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COMMENTARY

PROSTAGLANDINS AND CHRONIC INFLAMMATION

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It often happens that features of both acute and chronic inflammation appear in the same lesion.

F. A. Ward

The title of this commentary is obviously rather generalised and needs clarification in the light of our use of the terms *prostaglandins* and *chronic inflammation*. The name prostaglandin (PG) was originally limited to a group of fairly stable cyclic derivatives of prostanoic acid, first detected in seminal fluid. More recently, other short-lived intermediates and products in the complex PG biosynthetic pathway have been described, which also exhibit marked biological activity in relation to the inflammatory process [1]. Thus, any consideration of "prostaglandins" and chronic inflammation must include all implicated bioconversion products of arachidonic acid (or related essential fatty acids).

By custom, chronic inflammation is differentiated from acute inflammation on the basis of its time course. Such a division is inherently contradictory, since the features of both conditions frequently overlap (as indicated in the motto). Because of this overlap, the demarcation point between acute and chronic inflammation is rarely sharply defined. More objective criteria for the two types of inflammation are exudation as the hall-mark for the acute condition and tissue proliferation for chronic inflammation [2]. Such static criteria, however, do not take into consideration the dynamic characteristic of inflammation, which may, simultaneously, involve damage and repair processes. Because of this, inflammation has been referred to as a homeostatic process-loop system [3]. Bearing in mind this homeostatic function of inflammation ("chronic" inflammation implying some breakdown in the homeostatic loop), we shall consider interactions of PGs with individual components of this loop system, each component ultimately affecting one or more of the others. Rheumatoid arthritis will be considered as a clinical model, *par excellence*, since its chronicity has been proposed to result from a breakdown in the homeostatic function of PGs [4].

Prostaglandins: mediators or modulators? The failure to adequately differentiate between acute and chronic inflammation has, unfortunately, resulted in the application of far-reaching conclusions, drawn from studies on acute inflammatory models, to clinical conditions which are dominated by characteristics of chronic inflammation. The resulting ambiguities in the postulated roles of inflammatory mediators have also extended to PGs. Prostaglandins, particularly those of the E-type, have been shown to reproduce several signs of acute inflammation, including vasodilatation, increased permeability, local oedema and pain [5]. Using lower, more physiological doses, PGs have been shown to produce sensitization to the inflammatory properties of other mediators, such as histamine, 5HT and bradykinin [5, 6]. It should be noted that these mediators have been implicated in the very early phase of inflammation and PGs have been recovered in large amounts from inflammatory exudates which are a prominent feature of acute rather than chronic inflammation [2].

The discovery that several non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the formation of endogenous PGs from their precursor, arachidonic acid, provided a plausible explanation for the anti-inflammatory action of acidic NSAIDs and suggested an important role for PGs as mediators of inflammation [7]. This concept has since been strengthened by a large amount of *in vitro* and *in vivo* data [5]. Clinical data, from arthritic patients, also indicated that aspirin inhibits PGE production in the synovia [8, 9]. However, the presence of large amounts of PGs in arthritic synovia does not necessarily implicate them as pro-inflammatory mediators. Furthermore, while relieving several signs and symptoms, NSAIDs do not reverse rheumatoid arthritis. Recent data have indicated that all the anti-inflammatory activity of, at least, the salicylate group of NSAIDs cannot be entirely explained on the basis of inhibition of endogenous PG production [10, 11]. Indeed, a new compound, MK 447, though being anti-inflammatory in acute animal models, actually *promotes* the production of stable PGs [12]. MK-447 appears to be a scavenger of superoxide anion radical, which is produced during the conversion of the PG intermediate PGG_2 to PGH_2. These findings are supported by *in vivo* studies with antioxidants [13].

The earlier suggestions of an exclusively pro-

Abbreviations—PG, prostaglandin; 5HT, 5-hydroxytryptamine; NSAIDs, non-steroidal anti-inflammatory drugs; EFAD, essential fatty acid deficient; RA, rheumatoid arthritis; TxA_2, TxB_2, thromboxane A_2, B_2; RIA, radioimmunoassay; PMNLs, polymorphonuclear leucocytes; MAA, (rabbit) monoarticular arthritis; cAMP, adenosine-3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate; HETE, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid; PGI_2, prostacyclin; LK, lymphokine; MIF, macrophage migration inhibiting factor; GAG, glycosaminoglycan; O_2^-, superoxide anion; MK-477, 2 aminomethyl-4-t-butyl-6-iodophenol; L8027, 3-(2-isopropyl indolyl) 3-pyridyl ketone.

inflammatory role for PGE must now be tempered by the growing realisation that both pro- and anti-inflammatory actions are exerted by products of arachidonic acid metabolism. Anti-inflammatory effects of pharmacological doses of E-type PGs themselves have been reported [14–21]. Moreover, our results with essential fatty acid deficient (EFAD) rats, have shown that, when endogenous PG production is markedly reduced, the exudative component of inflammation is decreased (under both acute and chronic conditions), whereas the proliferative (chronic) component is enhanced [11, 22–24]. These data (a) invalidate the view that PGs are purely inflammagenic and (b) provide circumstantial evidence for a pro-inflammatory role of PGs in the acute phase and an anti-inflammatory, modulating role in the chronic phase of inflammation [25, 26].

Scope of the commentary. In this commentary we shall attempt to reconcile the apparently conflicting views on the pro- or anti-inflammatory role of PGs during chronic inflammation. The experimental animal data are drawn mainly from studies on models of connective tissue proliferation and of arthritic conditions. Aspects of clinical relevance will be centered around rheumatoid arthritis (RA), for the following reasons. (1) Since the clinical course of RA is easy to investigate [27], the majority of the work carried out on PGs and chronic inflammation in man has involved studies directly or indirectly related to RA. (2) Current anti-inflammatory drugs are not only frequently used on RA patients, but several of these drugs have been developed specifically for this purpose. (3) Joint inflammation, particularly RA, seems to involve most mechanisms which have been proposed on the basis of experimental studies, including immune mechanisms [27]. The proliferative lesion in RA is of particular interest, since it involves both connective and other joint tissues [28]. Obviously, conclusions based upon RA may apply to other clinical chronic inflammatory conditions, depending on the mechanisms involved. In this context the possible interactions of PGs with immune events are only considered briefly since this has been the subject of another recent review [29].

Presence of prostaglandins in chronic inflammation

Granulomatous tissue models. These studies have been conducted exclusively on rats. The commonest model, originally called the granuloma-pouch and in many recent papers re-named an air-bleb, involves the administration of an irritant into a subcutaneous pouch formed by air injection [30, 31]. Subsequently, a wall of proliferative tissue develops surrounding a pocket of exudate. Thus, the model displays the two pathological hall-marks of inflammation. The exudate can be collected at different time points after the irritant injection, varying from a few hr to 8 days, thus yielding qualitatively and quantitatively different cells and mediators (including PGs). Differences in the inducing irritant also explain wide variations and form a possible source of contradictions. With respect to the PG content, it is notable that the activity of 15α-hydroxy-dehydrogenase, the enzyme metabolizing stable PGs, in the granuloma exudate is very low [32]. Granulomata may also be produced by subcutaneous implantation of polyether sponge pieces, mostly soaked beforehand in carrageenin. After removal of the sponges (again, at widely varying times!) the fluid can be collected either by squeezing or by centrifuging [33].

PGE_2 and $PGF_{2\alpha}$ have been reported in 8–72 hr exudates of carrageenin-pouch granulomata, but in 24 hr and 8 day sponge granuloma exudates, only PGE-like material was detectable by bioassay [24, 32, 33]. One of the major products transformed from arachidonate *in vitro* by 8 day granulation tissue was identified as thromboxane B_2, a stable metabolite of thromboxane A_2 (TxA_2) with chemotactic activity [34, 35]. A new PG (9α, 11α, 15α-trihydroxy-6-oxo-prostenoic acid) was also detected, with, as yet, unknown biological activity [36]. The identification of these stable PGs may indicate the release of the more labile intermediate, PGG_2, which has also been implicated in the inflammatory response [12].

Quantitatively, extremely large variations have been observed in the PG contents of granulomata. These differences may partially be explained on the basis of the determination procedures used, either radioimmunoassay (RIA) [32, 37], or bioassay [24, 33], the latter giving much higher values.

It is regrettable that the time-dependency of the PG content has, so far, attracted little attention. In the kaolin-induced granuloma pouch PGE-like activity reaches a maximum at 6 hr, high levels being maintained for up to 96 hr [24, 38]. With carrageenin as the irritant, two PGE maxima have been found at 7–8 hr [33, 37, 39]. The source(s) of the PGs is unclear. Suggested sources include phagocytozing polymorphonuclear leucotytes (PMNLs) and skeletal muscle surrounding the pouch [40, 41]. In the latter case the PGs would, thus, appear as artefacts, since skeletal muscle cells are not involved in inflammation. PMNLs may not be important sources, since PG content does not seem to be related to leucocyte count, at least not in the early carrageenan granuloma pouch [39, 42]. Macrophages rather than PMNLs are the predominant invasive cells during the more chronic phase and macrophages release PGs in response to inflammatory stimuli [79, 159]. However, a possible relation between macrophage accumulation and PG concentration in the same granuloma has not been investigated.

In the kaolin-induced granuloma pouch, a positive correlation appears to exist between PG levels and exudate volume, but when carrageenin was used as the irritant, the two parameters were unrelated [24, 37]. Paradoxically, with sponge-induced granulomata, in PG-precursor-depleted rats, an inverse correlation was observed between PG levels and granuloma formation [24], a finding which will be discussed later.

Monoarthritis of rabbits. Monoarticular arthritis (MAA), produced by intra-articular injection of antigen into sensitized rabbits, is a chronic model closely resembling RA [43]. Levels of E-type PGs in the afflicted joint synovium reach a peak after 19 hr, declining to low levels again during the chronic

phase (7 and 46 days) [44]. Indomethacin, while inhibiting the early increase in PG levels only produced a moderate reduction in joint swelling. Thus, PGEs may be involved in the acute inflammatory stage of this model, but they do not contribute to chronicity or to joint histopathology.

In a surgically-induced chronic osteoarthritis model in rabbits, the concentration of PGE (measured after conversion to PGB) was lower in the synovial fluid of the operated knee than in that of the unoperated knee; but when taken together with the PG content of cartilage, the total amount of PG was equal in the normal and afflicted joints [45]. The conclusion that chronic, non-inflammatory degenerative joint changes are in no way correlated with the presence of prostaglandins is in agreement with clinical results [46].

Adjuvant polyarthritis of rats. This experimental disorder is one of the most extensively used models in investigating mechanisms and pharmacological aspects of RA, to which it shows several similarities [47]. Following injection of complete Freund's adjuvant into one hind limb, acute inflammation appears in the treated paw, followed by chronic inflammation and arthritic lesions in all four limbs. The difficulty in obtaining synovial material from this model has, in the past, discouraged investigators from measuring PG levels. However, using a perfusion technique, we have shown that the levels of PGE in perfusates of the non-injected hind paws of arthritic rats parallel the increase in paw volume, which was taken as an inflammatory parameter, over the initial period (between days 14–22) of the chronic phase [48]. PGF was not detectable (by bioassay). The increased PGE concentration was accompanied by decreasing cAMP levels from days 14 to 18, probably reflecting the infiltration into the joint of activated leucocytes, sources of lysosomal phospholipase A, the enzyme which releases PG-precursor fatty acids from cell membranes. cAMP levels increased between days 18–22, possibly due to stimulation of synovial cell adenylate cyclase by the increased amounts of PGE. In this context, Robinson *et al.* [49] have shown that the increased production of PGE by cultured RA synovial cells is associated with increased cAMP levels. Theoretical similarities of interactions between synovial PGE and cAMP in adjuvant arthritis of rats and clinical RA have been discussed in a recent paper [50].

Acute monoarthritis induced in rats by injection of a cell-free Streptococcus extract into knee joints is also associated with the subsequent release of 5–8 fold higher amounts of PGE by synovial tissue *in vitro* than by non-arthritic synovia [51].

Clinical rheumatoid arthritis. Several studies have been carried out on the PG content of synovial fluid withdrawn from joints of arthritic patients, including those treated with one or more NSAID. PGE_2 appears to predominate, though, in some cases, PGE_1 and $PGF_{2\alpha}$ have been reported as one of the major activities present [9, 46, 53–55]. Attempts to correlate these levels of PGs with leucocyte populations have produced conflicting results [46, 52, 54–56]. Furthermore, the PG content of synovial fluid does not seem to parallel the clinical course of RA [46].

Recently, several reports on PG production by cultured human synovial tissue have appeared. PGE_2 and $PGF_{2\alpha}$ have both been detected in cultured RA synovial tissue, though studies on the crude enzyme have shown that arachidonic acid is preferentially converted to PGE_2 *in vitro* [49, 57–59]. It has been suggested that platelets might be the major source of these PGs [46, 60]. However, cultured fibroblasts from rheumatoid synovium produce much larger amounts of PGE than those from a non-diseased source and it is probable that these cells constitute a major source of synovial PGE [49, 61, 62].

The studies conducted so far shed little light on the possible parallelism between synovial PG production and the clinical course of RA. Moreover, the more labile products of arachidonic acid (PGG_2, TxA_2 etc.) have not yet been detected, though TxB_2 levels in RA synovia are reported to be low [53]. However, the conversion of PGG_2 to PGH_2 is associated with the formation of superoxide anion and such tissue-damaging fragments as malondialdehyde, superoxide and hydroxyl radical formation having been shown to promote the degradation of synovial fluid [6, 12, 63]. As the detection of the short-lived arachidonate products in synovia is an imminent likelihood, it is possible that such intermediates, rather than classical PGs, will turn out to be the inflammatory mediators in rheumatoid conditions. The synthesis of PGE itself by rheumatoid synovial explants was found to be associated with augmented levels of intracellular cAMP, a phenomenon known to prevent the cellular discharge of inflammogenic substances [49, 64]. Elevation of cAMP levels by pharmacological doses of PGE seems to inhibit adjuvant arthritis of rats, and it has recently been shown that intra-articular elevation of cAMP or decrease of cGMP is associated with clinical improvement of RA [14, 16–21, 53]. The presence of PGE in the synovia might, thus, reflect an endogenous, cAMP-mediated, anti-inflammatory regulating mechanism (which may become refractory during arthritis [4]). Therefore, arachidonic acid conversion in inflamed synovia most probably provides substances, like PGG_2, TxA_2 and HETE, which contribute to destructive processes and also substances, like PGI_2 (prostacyclin, also an elevator of cAMP) and PGE, which form part of the regenerating functions.

Prostaglandins and vascular events

The literature on the acute vascular effects of PGs has been recently reviewed [5, 6]. Since PGs have been detected in many types of chronic inflammation, it would seem likely that these vascular effects of PGs still occur during such chronic conditions, even after tissue necrosis has taken place.

Vasodilatation. Apart from the relatively large number of studies carried out on the erythema-producing effects of PGs in the skin, PGs E_1 and E_2 were recently found to be potent dilators of the non-inflamed canine synovial microcirculation [65]. $PGF_{2\alpha}$ was less effective and $PGF_{1\alpha}$ inactive. Despite this well-documented, potent vasodilatory activity of PGEs in normal tissue, very little is known about the involvement of PGs in producing

local vasodilatation (redness) and heat during the chronic inflammatory response. A parallelism has been observed between the increase and subsequent slow decline in joint temperature and the levels of PGE in synovial fluid, following injection of ovalbumen into the joints of sensitized rabbits [44]. However, this temperature change was associated with a systemic pyrogenic mechanism as well as local vasodilatation. Recently, the PG endoperoxide, PGG_2, has been implicated as a mediator of inflammatory vascular changes [12, 66]. PGD_2, also, produces erythema and may play a role in the inflammatory response [67].

Vascular permeability. Although still imperfectly understood, the role of PGs in producing exudation during chronic inflammation has received somewhat more attention than their role in regulating blood flow and local temperature changes. Apart from the many other different sites at which the oedema-producing and potentiating properties of PGE have been demonstrated [68, 69], PGE_1, in sub-threshold doses, has also been shown to potentiate the actions of bradykinin and histamine on the non-inflamed canine synovial microcirculation [70]. As far as inflamed tissues are concerned, many studies have been carried out on the role of PGs in acute inflammatory oedema, but several studies have also been carried out on more chronic conditions. In rabbit MAA, joint swelling during the first week has been correlated with local increases in PGE levels [44]. Also, using different experimental granuloma models, several workers have observed the effects of exogenous PGs or the effects of drug-induced or dietary reduction of endogenous PGs on vascular permeability. These data are summarized in Table 1. It is clear that $PGF_{2\alpha}$ has no effect on vascular permeability under these conditions, whereas PGE_1 seems to be the most potent of the PGs in increasing vascular permeability. Surprisingly PGE_2, which occurs to a far greater extent in mammalian tissues than PGE_1, was only effective in increasing exudation in the carrageenin pouch model, but not in the croton oil pouch. The studies with indomethacin on carrageenin pouch granuloma (Table 1) suggest that vascular permeability changes may become refractory to endogenous PGE as the proliferative phase develops. Essential fatty acid (EFA) deficiency also has less effect on exudation as proliferation progresses [24]. In fact, Chang *et al.* [74] have suggested that PGE is unimportant in increasing exudation during the proliferative phase of inflammation. It is possible that the capillaries become desensitized to the vascular permeability-increasing effects of PGs, though whether this apparent refractoriness is applicable to the exudative component of other chronic inflammatory conditions remains to be seen. Certainly, indomethacin, aspirin and similar PG synthesis inhibitors are effective in suppressing joint swelling in rheumatoid arthritis, but, as far as aspirin is concerned, this effect need not necessarily be due to inhibition of PG synthesis [11]. An alternative explanation is that different components of the PG biosynthetic pathway such as PGD_2 and PGG_2 are partially responsible for increases in vascular permeability at different stages of inflammation [66, 67].

Prostaglandins and cellular events

One of the major characteristics of chronic inflammation is the involvement of several different types of white blood cells, which migrate into the inflamed area. These include the phagocytic neutrophils (or polymorphonuclear leucocytes—PMNLs) and macrophages and lymphocytes, which may be involved in both immunological and non-immunological inflammation [3]. In RA, both B lymphocytes (producing antibodies, leading to immune complex formation) and T lymphocytes (involved in cell-mediated immune mechanisms) have been implicated. The involvement of PGs in the regulation of the immune response has recently been reviewed and only data of direct relevance to chronic joint inflammation will be discussed here [29].

Systemic events. The aetiology of RA is unknown,

Table 1. Local prostaglandins and exudation in experimental granuloma models

Model	Treatment	Period of treatment (days)	Day of measurement	Response	References
Croton oil pouch	PGE_2	1 (with irritant)	10	N.E.†	15
	$PGF_{2\alpha}$			N.E.	
Croton oil or D-α-tocopherol pouch	PGE_1	1–8	8	Increased exudation	71
	PGE_2			N.E.	
	$PGF_{2\alpha}$			N.E.	
Carrageenan pouch	PGE_1	7*	7	Increased exudation	72
	PGE_2			Increased exudation	
	$PGF_{2\alpha}$			N.E.	
Carrageenan pouch	Indomethacin	1–5	5	Decreased exudation	73
	Indomethacin	5–8	9	N.E.	
Kaolin pouch	Essential fatty acid deficient diet	From birth	4	Decreased exudation	24
			8	Decreased exudation	
Carrageenan sponge	Essential fatty acid deficient diet	From birth	8	Decreased exudation	24

* Exudation was measured by the accumulation of ^{125}I-albumin over a 20 min period after the injection of the PG.

† Lack of effect may be due to very short period of treatment.

N.E. = no effect.

but it is generally assumed that an antigenic stimulus is involved. Thus, although the inflammatory signs appear in the joints, the antigen is likely to enter the lymphatic system and activate lymphocytes in the spleen and lymph nodes. Since antigenic stimulation of T cells increases PG production by the spleen, as shown in the mouse, it is probable that PGs regulate lymphocyte activation at these systemic sites [75].

T-cells. PGs, particularly those of the E series, inhibit several parameters of lymphocyte activation *in vitro* and this inhibition appears to be associated with an increase in intracellular cyclic 3',5'-adenosine monophosphate (cAMP) concentrations [29, 76]. The release of the pro-inflammatory lymphokines (LKs)—detectable in rabbit and human arthritic joints [77, 78]—from antigen-activated T lymphocytes, is also inhibited by PGEs [79]. Furthermore, PGE is released by phagocytosing macrophages in sufficient amounts to produce inhibition of LK release [79]. Morley [4] has, thus, suggested that antigen-induced PGE release by macrophages exerts a negative-feedback inhibition of the subsequent release of LKs by T cells. He has further proposed that the chronicity of RA may be due to defective reactivity of LK-secreting T cells to the inhibitory effects of macrophage PGEs. Although defective reactivity has been observed with peripheral blood lymphocytes from multiple sclerosis patients, no such effect has been observed in RA patients, despite apparent differences in the reactivity of peripheral and synovial RA monocytes to PGE_1 [80, 81]. In rats, treatment with either PGE_1 or PGE_2 markedly inhibits the development of adjuvant arthritis, independently of an action on the adrenals, and this effect is enhanced by theophylline, which would potentiate PGE-induced increases in intracellular cAMP [14, 16–21]. Since adjuvant arthritis is thought to involve cell-mediated mechanisms, this may indicate systemic inhibition of T cell activation by PGE, particularly since PGE also inhibited splenomegaly, though a local anti-inflammatory action cannot be ruled out [20, 21]. That removal of glass-adherent cells (macrophages?) from spleen cell suspensions of arthritic rats reverses the reduced responsiveness of splenic lymphocytes to T cell mitogens, may indirectly indicate that macrophage PGEs exert a negative-feedback effect on T cells in adjuvant arthritis [82]. It is unlikely that PGF affects lymphocyte responses *in vivo*, since macrophages produce predominantly PGE *in vitro* and $PGF_{2\alpha}$ does not alter LK production *in vitro*, although T-dependent antigens do increase splenic $PGF_{2\alpha}$ levels in mice *in vivo* [75, 79]. It is possible that other products of the PG biosynthetic pathway may affect lymphocyte function. In this context, it has not been possible to correlate drug-induced effects on lymphocyte activation with inhibition of PG synthesis in any clinical or experimental model of arthritis, since both antirheumatic drugs which *are* inhibitors of PG biosynthesis and those which *are not* inhibit lymphocyte activation *in vitro* [83].

B cells. Until very recently few studies had been carried out on the effects of PGs on B cell function, though these indicated that PGs inhibited B cell activity [29]. There is some *in vitro* evidence suggesting that B cells from mice spleens may exert a cAMP-mediated negative-feedback effect on their own activation through the release of PGE [84]. It remains to be seen whether this effect of PGE is involved in modulating antibody and subsequent immune complex formation during RA and related diseases.

Suppressor T cells. A growing amount of data indicate the presence of a sub-population of T cells, called suppressor T cells, which depress the activation of other T cells and their subsequent movement to the inflamed site [85]. Recently, it has been suggested, on the basis of indirect evidence, that PGE might enhance the activity of these suppressor T cells, particularly in diseases associated with depressed T cell function [86, 87]. More work is required before an effect of PGs on suppressor T cells can be accepted. The probable effects of endogenous PGEs on systemic antigen-induced cellular responses in the spleen and lymph nodes during chronic joint inflammation are summarized in Fig. 1, based on a previous discussion of the possible interactions between PGs and cyclic nucleotides under these conditions [50].

Local events. The two major areas of cellular function affected by PGs at the site of inflammation are cellular infiltration or mobilization and cellular metabolism and secretion. To a certain extent these areas overlap, but, for the sake of clarity, they may be considered separately.

Cellular mobilization. In chronic inflammation, PMNL infiltration into the inflammatory locus usually precedes macrophage infiltration, but there is considerable controversy over the timing of lymphocyte infiltration. In considering the effects of PGs on these processes, it is more important to consider *how* PGs might affect cellular mobilization rather than *when* each cell type is affected.

Both PMNLs and macrophages produce PGs, mainly of the E-series, but nothing is known of the

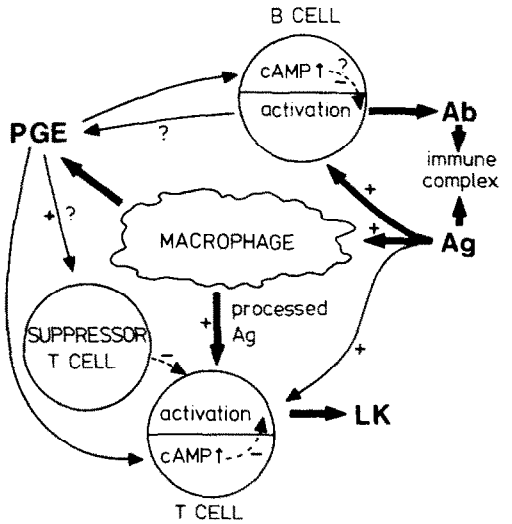

Fig. 1. Probable effects of PGE on systemic antigen-induced cellular events (in spleen and lymph nodes). The importance of the various effects will depend on the contribution of the different cell types to particular chronic inflammatory diseases.

direct effects of PGs on macrophage chemotaxis and the data on PGs and the chemotaxis of PMNLs is not very clear [40, 79]. PGE_1 is chemotactic for certain PMNLs *in vitro*, depending on the species and source, whereas contradictory data have been obtained for PGs E_2 and $F_{2\alpha}$ [40, 88–90]. It has been suggested that all PGs simply stimulate random movement of PMNLs rather than initiate chemotactic directional migration [90]. Certainly, there is no evidence for a chemotactic role for PGs in man [89]. HETE and thromboxane B_2 (TxB_2), arachidonic acid metabolites released by platelets, have both been shown to be chemotactic for PMNLs [35, 91]. The precursor of TxB_2, TxA_2, and two monohydroxy acid metabolites of arachidonic acid are also released by PMNLs [41, 92]. All these substances have been postulated as chemotactic agents for PMNLs during inflammation. However, studies *in vivo*, using PG synthetase inhibitors have not elucidated the possible role of any of these compounds in PMNL chemotaxis, since some of the inhibitors appear to have an additional, PG-independent, mechanism of action [11, 42]. The direct effects of PGs on lymphocyte mobilization are unknown. It is possible, however, that PGEs may inhibit lymphocyte mobilization because other agents, which also increase intracellular cAMP levels, inhibit spontaneous and antibody-induced T cell and to a greater extent, B cell motility *in vitro* [93].

PGE may also effect cellular mobilization indirectly, through inhibition of LK release, including those which stimulate macrophage and PMNL chemotaxis and a factor which inhibits macrophage migration (MIF) [79, 94]. According to Morley's [4] theory, that the sensitivity of LK-producing T cells to inhibition by PGE is lost during RA and thus contributes to the chronicity of this disease, the LK-mediated mobilization and trapping (by MIF) of phagocytic cells should be unaffected in arthritic joints. Zurier *et al.* [81], using RA patients, found that the increase in mononuclear cell cAMP levels, induced by PGE_1 *in vitro*, was smaller with synovial cells than with the corresponding cells from peripheral blood. This may indicate a pathological loss of sensitivity of synovial T cells to inhibition by PGE_1 but might equally reflect desensitization of the cells by prolonged exposure to the high levels of PGE present in rheumatoid synovia. PGEs also antagonise the effect of MIF on macrophage migration, apparently through increasing macrophage cAMP levels [95]. This would also serve as a negative feedback inhibition of LK-induced cellular mobilization.

Cellular secretion and metabolism. LK production by T lymphocytes (the predominant lymphocytes at the inflammatory site [3]) and phagocytosis by and lysosomal enzyme release from PMNLs are all affected by PGs. As far as LK production is concerned, the same mechanisms are likely to apply locally, at the inflamed site, as those which are involved systemically (see above). Thus, macrophage—and at the inflamed site, PMNL—PGE, released following infiltration of these cells, would exert a negative feedback effect on the release of the many pro-inflammatory LK factors. Apart from LK effects on phagocytes, lymphocyte factors have been detected which also damage tissues [96–98]. Interestingly, a LK which stimulates collagenase production also stimulates PGE_2 production by the same human rheumatoid synovial cells [62]. This represents a further possible negative-feedback route for PGE on LK-mediated tissue damage. As with systemic cellular events, these negative-feedback effects of PGs on LK release are probably mediated through stimulation of intracellular T cell cAMP.

The cyclic nucleotide-mediated effects of PGs on phagocytosis and the release of lysosomal enzymes from PMNLs *in vitro* are very well-documented and have been discussed in several recent reviews [99–101]. From the small number of studies carried out on macrophages, PGs do not appear to effect lysosomal enzyme release from these cells [101]. It is clear from the many *in vitro* studies that PGs of the E series inhibit phagocytosis by, and lysosomal enzyme release from PMNLs, through an increase in intracellular cAMP levels. $PGF_{2\alpha}$ stimulates phagocytosis and lysosomal enzyme release *in vitro*, through stimulation of intracellular cyclic guanosine 3′,5′-monophosphate (cGMP). Since PGEs predominate at inflamed sites, it is not clear whether $PGF_{2\alpha}$ plays any role in controlling lysosomal enzyme release *in vivo*. In fact PGE may also be unimportant because there is some doubt as to whether changes in intracellular cAMP levels are involved in regulating phagocytosis and lysosomal enzyme release by PMNLs *in vivo* [102]. No data are available on the possible effects of PGs on phagocytosis and enzyme release *in vivo* during chronic inflammation. Certainly, if PGE does prove to exert inhibitory actions on these parameters *in vivo* under chronic conditions, this would represent yet another negative-feedback effect of the endogenous PGs.

The probable interactions of PGs and related compounds with local cellular events are summarized in Fig. 2.

Prostaglandins and tissue events

During chronic proliferative joint inflammation, such as RA, three tissue events are of paramount importance. (1) The proliferation of infiltrating macrophages and synovial fibroblasts, with associated deposition of connective tissue (pannus). (2) Resorption of bone, and (3) destruction of cartilage by the invading pannus. The effects of PGs on these processes involve changes in cell growth, in collagen and glycosaminoglycan (GAG) metabolism and in calcium mobilization.

Proliferative tissue (granuloma)

Fibroblast growth. Increased intracellular cAMP results in inhibition of the growth of fibroblasts *in vitro* and both these effects are produced by PGE_1 [76, 103]. The cAMP-stimulating effect of exogenous PGE on human synovial fibroblasts is subject to desensitization after prolonged culture, though this may not apply *in vivo* because overproduction of PGE by rheumatoid synovial fibroblasts is associated with increased, rather than decreased, intracellular cAMP [49, 104]. Treatment with PGE_1 also inhibits pannus formation in adjuvant arthritic rats

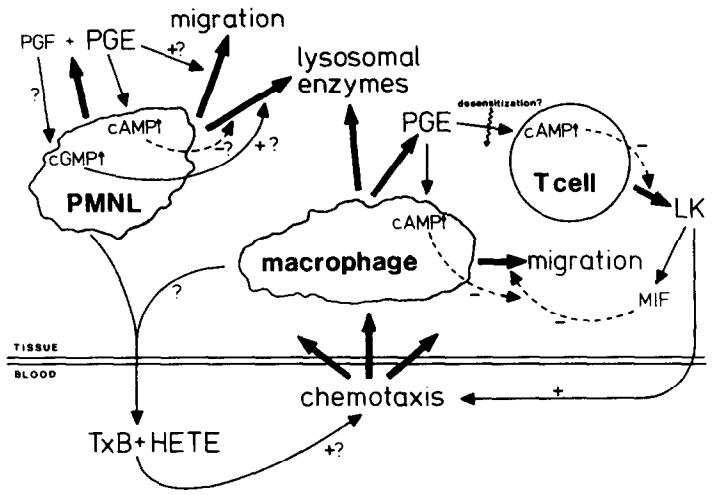

Fig. 2. Probable effects of prostaglandins on cellular events at the site of a chronic inflammatory response. It should be noted that the possible effects of PGs on lysosomal enzyme release only apply to PMNLs. PMNLs are more important in the early stages of the inflammatory response, whereas macrophages are more important in the later stages. Dotted lines indicate inhibitory effects, continuous lines indicate stimulatory effects.

and local PGE_2 administration inhibits cotton pellet granuloma in rats [15, 18]. These *in vivo* effects almost certainly involve other actions of PGE, as well as those on fibroblast growth. However, the possibility that PGE exerts a negative feedback effect on proliferating fibroblasts is strengthened by the recent finding that indomethacin inhibits human LK-induced growth suppression of the PGE_2 production by cultures rheumatoid synovial fibroblasts [62].

Collagen metabolism. Very little is known about the possible relationship between PGs and collagen metabolism in granulation tissue. This is surprising since marked changes occur in both the synthesis and breakdown of collagen in these proliferative tissues [105]. Several anti-inflammatory drugs, both steroidal and non-steroidal, have been shown to inhibit collagen synthesis *in vivo*, using experimental, sub-cutaneous granulomata induced in rats [73, 106]. However, a lower dose of indomethacin, a potent inhibitor of PG synthesis, stimulated collagen synthesis in granuloma tissue both *in vivo* and *in vitro* [106, 107]. The authors, however, failed to connect their drug-induced effect with PG synthesis inhibition. Recently, we observed that inflammatory tissue growth, induced in rats with carrageenan-impregnated sponges, is greater in EFAD rats than in normal rats, particularly when related to the much slower increase in body wt [24]. This greater tissue formation is associated with a marked increase in collagen synthesis [108]. We, therefore, suggested that endogenous PGs may exert a negative-feedback effect on collagen synthesis in proliferating inflammatory tissue [108]. This suggestion might also partially account for the inhibitory effects of PGE_2 on cotton pellet granuloma in the rat and the apparently more severe chronic adjuvant arthritic response seen in EFAD rats when compared with normal animals [15, 109]. In contrast, collagen synthesis is increased in several collagen-rich, non-inflamed tissues of EFAD rats and PGs E_1 and $F_{1\alpha}$ have been shown to stimulate collagen synthesis in normal chick embryo skin [110, 111]. It is possible that, as with vascular permeability (see above), the sensitivity of collagen to PGs may be altered during chronic inflammation, when compared with normal tissues. As far as data from EFAD rats is concerned, it is conceivable that some effects attributed to reduction of endogenous PGs may partially have been due to the altered production of adrenal corticosteroids in these animals [112].

Glycosaminoglycan metabolism. All the available data indicate that PGs E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ and D_2 increase and PG synthesis inhibitors decrease GAG synthesis in proliferating and non-proliferating loose connective tissue, both *in vitro* and *in vivo* [106, 107, 113–117]. The actions of PGE are probably mediated through increased intracellular cAMP [117]. Paradoxically, mefenamic acid increases GAG synthesis by mouse embryonic fibroblasts *in vitro* [117]. This effect was due to stimulation of cAMP production through a direct effect on cAMP-dependent and independent protein kinases, i.e. by an effect which was unrelated to inhibition of PG biosynthesis. It might, thus, be concluded that PGs released at the site of a chronic proliferative inflammation probably facilitate the proliferation through cAMP-mediated increases in fibroblast GAG production. Therefore, the effects of PGs on proliferating granulation tissue in joint disease, such as RA, will depend, to a large extent, on the relative contribution of fibroblast growth, collagen synthesis and GAG synthesis to the granuloma. Since both collagen and GAG synthesis ultimately depend on fibroblast activity, the end-effect of PGs is likely to be a negative-feedback inhibition of granuloma (pannus) formation.

Cartilage

Discussion of the effects of PGs on cartilage is, necessarily, similar to consideration of the effects

of PGs on granulation tissue, since cartilage consists largely of GAGs and smaller amounts of collagen. However, cartilage constituents are, chemically, slightly different to those of loose connective tissue and thus warrant separate consideration.

Collagen metabolism. Almost nothing is known of the effects of PGs on cartilage collagen metabolism. Denko[110] has shown that collagen synthesis is reduced in non-inflamed cartilage of EFAD rats, but whether this has any bearing on the pathological effects of PGs is unknown. It is likely, however, that PGE_2 affects cartilage collagen in joint inflammation indirectly, through inhibition of the release of collagenase from proliferating synovial cells[61]. Furthermore, PGG_2, by forming the highly reactive superoxide radical (O_2^-), may possibly be involved in the breakdown of cartilage collagen[12, 118, 119].

Glycosaminoglycan metabolism. PGs, particularly those of the A series and, to a lesser extent, PGEs, inhibit GAG synthesis and substrate uptake in cultured cartilage or chondrocytes[120–122]. These observations contrast with the finding that rats fed an EFAD diet also exhibit reduced cartilage GAG synthesis[110]. Cartilage GAG catabolism is not affected by PGs *in vitro*[121], but intra-articular PGE_1 causes marked cartilage damage in the rabbit *in vivo*[123]. It is possible that the latter may be an indirect effect, though it is difficult to reconcile with the suggested inhibition of cartilage degradation by PGs E and A through inhibition of lysosomal enzyme release[124]. PGG_2, may stimulate cartilage GAG breakdown through formation of O_2^-[12, 118, 119]. Because of these conflicting data it is not possible to determine exactly how PGs might affect cartilage GAG in chronic joint disease, though they probably contribute, ultimately, to its breakdown.

Bone

Bone consists of closely packed collagen fibres in a mineral matrix, mostly calcium and phosphate. Thus, any effects of PGs on bone are likely to involve actions on collagen metabolism and calcium mobilization.

Calcium-mobilization. Before the breakdown of collagen and bone resorption, which are characteristic of RA, can occur, it is necessary for the mineral matrix to be removed so that the degradative enzymes can act on the fibrous bone structure[125]. PGE, including that released by rheumatoid synovial tissue, stimulates the release of ^{45}Ca from pre-labelled rat and mouse foetal bone *in vitro*[57, 126, 127]. The endoperoxide PG intermediates, PGG_2 and PGH_2, and the 13,14-dihydro PGE metabolites were also found to be potent stimulators of Ca release in the same *in vitro* model, though synthetic PG endoperoxide analogues were almost inactive[128, 129]. The Ca-releasing action of collagenase too may be mediated by PGs[130]. These data suggest that several components of the PG biosynthetic pathway, released locally, are probably involved in calcium release and bone resorption during proliferative joint disease. However, indomethacin administered *in vivo*, has little effect on bone erosion in rabbit MAA, in which PGs are only involved in the early stages, or in adjuvant arthritis in the rat[44, 131]. Thus, a bone-resorbing role for locally-produced PGs has yet to be clearly demonstrated *in vivo*. Paradoxically, exogenous PGE_1, administered with or without theophylline, in pharmacological doses, almost completely prevents bone destruction in adjuvant arthritis rats[20, 21]. This is probably an indirect effect, mediated through inhibition of LK release from T cells (see above).

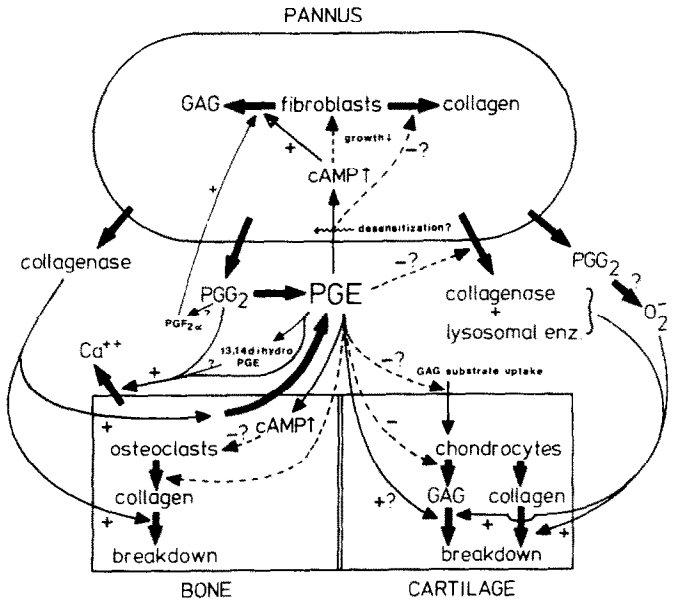

Fig. 3. Probable effects of prostaglandins on tissue events in chronic proliferative joint inflammation. PGE and collagenase are produced by both fibroblasts and phagocytes but, for the sake of clarity, they are shown as released by the pannus of granulomatous tissue. PGE also inhibits bone and cartilage destruction indirectly, by inhibiting lymphokine release from T lymphocytes (not shown). Dotted lines indicate inhibitory effects, continuous lines indicate stimulatory effects.

though an effect on calcitonin release cannot be ruled out. Thus, while local PGs may exert a positive bone-resorbing effect, PGs released systemically may function as an indirect negative-feedback on further resorption.

Collagen metabolism. PGE has been shown to be effective in inhibiting the synthesis of collagen in rat foetal bone *in vitro*, an action which would prevent new bone formation and thus exacerbate collagen breakdown by collagenase [132]. In fact, collagenase has been shown to release PGE from cultured mouse bone [130]. As with many of the other actions of PGE, its bone-resorbing effects are associated with an increase in bone cell cAMP, which may be related to inhibition of collagen synthesis and/or osteoclast growth [13]. The possible effects of PGs on tissue events during chronic proliferative joint inflammation are summarized in Fig. 3.

Prostaglandins and other aspects of chronic inflammation

Pain. In pharmacological doses PGE produces long-lasting pain, following intradermal injection in man [134]. However, in low, sub-threshold doses, PGE only potentiates the pain-producing properties of other agents, such as histamine and bradykinin, at the same site in man, in the dog kneejoint, and in the rabbit ear [134–136]. Studies with indomethacin indicate that, *in vivo*, bradykinin increases its own pain-producing properties through stimulating PGE release [135, 136]. It is likely that sensitization of pain receptors by PGE, to other pain-producing substances, probably contributes to the pain experienced in chronic joint inflammation. This action of PGE_2 might be antagonized by $PGF_{2\alpha}$ [137]. The importance of PGs in producing pain in inflamed joints is lessened when one considers the large contribution of mechanically-induced pain due to the gross malfunction of the articulation.

Platelets. Aggregating platelets release a large number of products of the PG biosynthetic pathway, including the classical PGs. Other, less stable, products, such as HETE and TxB_2, which are chemotactic for PMNL's, and PGG_2 and PGH_2, which stimulate bone resorption [35, 91, 128], also exert pro-inflammatory actions *in vitro*. It has been suggested that aggregating platelets may, therefore, play a role in inflammation [138]. In this context, a novel metabolite of arachidonic acid, prostacyclin (PGX, PGI_2), released by arterial walls, was found to be much more potent than PGE_1 in inhibiting platelet aggregation and raising intracellular cAMP levels [139, 140]. It is possible that PGI_2 may be involved in a negative-feedback inhibition of certain chronic inflammatory processes, since it can also be formed by fibroblasts *in vitro* and appears to stimulate the adenylate cyclase in these cells [141].

Non-articular inflammation. Prolonged treatment of New Zealand black (NZB) mice with pharmacological doses of PGE_1 markedly suppresses the spontaneous development of immune-complex-mediated glomerulonephritis, to which these animals are genetically susceptible [86]. It is possible that this treatment may correct a defect in PG- and cAMP-mediated control of a sub-population of splenic lymphocytes [142]. These data suggest that PGs may be involved in controlling the development of non-articular immune complex disease.

A defect in endogenous PG production has also been postulated as the underlying mechanism in cystic fibrosis though this suggestion is based on studies on endogenous fatty acid levels and fatty acid supplementation rather than on PG biosynthesis *per se* [143, 144].

Implications for pharmacological control of chronic inflammation

The acidic NSAIDs and the corticosteroids form the two main classes of drugs, which are, more or less, successfully employed for the clinical control of such chronic inflammatory conditions as RA. Following the discovery by Vane and his colleagues that acidic NSAIDs inhibit the biosynthesis of PGs, it appeared that inhibition of cyclo-oxygenase satisfactorily explains all actions (therapeutic and adverse effects) exerted by NSAIDs [5–7]. Recent findings suggest that corticosteroids may act through inhibition of the release of PGs, thus preventing their extracellular accumulation, though the precise mechanism is subject to considerable discussion [51, 145–147]. Thus, inhibition of the extracellular accumulation of arachidonic acid products would seem to explain the main clinical actions of both NSAIDs and anti-inflammatory steroids. This concept, however, is not as strong as it would appear at first glance. Since the arachidonate conversion pathway appears to be a modulatory mechanism, providing endogenous pro- and anti-inflammatory substances, it follows that any drug, irrespective of its locus of action, which prevents the interstitial enhancement of PG levels would not only provide therapeutic benefit but also counteract it. In the latter sense, such drugs should be considered as camouflaged traitors of therapy. Common sense, however, dictates that this is not the case, because both classes of drugs, NSAIDs and corticosteroids, are dominantly anti-inflammatory in animal models and of real therapeutic value in clinical RA. This seeming paradox may be resolved by the time-honoured pharmacological paradigm that every drug has more than one action. Certainly, amongst other actions, corticosteroids are stabilizers of membranes, thus counteracting the release of lysosomal enzymes, and they also inhibit the activity of mononuclear phagocytes [148, 149]. While these effects may ultimately lead to prevention of PG output, both effects may, *per se*, account (at least partially) for the anti-inflammatory effect of corticosteroids. Since sodium salicylate and aspirin are equally effective as anti-inflammatory drugs, but totally different as cyclo-oxygenase inhibitors, it has been proposed that these two effects are not necessarily related [10]. Suggested alternative mechanisms of action for NSAIDs include inhibition of phosphodiesterase, leading to increased intracellular cAMP levels [150], thus reinforcing the anti-inflammatory, cAMP-mediated effect of PGE and inhibition of leucocyte mobilization [42]. Furthermore, when arachidonic acid is lacking, i.e. in EFAD rats, the anti-inflammatory effects of aspirin and dexamethasone are still readily demonstrable [11]. Thus, prevention of PG release is not necessarily a

prerequisite for anti-inflammatory drug action, an element of caution advised in other recent reports [10, 151–154].

In contrast to the inhibitory effects of NSAIDs and steroids on PG output, gold salts, commonly used as anti-rheumatic agents, have been shown to stimulate the production of PGE_2, *in vitro*, whilst inhibiting $PGF_{2\alpha}$ synthesis [155]. The authors suggested that this might explain why gold salts are useful in chronic inflammatory conditions in which conventional drugs are ineffective. Other authors, however, observed inhibition of PGE_2 synthesis by gold salts [160]. The phenolic anti-oxidant, MK-447, has also been shown to stimulate PGE_2 production, its acute, anti-inflammatory effect also being associated with inhibition of PGG_2 formation [12]. More recently the chronic anti-inflammatory effect (inhibition of granuloma formation) of an anti-oxidant polyether sponge constituent was found to be accompanied by increased PGE concentrations at the inflamed site [13]. Anti-oxidation is, in fact, the mode of action of endogenous superoxide dismutase, which protects synovial fluid from disintegration *in vitro* [63]. As discussed elsewhere in this article, PGE exerts several anti-inflammatory actions. Thus, anti-oxidation and stimulation of PGE production may be mutually reinforcing anti-inflammatory effects.

One possible method of increasing endogenous PGE production is to inhibit an alternative pathway of arachidonic acid metabolism. Thus, the anti-inflammatory compound, L-8027, which inhibits PG synthesis in higher doses, appears to be a selective inhibitor of TxA_2 synthesis [156, 157]. Imidazole is also reported to be a selective inhibitor of TxA_2 production, but whether these observations indicate a role for TxA_2 or B_2 in inflammation has yet to be established [158].

The anti-inflammatory action of exogenous PGE (with or without a phosphodiesterase inhibitor) could be thought to have little relevance for possible clinical application because of its rapid metabolism *in vivo* and because of the large number of possible side-effects. The first objection could be overcome by using a PGE analogue which is resistant to metabolism, though it is possible that this may prevent the formation of an (as yet undiscovered) anti-inflammatory metabolite. With regard to side-effects, it is conceivable that the more potent adenylate cyclase-stimulating PGI_2 (prostacyclin), or a suitable analogue, might exert less adverse clinical effects than PGE itself. In this respect, recent data indicate that intrasynovial elevation of cAMP leads to significant improvement in RA patients [53].

Final conclusions

The role of PGs in chronic inflammation, as in many other pathophysiological processes in which they have been implicated, appears to be homeostatic. Thus, in the early stages of the inflammatory response PGs potentiate the process, causing vasodilatation, increasing capillary permeability and, to a certain extent, facilitating cellular mobilization. Under acute conditions, these effects would facilitate the breakdown or removal of a foreign body or irritant. However, as the inflammatory condition becomes chronic, some of these pro-inflammatory phenomena appear (at least as far as capillary permeability is concerned) to become desensitized to PGs. Furthermore, where immune mechanisms are involved, PGEs, accumulating at the site of inflammation, would inhibit lymphocyte activation and, systemically, PGEs may regulate further lymphocyte activation. These negative-feedback regulatory effects may also extend to the inhibition of other leucocyte pro-inflammatory products, such as lysosomal enzymes. While regulating cellular events, the increasing levels of PGs at the inflammatory site may also facilitate tissue damage, such as cartilage and bone destruction, though, without sufficient cellular stimulation these processes would slow down. Where proliferative tissue is involved, PGs may regulate granuloma formation through actions on fibroblast growth and collagen synthesis.

In the light of this apparent homeostatic function of PGs in chronic inflammation, this group of substances cannot be referred to as either pro- or anti-inflammatory agents, particularly since different PGs (in the sense of arachidonic acid metabolites) often have opposing activities. Whereas recent efforts have been directed towards development of PG synthetase inhibitors as anti-inflammatory agents, it is now necessary to develop drugs which act at certain points along the PG biosynthetic pathway, thus removing the relevant pro-inflammatory and not an anti-inflammatory effect. Further elucidation of the relative roles of the various products of the PG biosynthetic pathway in chronic inflammation will serve to highlight the specific areas of therapy required.

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The new PG produced by carrageenin-induced granulation tissue *in vitro* [see ref 36] is now known to be 6-keto $PGF_{1\alpha}$, the metabolite of PGI_2. Other studies on carrageenin-induced granulomata have shown that, when administered *in vivo* in the acute phase of the inflammatory response, PGE_1 stimulates granuloma formation, whereas administration of PGE_1 during the chronic phase inhibits granuloma formation [I. L. Bonta and M. J. Parnham, *Br. J. Pharmac.* (in press) (1978)]. This confirms the acute pro-inflammatory and chronic anti-inflammatory role of PGE. In this context, PGE_2 also stimulates the initial phase of endotoxin-induced release of collagenase from guinea-pig macrophages *in vitro* [L. M. Wahl, C. E. Olsen, A. L. Sandberg and S. E. Mergenhagen, *Proc. natn Acad. Sci. U.S.A.* **74**, 4955 (1977)]. This acute stimulation of collagenase release by PGE may alter to an inhibitory action during chronic inflammation [see ref 62].

REFERENCES

1. C. R. Pace-Asciak, *Prostaglandins* **13**, 811 (1977).
2. F. A. Ward, in *A Primer of Pathology*, 2nd Edition, Butterworths, London (1967).
3. J. Oort and R. J. Scheper, in *Inflammation—Mechanisms and their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3**, 25 (1977).
4. J. Morley, *Prostaglandins* **8**, 315 (1974).
5. J. R. Vane, *J. Allerg. clin. Immunol.* **58**, 691 (1976).

6. R. J. Flower, in *Inflammation—Mechanisms and their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3**, 99 (1977).
7. R. J. Flower and J. R. Vane, *Biochem. Pharmac.* **23**, 1439 (1974).
8. F. D. Hart, in *Prostaglandin Synthetase Inhibitors—Their Effects on Physiological Functions and Pathological States* (Eds H. J. Robinson and J. R. Vane), p. 219. Raven Press, NY (1974).
9. D. R. Robinson and L. Levine, in *Prostaglandin Synthetase Inhibitors—Their Effects on Physiological Functions and Pathological States* (Eds H. J. Robinson and J. R. Vane), p. 223. Raven Press, NY (1974).
10. M. J. H. Smith, *Ag. Act.* **5**, 315 (1975).
11. I. L. Bonta, H. Bult, J. E. Vincent and F. J. Zijlstra, *J. Pharm. Pharmac.* **29**, 1 (1977).
12. F. A. Kuehl, J. L. Humes, R. W. Egan, E. A. Ham, G. C. Beveridge and C. G. Van Arman, *Nature, Lond.* **265**, 170 (1977).
13. M. J. Parnham, I. L. Bonta, M. J. P. Adolfs and P. Bragt, *Ag. Act.* **7**, 539 (1977).
14. R. L. Aspinall and P. S. Cammarata, *Nature, Lond.* **224**, 1320 (1969).
15. G. Di Pasquale, C. Rassaert, R. Richter, P. Welaj and L. Tripp, *Prostaglandins* **3**, 741 (1973).
16. R. B. Zurier and F. Quagliata, *Nature, Lond.* **234**, 304 (1971).
17. E. M. Glenn and N. Rohloff, *Proc. Soc. exp. Biol. Med.* **139**, 290 (1972).
18. R. B. Zurier and M. Ballas, *Arth. Rheum.* **16**, 251 (1973).
19. R. B. Zurier, S. Hoffstein and G. Weissmann, *Arth. Rheum.* **16**, 606 (1973).
20. I. L. Bonta, M. J. Parnham, L. van Vliet and J. E. Vincent, *Br. J. Pharmac.* **59**, 438P (1977).
21. I. L. Bonta, M. J. Parnham and L. van Vliet, *Ann. Rheum. Dis.* in press (1978).
22. I. L. Bonta, H. Chrispijn, J. Noordhoek and J. E. Vincent, *Prostaglandins* **5**, 495 (1974).
23. I. L. Bonta, H. Bult, L. L. M. v.d. Ven and J. Noordhoek, *Ag. Act.* **6**, 154 (1976).
24. I. L. Bonta, M. J. Parnham and M. J. P. Adolfs, *Prostaglandins*, **14**, 295 (1977).
25. I. L. Bonta, in *Inflammation—Mechanisms and Their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3**, 121 (1977).
26. I. L. Bonta, in *Inflammation and Anti-Inflammatory Drugs*, Handbook of Experimental Pharmacology (Eds S. H. Ferreira and J. R. Vane), Springer-Verlag, Berlin (in press).
27. A. Cats and L. van de Putte, in *Inflammation—Mechanisms and Their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3**, 133 (1977).
28. E. D. Harris, *Arth. Rheum.* **19**, 68 (1976).
29. L. M. Pelus and H. R. Strausser, *Life Sci.* **20**, 903 (1977).
30. H. Selye, *Proc. Soc. exp. Biol. Med.* **82**, 328 (1953).
31. A. L. Willis, *J. Pharm. Pharmac.* **21**, 126 (1969).
32. K. Ohuchi, H. Sato and S. Tsurufuji, *Biochim. biophys. Acta* **424**, 439 (1976).
33. G. A. Higgs, E. A. Harvey, S. H. Ferreira and J. R. Vane, in *Advances in Prostaglandin and Thromtoxane Research* (Eds B. Samuelsson and R. Paoletti), 1, p. 105. Raven Press, NY (1976).
34. W. Ch. Chang, S. Murota and S. Tsurufuji, *Prostaglandins* **13**, 17 (1977).
35. J. R. Boot, W. Dawson and E. A. Kitchen, *J. Physiol. Lond.* **257**, 47P (1976).
36. W. C. Chang, S. Murota, M. Matsuo and S. Tsurufuji, *Biochem. biophys. Res. Commun.* **72**, 1259 (1976).
37. W. C. Chang, S. Murota and S. Tsurufuji, *Jap. J. Pharmac.* **25**, 219 (1975).
38. A. J. Lewis, J. Cottney and D. J. Nelson, *Eur. J. Pharmac.* **40**, 1 (1976).
39. S. S. Adams, C. A. Burrows, N. Skeldon and D. B. Yates, in *Abstracts XIVth Int. Congr. Rheumatology*, p. 170. San Francisco (1977).
40. G. A. Higgs, E. McCall and L. J. F. Youlten, *Br. J. Pharmac.* **53**, 539 (1975).
41. G. A. Higgs, S. Bunting, S. Moncada and J. R. Vane, *Prostaglandins* **12**, 749 (1976).
42. J. R. Walker, M. J. H. Smith and A. W. Ford-Hutchinson, *Ag. Act.* **6**, 602 (1976).
43. D. C. Dumonde and L. E. Glynn, *Br. J. exp. Path.* **43**, 373 (1962).
44. A. Blackham, J. B. Farmer, H. Radziwonik and J. Westwick, *Br. J. Pharmac.* **51**, 35 (1975).
45. E. W. Gold, L. B. Anderson, E. R. Schwartz and C. W. Miller, *Prostaglandins* **12**, 837 (1976).
46. D. R. Swinson, A. Bennett and E. B. D. Hamilton, in *The Role of Prostaglandins in Inflammation* (Ed. G. P. Lewis), p. 41. Hans Huber, Bern (1976).
47. C. M. Pearson, *Arth. Rheum.* **7**, 80 (1964).
48. M. J. Parnham, I. L. Bonta and M. J. P. Adolfs, *Ann. Rheum. Dis.* in press (1978).
49. D. R. Robinson, M. B. McGuire and L. Levine, *Ann. N.Y. Acad. Sci.* **256**, 318 (1975).
50. M. J. Parnham, I. L. Bonta and M. J. P. Adolfs, in *Perspectives in Inflammation: Future Trends and Developments* (Eds D. A. Willoughby, J. P. Giroud and G. P. Velo), p. 279. MTP Press, Lancaster (1977).
51. Y. Floman and U. Zor, *Prostaglandins* **12**, 403 (1976).
52. J. P. Giroud, G. P. Velo, C. J. Dunn, J. Timsit and D. A. Willoughby, in *Future Trends in Inflammation* (Eds G. P. Velo, D. A. Willoughby and J. P. Giroud), p. 19. Piccin Medical Books, Padua (1974).
53. L. E. Trang, E. Granström, O. Lövgren, A. E. Roch-Nordlund, R. Horn and O. Walaas, in *Abstracts XIVth Int. Congr. Rheumatology*, p. 234. San Francisco (1977).
54. G. A. Higgs, J. R. Vane, F. D. Hart and J. A. Wojtulewski, in *Prostaglandin Synthetase Inhibitors—Their Effects on Physiological Functions and Pathological States* (Eds H. J. Robinson and J. R. Vane), p. 165. Raven Press, NY (1974).
55. C. Patrono, S. Bombardieri, O. di Munno, G. P. Pasero, F. Greco, D. Grossi-Belloni and G. Ciabattoni, in *The Role of Prostaglandins in Inflammation* (Ed. G. P. Lewis), p. 122. Hans Huber, Bern (1976).
56. G. Husby, A. D. Bankhurst and R. C. Williams, in *Abstracts XIVth International Congress Rheumatology*, p. 232. San Francisco (1977).
57. D. R. Robinson, A. H. Tashjian and L. Levine, *J. clin. Invest.* **56**, 1181 (1975).
58. D. Crook and A. J. Collins, *Prostaglandins* **9**, 857 (1975).
59. A. J. Collins, D. Crook, R. Chan and P. A. Bacon, in *The Role of Prostaglandins in Inflammation* (Ed. G. P. Lewis), p. 138. Hans Huber, Bern (1976).
60. A. J. Collins, in *The Role of Prostaglandins in Inflammation* (Ed. G. P. Lewis), p. 146. Hans Huber, Bern (1976).
61. J.-M. Dayer, S. M. Krane, R. Graham, H. Russell and D. R. Robinson, *Proc. natn. Acad. Sci. U.S.A.* **73**, 945 (1976).
62. J.-M. Dayer, D. R. Robinson and S. M. Krane, *J. exp. Med.* **145**, 1399 (1977).
63. J. M. McCord, *Science, NY* **185**, 529 (1974).
64. H. R. Bourne, L. M. Lichtenstein, K. L. Melmon, C. S. Henney, Y. Weinstein and G. M. Shearer, *Science, NY* **184**, 19 (1974).
65. W. C. Dick, D. M. Grennan and I. J. Zeitlin, *Br. J. Pharmac.* **56**, 313 (1976).
66. G. P. Lewis, J. Westwick and T. J. Williams, *Br. J. Pharmac.* **59**, 442P (1977).

67. R. J. Flower, E. A. Harvey and W. P. Kingston, *Br. J. Pharmac.* **56**, 229 (1976).
68. T. J. Williams and J. Morley, *Nature, Lond.* **246**, 215 (1973).
69. S. Moncada, S. H. Ferreira and J. R. Vane, *Nature, Lond.* **246**, 217 (1973).
70. D. M. Grennan, W. Mitchell, W. Miller and I. J. Zeitlin, *Br. J. Pharmac.* **60**, 251 (1977).
71. E. M. Glenn, B. J. Bowman and N. A. Rohloff, in *Prostaglandins in Cellular Biology* (Eds P. W. Ramwell and B. B. Pharris), p. 329. Plenum Press, NY (1972).
72. W.-C. Chang and S. Tsurufuji, *Eur. J. Pharmac.* **36**, 7 (1976).
73. M. Fukuhara and S. Tsurufuji, *Biochem. Pharmac.* **18**, 475 (1969).
74. W.-C. Chang, S. Murota and S. Tsurufuji, *Biochem. Pharmac.* **25**, 2045 (1976).
75. D. R. Webb and P. L. Osheroff, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1300 (1976).
76. I. H. Pastan, G. S. Johnson and W. B. Anderson, *Ann. Rev. Biochem.* **44**, 491 (1975).
77. P. Stastny, M. Rosenthal, M. Andreis and M. Ziff, *Arth. Rheum.* **18**, 237 (1975).
78. P. Stastny, T. D. Cooke and M. Ziff, *Clin. exp. Immun.* **14**, 141 (1973).
79. D. Gordon, M. A. Bray and J. Morley, *Nature, Lond.* **262**, 401 (1976).
80. P. J. Kirby, J. Morley, J. R. Ponsford and W. I. McDonald, *Prostaglandins* **11**, 621 (1976).
81. R. B. Zurier, J. Doty and A. Goldberg, *Prostaglandins* **13**, 25 (1977).
82. L. Kourounakis and M. A. Kapusta, *J. Rheum.* **3**, 346 (1976).
83. R. S. Panush, *Arth. Rheum.* **19**, 907 (1976).
84. M. Zimecki and D. R. Webb, *J. Immun.* **117**, 2158 (1976).
85. G. L. Asherson, in *Future Trends in Inflammation* (Eds G. P. Velo, D. A. Willoughby and J.-P. Giroud), p. 331. Piccin Medical Books, Padua (1974).
86. R. B. Zurier, D. M. Sayadoff, S. B. Torrey and N. F. Rothfield, *Arth. Rheum.* **20**, 723 (1977).
87. R. B. Zurier, P. Dore-Duffy and M. V. Viola, *New Eng. J. Med.* **296**, 1443 (1977).
88. G. Kaley and R. Weiner, *Ann. N.Y. Acad. Sci.* **180**, 338 (1971).
89. J. R. Walker, M. J. H. Smith and A. W. Ford-Hutchinson, *J. Pharm. Pharmac.* **28**, 745 (1976).
90. E. Shibuya, K. Masuda and Y. Izawa, *Prostaglandins* **12**, 165 (1976).
91. S. R. Turner, J. A. Tainer and W. S. Lynn, *Nature, Lond.* **257**, 680 (1975).
92. P. Borgeat, M. Hamberg and B. Samuelsson, *J. biol. Chem.* **251**, 7816 (1976).
93. G. F. Schreiner and E. R. Unanue, *J. Immun.* **114**, 802 (1975).
95. E. Pick and J. L. Turk, *Clin. exp. Immun.* **10**, 1 (1972).
95. W. J. Koopman, M. H. Gillis and J. R. David, *J. Immun.* **110**, 1609 (1973).
96. J.-M. Dayer, R. Graham, G. Russell and S. M. Krane, *Science, NY* **195**, 181 (1977).
97. J. H. Herman, D. S. Musgrave and M. V. Dennis, *Arth. Rheum.* **20**, 922 (1977).
98. R. L. Johnson and M. Ziff, *J. clin. Invest.* **58**, 240 (1976).
99. L. J. Ignarro, *Ag. Act.* **4**, 241 (1974).
100. G. Weissmann, I. Goldstein and S. Hoffstein, in *Advances in Prostaglandin and Thromboxane Research* (Eds B. Samuelsson and R. Paoletti), p. 803, Raven Press, NY (1976).
101. P. Davies, in *Inflammation—Mechanisms and their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3**, 107 (1977).
102. D. A. Deporter, P. A. Dieppe, M. Glatt and D. A. Willoughby, *J. Pathol.* **121**, 129 (1977).
103. G. S. Johnson and I. Pastan, *J. natl. Cancer Inst.* **47**, 1357 (1971).
104. D. S. Newcombe, C. P. Ciosek, Y. Ishikawa and J. V. Fahey, *Proc. natn. Acad. Sci. U.S.A.* **72**, 3124 (1975).
105. S. Bazin, M. Le Lous and A. Delaunay, *Ag. Act.* **6**, 272 (1976).
106. E. Kulonen and M. Potila, *Biochem. Pharmac.* **24**, 219 (1975).
107. E. Kulonen and M. Potila, *Biochem. Pharmac.* **24**, 1671 (1975).
108. M. J. Parnham, S. Shoshan, I. L. Bonta and S. Neiman-Wollner, *Prostaglandins* **14**, 709 (1977).
109. I. L. Bonta, M. J. Parnham, M. J. P. Adolfs and L. van Vliet, in *Perspectives in Inflammation: Future Trends and Developments* (Eds D. A. Willoughby and G. P. Velo), p. 265. MTP Press, Lancaster (1977).
110. C. W. Denko, *J. Rheum.* **3**, 205 (1976).
111. N. Blumenkrantz and J. Søndergaard, *Nature, New Biol.* **239**, 246 (1972).
112. M. J. Parnham, M. J. P. Adolfs and I. L. Bonta, *J. Pharm. Pharmac.* **29**, 670 (1977).
113. J. Søndergaard, P. Helin and H. P. Jorgensen, *J. Pathol.* **109**, 239 (1973).
114. C. W. Castor, *J. Lab. clin. Med.* **85**, 392 (1975).
115. S. Murota, M. Abe, L. Otsuka and W.-C. Chang, *Prostaglandins* **13**, 711 (1977).
116. M. Yaron, I. Yaron, O. Smetana, E. Eylan and M. Herzberg, *Arth. Rheum.* **19**, 1315 (1976).
117. H. D. Peters, V. Dinnendahl and P. S. Schönhöfer, *Naunyn-Schmied. Arch. Pharmac.* **289**, 29 (1975).
118. R. A. Greenwald, W. W. Moy and D. Lazarus, *Arth. Rheum.* **19**, 799 (1976).
119. Y. Ōyanagui, *Biochem. Pharmac.* **25**, 1465 (1976).
120. G. S. Eisenbarth, S. C. Beuttel and H. E. Lebovitz, *J. Pharmac. exp. Ther.* **189**, 213 (1974).
121. C. J. Malemud and L. Sokoloff, *Prostaglandins* **13**, 846 (1977).
122. L. Lippiello, D. Mitrovic and H. J. Mankin, *Arth. Rheum.* **20**, 125 (1977).
123. C. C. Teitz and O. D. Chrisman, *Clin. Orthop. relat. Res.* **108**, 264 (1975).
124. L. J. Ignarro and S. Y. Cech, *Arth. Rheum.* **18**, 406 (1975).
125. S. M. Krane, *Arth. Rheum.* **17**, 306 (1974).
126. D. C. Klein and L. G. Raisz, *Endocrinology* **86**, 1436 (1970).
127. J. W. Dietrich, J. M. Goodson and L. G. Raisz, *Prostaglandins* **10**, 231 (1975).
128. L. G. Raisz, J. W. Dietrich, H. A. Simmons, H. W. Seyberth, W. Hubbard and J. A. Oates, *Nature, Lond.* **267**, 532 (1977).
129. A. H. Tashjian, J. E. Tice and K. Sides, *Nature, Lond.* **266**, 645 (1977).
130. M. Dowsett, A. R. Eastman, D. M. Easty, G. C. Easty, T. J. Powles and A. M. Neville, *Nature, Lond.* **263**, 72 (1976).
131. A. Blackham, J. W. Burns, J. B. Farmer, H. Radziwonik and J. Westwick, *Ag. Act.* **7**, 145 (1977).
132. L. G. Raisz and A. R. Koolemans-Beynen, *Prostaglandins* **8**, 377 (1974).
133. J.-H. Yu, H. Wells, W. J. Ryan and W. S. Lloyd, *Prostaglandins* **12**, 501 (1976).
134. S. H. Ferreira, *Nature, New Biol.* **240**, 200 (1972).
135. S. Moncada, S. H. Ferreira and J. R. Vane, in *Prostaglandin Synthetase Inhibitors—Their Effects on Physiological Functions and Pathological States* (Eds H. J. Robinson and J. R. Vane), p. 189. Raven Press, NY (1974).
136. F. Lembeck and H. Juan, *Naunyn-Schmied. Arch. Pharmac.* **285**, 301 (1974).

137. H. Juan and F. Lembeck, *Br. J. Pharmac.* **59,** 385 (1977).
138. B. B. Vargaftig, in *Inflammation—Mechanisms and Their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3,** 75 (1977).
139. R. R. Gorman, S. Bunting and O. V. Miller, *Prostaglandins* **13,** 377 (1977).
140. J. E. Tateson, S. Moncada and J. R. Vane, *Prostaglandins* **13,** 389 (1977).
141. S. Hammarström, H.-K. Claesson and J. Å. Lindgren, *Prostaglandins* **13,** 1009 (1977).
142. D. R. Webb, I. Nowowiejski, M. Dauphinée and N. Talal, *J. Immun.* **118,** 446 (1977).
143. R. B. Elliott, *Pediatrics* **57,** 474 (1976).
144. I. M. Campbell, D. N. Crozier and R. B. Caton, *Pediatrics* **57,** 480 (1976).
145. G. P. Lewis and P. J. Piper, *Nature, Lond.* **254,** 308 (1975).
146. R. J. Gryglewski, *Pharmac. Res. Commun.* **8,** 337 (1976).
147. F. P. Nijkamp, R. J. Flower, S. Moncada and J. R. Vane, *Nature, Lond.* **263,** 479 (1976).
148. G. Weissmann and L. Thomas, *Recent Prog. Horm. Res.* **20,** 215 (1964).
149. J. Thompson and R. van Furth, *J. exp. Med.* **131,** 429 (1970).
150. C. P. Cioseik, R. W. Ortel, N. M. Thanassi and D. S. Newcombe, *Nature, Lond.* **251,** 148 (1974).
151. W. E. Brocklehurst and W. Dawson, in *Future Trends in Inflammation* (Eds G. P. Velo, D. A. Willoughby and J. P. Giroud), p. 37. Piccin Medical Books, Padua (1974).
152. D. A. Willoughby and P. Dieppe, in *The Role of Prostaglandins in Inflammation* (Ed. G. P. Lewis), p. 14. Hans Huber, Bern (1976).
153. J. Morley, in *Inflammation—Mechanisms and Their Impact on Therapy* (Eds I. L. Bonta, J. Thompson and K. Brune), *Ag. Act. Suppl.* **3,** 71 (1977).
154. C. H. Cashin, W. Dawson and E. A. Kitchen, *J. Pharm. Pharmac.* **29,** 330 (1977).
155. K. J. Stone, S. J. Mather and P. P. Gibson, *Prostaglandins* **10,** 241 (1975).
156. C. Deby, M. Descamps, F. Binon and Z. M. Bacq, *Biochem. Pharmac.* **24,** 1089 (1975).
157. R. J. Gryglewski, A. Zmuda, R. Korbut, E. Krecioch and K. Bieron, *Nature, Lond.* **267,** 627 (1977).
158. S. Moncada, S. Bunting, K. Mullane, P. Thorogood and J. R. Vane, *Prostaglandins* **13,** 611 (1977).
159. J. L. Humes, R. J. Bonney, L. Pelus, M. E. Dahlgren, S. J. Sadowski, F. A. Kuehl and P. Davies, *Nature, Lond.* **269,** 149 (1977).
160. C. Deby, Z. M. Bacq and D. Simon, *Biochem. Pharmac.* **22,** 3141 (1973).