 6% dextran/saline as the perfusion fluid. However, recovery of labelled PGs, after extraction from the perfusate, was low, presumably due to binding of PGs to the dextran. Thus our results on PG levels in the perfusates were obtained using Krebs solution (composition g/l: NaCl 6.9, KCl 0.35, CaCl₂ 0.27, KH₂PO₄ 0.16, MgSO₄·7H₂O 0.29, NaHCO₃ 2.1, glucose 1.0) as the perfusion fluid.

CYCLIC AMP ASSAY

Four ml of the 2-hour perfusate were shaken with 10 ml chloroform:methanol (2:1), to remove total lipids. The dextran was then emulsified by the addition of 2 × 10 ml absolute ethanol and the mixture centrifuged at 2500 rpm for 5 minutes. The

supernatant was evaporated to dryness under vacuum on a rotary evaporator (Büchi, Flawil, Switzerland) and resuspended in 350 µl distilled water. Each perfusate extract was then assayed for cAMP, in duplicate, by the protein-binding method of Gilman (1970), using a commercially-available kit (Boehringer-Mannheim). The mean recovery of ³H-cAMP (7500 cpm), added to the perfusate, through the extraction procedure was 82.3%. The mean difference between duplicate samples was 29.7%. cAMP binding was undetectable after incubation of the samples with phosphodiesterase (Sigma, London) for 1 hour at 37°C.

Results were expressed as pmol cAMP/2 hours, i.e. the total amount of cAMP present in the perfusate after a 2-hour perfusion (approx 25 ml). All values were corrected for recovery.

PG EXTRACTION, SEPARATION, AND BIOASSAY

1 ml of the 30-minute perfusate was removed for protein determination. The remainder was acidified to pH 3 with 1 M HCl and the PGs extracted with 3 × 1 volume ethyl acetate. The acetate layers were removed with a Pasteur pipette and evaporated to dryness on a rotary evaporator. After storage overnight at -21°C, the dried extracts were resuspended in 1 ml redistilled chloroform and subjected to chromatography on silicic acid minicolumns using increasing concentrations of methanol in chloroform as successive eluants (Parnham and Sneddon, 1975). The fractions containing the PGEs and PGFs were each resuspended in 0.5 ml saline for bioassay. Mean recoveries of ³H-PGE₂ (17 000 cpm, Radiochemical Centre, Amersham) and ³H-PGF_{2α} (15 000 cpm, Radiochemical Centre) through extraction and chromatography were 65.8% and 66.3% respectively.

PGE column fractions were assayed against authentic PGE₂ (Upjohn, Kalamazoo) on the isolated rat stomach strip (Vane, 1957) and PGF fractions were assayed against authentic PGF_{2α} (Upjohn) on the isolated rat colon (Regoli and Vane, 1964). All assays were carried out by bracketing. The isolated tissues were suspended in an organ bath containing liquid paraffin at 37°C (Ferreira and De Souza Costa, 1976) and superfused at 0.2 ml/min with Krebs solution containing a mixture of antagonists (Gilmore *et al.*, 1968) plus indomethacin (Merck, Sharpe and Dohme) (2 µg/ml). Samples and standards were injected directly over the tissues in constant volumes of 50 or 100 µl (Bult *et al.*, 1977). Results were expressed as total PGE (ng) present in the 30-minute perfusate (approx 6 ml).

PROTEIN DETERMINATION

One ml samples of perfusates used for PG assay were

assayed for protein by the method of Lowry *et al.* (1951) using egg albumin as a standard. Results were expressed as total protein (mg) present in the 30-minute perfusate (approx 6 ml).

STATISTICAL ANALYSIS

The significance of differences between FIA- and FCA-injected animals was determined by the one-tailed Mann-Whitney U test. For cAMP levels, the significance of differences from the FIA-injected control group, taken on day 22, was determined for all FCA-injected groups, irrespective of the length of time from FCA injection. With the remaining data, the significance of differences was determined between FIA-injected and FCA-injected groups perfused on the same day after injection.

Results

CYCLIC AMP LEVELS

The mean cAMP levels in perfusates from paws taken at different stages during the development of the chronic arthritic response are shown in Fig. 2. cAMP levels in perfusates from day 10 FCA-injected rats were not significantly different from day 22 FIA-injected controls, although by day 14 cAMP levels were markedly reduced when compared with controls. This persisted in day 18 perfusates, but by day 22, although still significantly reduced, cAMP levels had increased towards control levels once again.

Fig. 2 Mean cyclic AMP levels in 2-hour perfusates of uninjected hind paws of adjuvant-treated rats. ● Animals treated with Freund's incomplete adjuvant; ○ animals treated with Freund's complete adjuvant. All points are the mean values from 5 rats. Significance of differences (FIA vs FCA): * $P < 0.05$, ** $P < 0.01$.

CYCLIC AMP LEVELS AFTER PGE₁ TREATMENT
After treatment with PGE₁ (500 µg/kg per day subcutaneously) on days 16–22, the mean uninjected hind paw volume of FCA-injected rats, measured on day 22 (2.38 ± 0.05 ml, 5 rats), was significantly ($P < 0.01$) larger than the mean volume of the same paws of saline-treated, FCA-injected rats ($2.13 \pm$

Fig. 3 Mean PGE levels and protein levels in perfusates and mean changes in volume of uninjected hind paws of adjuvant-injected rats. (a) Mean PGE levels; (b) mean changes in paw volume. Values were calculated by subtracting paw volume/100 g body weight on the day of adjuvant injection from paw volume/100 g weight on the day of perfusion. (c) Mean protein levels. ● FIA-injected animals, ○ FCA-injected animals. The numbers indicate the number of perfusions per mean value. Significance of differences (FIA vs FCA): * $P < 0.05$, *** $P < 0.001$.

0.02 ml, 5 rats). However, mean cAMP levels in 2-hour perfusates from these same paws were not significantly different ($P > 0.05$), though the mean levels in perfusates of PGE₁-treated paws (902.2 ± 311.8 pmol/2 h) were somewhat higher than those in perfusates of saline-treated paws (593.2 ± 168.2 pmol/2 h).

PG AND PROTEIN LEVELS

The changes in mean PGE and protein levels and in mean paw volumes during the development of the arthritic response are shown in Fig. 3 *a, b, c*. PGF levels in perfusates from both FIA- and FCA-injected animals were below the detection limit of our bioassay (500 pg PGF_{2α}/100 μl, i.e. 2.5 ng PGF/30-min perfusate). The detection limit for the PGE bioassay was 100 pg PGE₂/100 μl, i.e. 500 pg PGE/30-min perfusate. Mean PGE levels in perfusates from FCA-injected animals, taken 10 and 14 days after adjuvant injection, were not significantly different from those in perfusates from FIA-injected controls (Fig. 3*a*). However, PGE levels in FCA-injected rats had increased significantly by day 18 and further by day 22.

This time pattern was reflected in the increase in the mean volume of the uninjected hind paws of the FCA-injected animals when compared with FIA-injected controls (Fig. 3*b*), confirming earlier paw volume measurements in similarly-treated Lewis rats (Bonta *et al.*, 1977*a, b*, 1978). However, the increase seen on day 18 was not significant, as one of the 4 FCA-treated rats perfused on this day exhibited no marked arthritic symptoms.

Protein levels in perfusates from FCA-injected rats also increased between days 14 and 22 (Fig. 3*c*), achieving a significant difference from FIA-injected controls on day 22 only. Similar PGE and protein levels were also obtained in initial experiments using 6% dextran/saline as the perfusing fluid.

Discussion

Our results indicate that while PGE and protein release from chronic arthritic rat paws increases with paw swelling, cAMP release falls initially and then rises again as the paw swelling reaches a peak. Random sampling of perfusates, obtained for both cAMP and PG assay, showed that the fluid was cell-free at all stages of the arthritic response. It is possible that cells adhered to both the tissue and the inside of the steel catheter used for the perfusions. cAMP release into the culture medium has been reported with several isolated cell types, including rat macrophages (Gemsä *et al.*, 1975) and human fibroblasts (Franklin and Foster, 1973), and in both cases this release was stimulated by PGE. Thus, the

cAMP detected in the perfusates in our study was probably released into the extracellular fluid of cells in and around the tibiotarsal joint.

There are doubts as to which leucocytes are the first to migrate into the joints of uninjected limbs of FCA-injected rats (Jones and Ward, 1963; Burnstein and Waksman, 1964; Perper *et al.*, 1975). However, pharmacological inhibition *in vitro* of the function of all leucocytes detectable in arthritic joints is associated with an increase in intracellular cAMP (Bourne *et al.*, 1974). It is likely then that activation *in vivo* is associated with a decrease in cAMP levels. This decrease, observed between days 10 and 18 in our experiments, probably reflects the infiltration into the joint of activated leucocytes.

Although cAMP levels in perfusates of day 22 arthritic joints were still significantly lower than control perfusates, they were higher than in day 10 perfusates, probably reflecting the changes in intracellular cAMP which occur in different cell types in established chronic joint disease (see Parnham *et al.*, 1977). Our results also show that by day 22 PGE levels in arthritic paws are markedly higher, an increase which is also seen in the synovia of RA patients (Higgs *et al.*, 1974; Robinson and Levine, 1974). Robinson *et al.* (1975*a*) have shown that the increased production of PGE by cultured RA synovial cells is associated with increased cAMP production. Thus, a large component of the increase in cAMP towards control levels, seen in our day 22 arthritic rat paw perfusates, may have been due to stimulation of synovial cell adenylate cyclase by the increased amounts of PGE.

The complex nature of the changes in cAMP occurring in arthritic rat joints by day 22 is indicated by the results of the short series of experiments in which rats were treated with PGE₁ on days 16–22. Surprisingly, at the relatively low dose used, PGE₁ increased paw volume as measured on day 22. This is in contrast to previous results (Aspinall and Cammarata, 1969; Zurier and Quagliata, 1971; Bonta *et al.*, 1977*b*, 1978). However, there is some indication that low doses of PGE₁ increase lysosomal enzyme release from granulocytes *in vitro* (Weissmann, 1972) and this may have accounted for the increase in paw volume in our study. cAMP levels in perfusates from the PGE₁-treated animals showed large variations, suggesting that the PGE₁ may have affected several independent cAMP-mediated processes in the arthritic joints, such as fibroblast, leucocyte, and osteoclast activity (Parnham *et al.*, 1977). Consequently, cAMP levels in PGE₁-treated animals were not significantly different from saline-treated controls.

The gradual increase in PGE levels in arthritic rat paw perfusates, occurring between days 14 and 22, closely paralleled the increase in paw volume.

Since relatively low doses of PGE potentiate the oedema-producing activity of other mediators, such as histamine and bradykinin (see Vane, 1976), this sensitising activity of PGE is probably involved in the production of the early inflammatory symptoms in adjuvant arthritis. It has been suggested that phospholipase A, released by lysosomes, is the main trigger to the release of PGs in inflammation (Kunze and Vogt, 1971; Anderson *et al.*, 1971). Certainly, lysosomal enzyme activity in uninjected hind paws of FCA-injected animals starts to increase about 8 to 9 days after FCA injection (Anderson 1970; Ignarro and Slywka, 1972). Since we were unable to determine the efficiency of our perfusion in extracting PGs from the joints, the true increase in PGE production in the inflamed paws may have begun earlier than we were able to detect.

Thus, it is highly probable that release of lysosomal phospholipase A was responsible for the increased PGE release. PGF_{2x} may also be involved in inflammatory processes (see Willoughby and Dieppe, 1976), but we detected no PGF in our rat paw perfusates. Recently, it has been suggested that PGG₂, an unstable intermediate in PG biosynthesis, may be more important than the classical PGs in inflammatory processes (Kuehl *et al.*, 1977). Thus, although we measured PGE-like activity in perfusates of arthritic rat paws, with regard to pro-inflammatory actions this may simply be a more stable metabolite of the more active PGG₂.

The increase in PGE levels was followed by a significant increase in protein release into paw perfusates between 18 and 22 days after FCA injection. This was taken to indicate the necrotic changes occurring in the joint at this time. Since PGE released by rheumatoid synovial tissue has been shown to induce bone resorption *in vitro* (Robinson *et al.*, 1975b) and intra-articular PGE₁ causes marked cartilage damage in the rabbit *in vivo* (Teitz and Chrisman, 1975), it is likely that the increased PGE released by adjuvant arthritic paws also contributes to bone and cartilage necrosis.

This study was supported by the Nederlandse Vereniging tot Rheumatiekbestrijding. We thank Dr J. E. Pike (Upjohn Co., Kalamazoo) and Dr D. H. Nugteren (Unilever Research, Vlaardingen) for kindly providing us with prostaglandins.

References

- Adolfs, M. J. P., Bonta, I. L., and Parnham, M. J. (1977). Cyclic AMP production during adjuvant-induced arthritis in rats. *British Journal of Pharmacology*, **59**, 439P-440P.
- Anderson, A. J. (1970). Lysosomal enzyme activity in rats with adjuvant-induced arthritis. *Annals of the Rheumatic Diseases*, **29**, 307-313.
- Anderson, A. J., Brocklehurst, W. E., and Willis, A. L. (1971). Evidence for the role of lysosomes in the formation of prostaglandins during carrageenin induced inflammation in the rat. *Pharmacological Research Communications*, **3**, 13-19.
- Aspinall, R. L., and Cammarata, P. S. (1969). Effect of prostaglandin E₂ on adjuvant arthritis. *Nature*, **224**, 1320-1321.
- Bertè, F., and Crema, A. (1962). Sul significato di alcuni metodi di valutazione dell'attività antiedemigena dei farmaci. *Archivio Italiano di Scienze Farmacologiche*, **12**, 170-180.
- Bonta, I. L., Parnham, M. J., Adolfs, M. J. P., and Van Vliet, L. (1977a). Dual function of E-type prostaglandins in models of chronic inflammation. *Perspectives in Inflammation: Future Trends and Developments*, p. 265. Ed. by D. A. Willoughby, J-P. Giroud, and G. P. Velo. MTP Press, Lancaster.
- Bonta, I. L., Parnham, M. J., Van Vliet, L., and Vincent, J. E. (1977b). Mutual enhancement of the effects of prostaglandin E₁ and theophylline on the Freund adjuvant-induced arthritis syndrome of rats. *British Journal of Pharmacology*, **59**, 438P-439P.
- Bonta, I. L., Parnham, M. J., and Van Vliet, L. (1978). Combination of theophylline and prostaglandin E₁ as inhibitors of the adjuvant-induced arthritis syndrome of rats. *Annals of the Rheumatic Disease*, **37**, 212-217.
- Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y., and Shearer, G. M. (1974). Modulation of inflammation and immunity by cyclic AMP. *Science*, **184**, 19-28.
- Bult, H., Parnham, M. J., and Bonta, I. L. (1977). Bioassay by cascade superfusion using a highly sensitive laminar flow technique. *Journal of Pharmacy and Pharmacology*, **29**, 369-370.
- Burnstein, N. A., and Waksman, B. H. (1964). The pathogenesis of adjuvant disease in the rat. I. A histologic study of early lesions in the joints and skin. *Yale Journal of Biology and Medicine*, **37**, 177-194.
- Ferreira, S. H., and De Souza Costa, F. (1976). A laminar flow superfusion technique with much increased sensitivity for the detection of smooth muscle-stimulating substances. *European Journal of Pharmacology*, **39**, 379-381.
- Franklin, T. J., and Foster, S. J. (1973). Leakage of cyclic AMP from human diploid fibroblasts in tissue culture. *Nature New Biology*, **246**, 119-120.
- Gemsa, D., Steggemann, L., Menzel, J., and Till, G. (1975). Release of cyclic AMP from macrophages by stimulation with prostaglandins. *Journal of Immunology*, **114**, 1422-1424.
- Gilman, A. G. (1970). A protein binding assay for adenosine 3':5'-cyclic monophosphate. *Proceedings of the National Academy of Sciences of the USA*, **67**, 305-312.
- Gilmore, N. J., Vane, J. R., and Wyllie, J. H. (1968). Prostaglandins released by the spleen. *Nature*, **218**, 1135-1140.
- Higgs, G. A., Vane, J. R., Hart, F. D., and Wojtulewski, J. A. (1974). Effects of anti-inflammatory drugs on prostaglandins in rheumatoid arthritis. *Prostaglandin Synthetase Inhibitors—Their Effects on Physiological Functions and Pathological States*, p. 165. Ed. by H. J. Robinson and J. R. Vane. Raven Press, New York.
- Ignarro, L. J., and Slywka, J. (1972). Changes in liver lysosome fragility, erythrocyte membrane stability, and local and systemic lysosomal enzyme levels in adjuvant-induced polyarthritis. *Biochemical Pharmacology*, **21**, 875-886.
- Jones, R. S., and Ward, J. R. (1963). Studies on adjuvant-induced polyarthritis in rats. II. Histogenesis of joint and visceral lesions. *Arthritis and Rheumatism*, **6**, 23-35.

- Kuehl, F. A., Humes, J. L., Egan, R. W., Ham, E. A., Beveridge, G. C., and Van Arman, C. G. (1977). Role of prostaglandin endoperoxide PGG_2 in inflammatory processes. *Nature*, **265**, 170–173.
- Kunze, H., and Vogt, W. (1971). Significance of phospholipase A for prostaglandin formation. *Annals of the New York Academy of Sciences*, **180**, 123–125.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.
- Parnham, M. J., and Sneddon, J. M. (1975). The influence of metyrapone on the synthesis and release of prostaglandins from the pregnant rat uterus *in vitro*. *British Journal of Pharmacology*, **55**, 535–539.
- Parnham, M. J. Bonta, I. L., and Adolfs, M. J. P. (1977). Interactions between prostaglandin E and cyclic nucleotides in chronic inflammatory diseases. *Perspectives in Inflammation; Future Trends and Developments*, p. 279. Ed. by D. A. Willoughby, J-P. Giroud, and G. P. Velo. MTP Press, Lancaster.
- Pearson, C. M. (1964). Experimental models in rheumatoid disease. *Arthritis and Rheumatism*, **7**, 80–86.
- Perper, R. J., Sanda, M., Stecher, V. J., and Oronsky, A. L. (1975). Physiologic and pharmacologic alterations of rat leucocyte chemotaxis (Cx) *in vivo*. *Annals of the New York Academy of Sciences*, **256**, 190–209.
- Regoli, D., and Vane, J. R. (1964). A sensitive method for the assay of angiotensin. *British Journal of Pharmacology and Chemotherapy*, **23**, 351–359.
- Robinson, D. R., and Levine, L. (1974). Prostaglandin concentrations in synovial fluid in rheumatic diseases: action of indomethacin and aspirin. *Prostaglandin Synthetase Inhibitors—Their Effects on Physiological Functions and Pathological States*, p. 223. Ed. by H. S. Robinson and J. R. Vane. Raven Press, New York.
- Robinson, D. R., McGuire, M. B., and Levine, L. (1975). Prostaglandins in the rheumatic diseases. *Annals of the New York Academy of Sciences*, **256**, 318–329.
- Robinson, D. R., Tashjian, A. H., and Levine, L. (1975). Prostaglandin-stimulated bone resorption by rheumatoid synovia. *Journal of Clinical Investigation*, **56**, 1181–1188.
- Rocha e Silva, M., and Antonio, A. (1960). Release of bradykinin and the mechanism of production of a 'thermic edema (46.5°C)' in the rat's paw. *Medicina Experimentalis*, **3**, 371–382.
- Teitz, C. C., and Chrisman, O. D. (1975). The effect of salicylate and chloroquine on prostaglandin-induced articular damage in the rabbit knee. *Clinical Orthopaedics and Related Research*, **108**, 264–275.
- Vane, J. R. (1957). A sensitive method for assay of 5-hydroxytryptamine. *British Journal of Pharmacology and Chemotherapy*, **12**, 344–349.
- Vane, J. R. (1976). The mode of action of aspirin and similar compounds. *Journal of Allergy and Clinical Immunology*, **58**, 691–712.
- Weissmann, G. (1972). Discussion. *Prostaglandins in Cellular Biology*, p. 172. Ed. by P. W. Ramwell and B. B. Pharriss. Plenum Press, New York.
- Willoughby, D. A. and Dieppe, P. (1976). Prostaglandins in the inflammatory response—pro or anti? *The Role of Prostaglandins in Inflammation*, p. 14. Ed. by G. P. Lewis. Huber, Bern.
- Zurier, R. B. (1974). Prostaglandins. *Mediators of Inflammation*, p. 163. Ed. by G. Weissmann. Plenum Press, New York.
- Zurier, R. B., and Quagliata, F. (1971). Effect of prostaglandin E_1 on adjuvant arthritis. *Nature*, **234**, 304–305.