ample CUC to UUA, for instance, contains silent changes of C to U and C to A; and UCU to AGC is assumed to have evolved from a single-nucleotide replacement ACC to AGC. Minimum base differences are the smallest number of nucleotide substitutions that will bring about the designated amino acid replacements, and substitutions in excess of these numbers are counted as silent. Most of the silent changes are synonymous (degenerate) third-base substitutions, but in some cases the steps in silent substitutions cannot be precisely identified. For example, AGA→UUG may have been AGA→AGG→CGG→CUG→UUG or AGA→CGA→UUA→UUG. For this reason, the totals in Table 3 are lower than the totals of silent nucleotide substitutions in Table 2. In column 6, Table 3, each item contains the sum of three pairwise comparisons of three globin genes. The values in Table 2, lines 2 to 6, have been rounded to the nearest integer, for simplification. More precise values may be calculated from the data in Table 1.

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Prostaglandins, Arachidonic Acid, and Inflammation

Frederick A. Kuehl, Jr., and Robert W. Egan

The prostaglandins (PG's) are a complex group of oxygenated fatty acids that have been detected in virtually all mammalian tissues thus far examined. They include some of the most potent natural substances known, and are important both as bioregulators and as participants in pathological states. The prostaglandins are not stored free in tissues, but are synthesized as a result of membrane perturbations that cause the release of free fatty acids, generally arachidonic acid, from esterified lipid sources. The release of arachidonic acid can be brought about by a wide variety of hormones either directly or indirectly (*1*), as well as by inflammatory or immunological stimuli (*2*, *3*), calcium ionophores (*4*), ultraviolet light (*5*), melittin, the membrane active component of bee venom (*6*), tumor promoting agents (*7*), and even mechanical agitation (*8*). The free arachidonic acid then reacts with prostaglandin cyclooxygenase, the first enzyme of the prostaglandin biosynthetic sequence. This enzyme oxygenates arachidonic acid to the endoperoxide intermediate, PGG_2, which is then converted to a variety of other biologically active products, the nature of which is determined by the enzyme content of the tissue under consideration. For example, platelets make primarily thromboxane A_2 (TXA_2) whereas the aorta forms prostacyclin (PGI_2).

The nature of these products is depicted in Fig. 1 with special reference to PGE_2, an oxygenated fatty acid with a *cis* double bond, a *trans* double bond, two hydroxyl groups, a carbonyl, and a five-membered ring joined to the two side chains at carbons-8 and -12. Prostaglandins E_3 and E_1 are derived from other eicosanoids (fatty acids with 20 carbon atoms), with PGE_3 having an additional *cis* double bond at carbon-17, and PGE_1 being devoid of the double bond at carbon-5. However, the E_2 prostaglandins, derived from arachidonic acid, are the most abundant. Variations on the ring give PGD_2 by interchanging the carbonyl and hydroxyl or $PGF_{2\alpha}$ with a hydroxyl group at carbon-9. Compounds with other rings and similar side chains (Fig. 1) such as the endoperoxides (PGG_2 and PGH_2), TXA_2, and PGI_2, are also physiologically active. Strictly speaking, TXA_2, devoid of a prostanoic acid skeleton, is not a prostaglandin. These structures are reviewed elsewhere in detail (*9*).

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Biosynthetic Pathways for Arachidonic Acid Oxygenation

Cyclooxygenase. The precise mechanism for releasing free fatty acid precursors of prostaglandins has not been elucidated. It is generally agreed, however, that they originate largely from phospholipid reserves in cell membranes. Although phospholipase A_2 has been recognized as an important enzyme in the release of these precursor acids, recent studies with platelets implicate a phosphatidyl inositol-specific phospholipase C, yielding diacylglycerides and, subsequently, arachidonic acid (*10*). However, the importance of this new pathway in other cell types has not yet been established. Of the three substrate fatty acids, *cis*-5,8,11,14-eicosatetraenoic acid (arachidonic acid), *cis*-8,11,14-

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eicosatrienoic acid, and *cis*-5,8,11,14,17-eicosapentaenoic acid, the first (arachidonic acid) is most relevant to prostaglandin physiology. It is the only one that is converted enzymatically to an active thromboxane and prostacyclin. As shown in Fig. 2, arachidonic acid is oxygenated to PGG_2 by prostaglandin cyclooxygenase, and this hydroperoxy acid is largely converted to PGH_2 by a hydroperoxidase. PGH_2 is then a substrate for thromboxane synthetase, PGI_2 synthetase, PGD_2 isomerase, or PGE_2 isomerase, and this pathway appears to predominate in most cells. The alternative pathway, the direct conversion of PGG_2 to 15-hydroperoxy PGE_2 (*11*) and hydroperoxy TXA_2 (*12*), has been demonstrated with isolated enzymes but does not appear to be important in vivo.

The action of nonsteroidal anti-inflammatory drugs (NSAID's) on the cyclooxygenase, the first enzymatic step in the oxygenation sequence, necessarily regulates the synthesis of all subsequent compounds. When prostaglandins were first associated with inflammation, only the PGE's and PGF's were recognized as biologically active (*13*). Consequently, these substances, sometimes referred to as primary prostaglandins, were implicated as inflammatory mediators. However, in 1973, PGG_2 and PGH_2, previously thought to be ephemeral intermediates, were isolated (*14*). Despite having a half-life of only a few minutes, these substances have biological activities exceeding those of the primary prostaglandins.

Although PGE_2 and PGF_2 have opposing actions in special instances, with the discovery of TXA_2 and PGI_2 (*15, 16*), the possibility of other prostaglandins having opposing effects surfaced. TXA_2 has a half-life of seconds and is most potent of the eicosanoids in contracting aortic tissue and triggering platelet aggregation. In fact, there is substantial evidence that TXA_2 is the major prostaglandin-related mediator of platelet aggregation (*15*). In contrast, PGI_2 relaxes aortic tissue and prevents platelet aggregation by antagonizing the actions of TXA_2 in these tissues (*16*). Since NSAID's inhibit cyclooxygenase and, thereby, depress the formation of these products, those mentioned above must now also be considered as potential inflammatory mediators. However, despite the isolation of the biologically active compounds PGG_2 and PGH_2, the original concept of the endoperoxides being intermediates in inflammation appears to be correct. Addition of these precursor endoperoxides to various tissues gives responses characteristic of the enzymatic makeup of that tissue, that is, they cause TXA_2 synthesis in platelets and PGI_2 synthesis in aorta. Further evidence for the obligatory metabolism of PGH_2 to such active products is the finding that the potent TXA_2 synthetase inhibitor OKY-1555 (*17*) inhibits the ability of PGH_2 to trigger platelet aggregation (*18*).

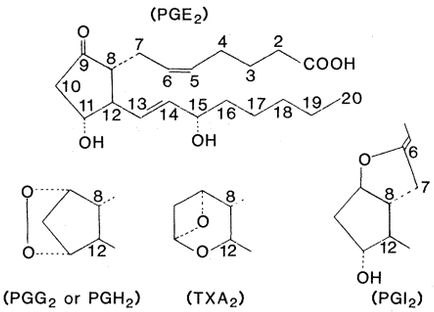

Fig. 1. Structures of prostaglandins.

Summary. The enzymatic oxidation of arachidonic acid has been shown to yield potent pathological agents by two major pathways. Those of the prostaglandin (PG) pathway, particularly PGE_2, have been implicated as inflammatory mediators for many years. The discovery and biological activities of thromboxane A_2 and prostacyclin as well as a destructive oxygen-centered radical as additional products of this biosynthetic pathway now require these to be considered as potential inflammatory mediators. Like PGE_2, their biosynthesis is prevented by nonsteroidal anti-inflammatory agents. More recently, the alternative metabolic route, the lipoxygenase pathway, has been shown to yield a new class of arachidonic acid oxygenation products, called the leukotrienes, which also appear to be important inflammatory mediators. Unlike the prostaglandins, some of which play important roles as biological regulators, the actions of the lipoxygenase products appear to be exclusively of a pathological nature.

Lipoxygenases. Mammalian lipoxygenase enzymes provide a competitive route for oxygenation of free arachidonic acid (Fig. 2) (*19*). Many cells possess both lipoxygenase and cyclooxygenase; however, despite their identical substrate requirements, the former is insensitive to NSAID's. The primary products of arachidonic acid and lipoxygenases are hydroperoxyeicosatetraenoic acids (HPETE's). Although the example in Fig. 2 (5-HPETE) has substitution at the fifth carbon, the location varies among cell types with substitution also being possible at the 5, 8, 9, 11, 12, or 15 positions. Whereas polymorphonuclear leukocytes (PMN's) can form HPETE's with substitution at each of the first five positions concurrently (*20*), other cells such as platelets, which are reported to generate only 12-HPETE, are more selective (*21*). These hydroperoxides can be metabolized to either the analogous alcohol, or to leukotrienes. The alcohols result from peroxidatic reduction which occurs rapidly with any of the hydroperoxides. In contrast, the leukotrienes are formed only from 5-HPETE by epoxide formation (leukotriene A) with subsequent ring opening either with water to give a diol (leukotriene B) (*22*) or with glutathione to give the slow-reacting substance of anaphylaxis (leukotriene C) (*23*). Since the various compounds in this class are only now being elucidated, and variations on these basic structures have already been identified, it appears likely that a large number of leukotrienes will eventually be found.

Prostaglandin Biosynthesis and Anti-inflammatory Activity

The discovery that aspirin and indomethacin block the synthesis of prostaglandins (*24*) led many laboratories to investigate the involvement of prostaglandins in inflammation. As is evident from the scheme in Fig. 2, there are several points in the biosynthetic pathway at which it might be possible to suppress the formation of primary prostaglandins. The cyclooxygenase was established as the site of action of NSAID's because the uptake of molecular oxygen was blocked by these compounds (*25*).

Acute inflammation is commonly measured by the edema induced in a rat paw by subplantar injection of carrageenan (*26*). It is also possible to measure edema in a mouse ear caused by topical application of phorbol esters (*27*). In both tests they effected increases in tissue weight within a few hours. The anti-inflammatory activity of a compound is assessed by administering it topically, intraperitoneally, or orally and measuring the decrease in the edematous weight gain.

Nonsteroidal anti-inflammatory drugs. A good correlation has been demonstrated between the capacity of a variety of structures to inhibit prostaglandin synthesis in vitro and their ability to suppress inflammation in the rat paw edema

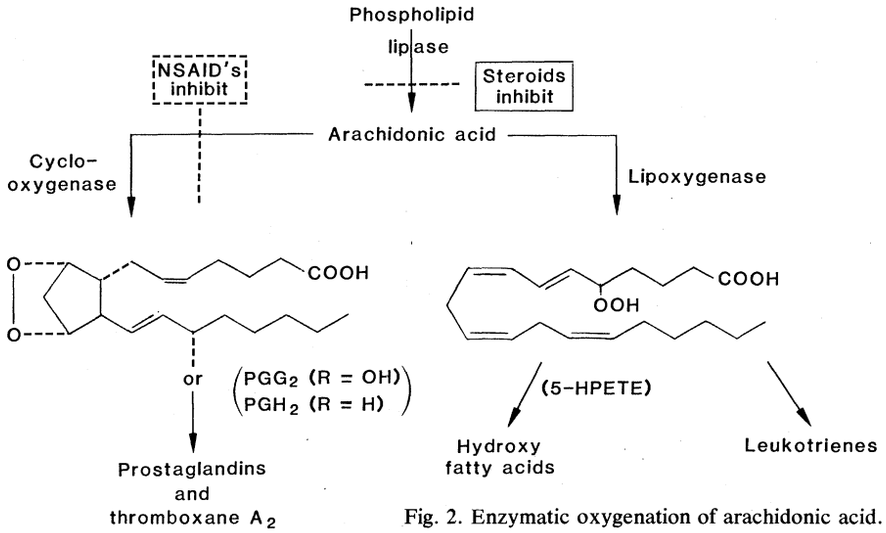

Fig. 2. Enzymatic oxygenation of arachidonic acid.

assay (*28*). Particularly convincing is the correlation between optical isomers of two pairs of NSAID's, one pair structurally related to indomethacin and the other belonging to the aryl acetic acid series. In both instances, the (+) isomer was significantly more active than the (−) isomer on cyclooxygenase and foot edema, and clinical studies on the indomethacin-related pair gave similar results (*28*). Several other laboratories have further strengthened the correlation (*29*). Nevertheless, there are also instances in which inhibitors of cyclooxygenase are not effective anti-inflammatory agents. However, metabolic stability and appropriate pharmacokinetic properties are essential to the action of any drug in vivo, and the accumulation after ingestion of effective NSAID's in inflamed joints has been determined by radiographic studies (*30*). Considering these added factors, the correlation between the activity of NSAID's as cyclooxygenase inhibitors in vitro and their anti-inflammatory activity in vivo is very convincing.

Glucocorticoids. Glucocorticoids express anti-inflammatory activity by means of a complex series of cellular responses. In general, they interact with a specific cytosolic receptor and ultimately alter protein synthesis. A host of effects results from this action, among which is decreased release of arachidonic acid from phospholipids (*31*). Since only unesterified fatty acids are used by the cyclooxygenase (Fig. 2), this depresses all cyclooxygenase-derived products. For example, bradykinin-stimulated synthesis of prostaglandins in isolated 3T3 cells was blocked by dexamethasone which thus acted as an inhibitor of arachidonic acid release (*1*). It appears that glucocorticoids effect this inhibition by steroid-directed synthesis of a specific protein (*32*).

The superior efficacy of steroidal compared to nonsteroidal anti-inflammatory drugs is consistent with the ability of the former to induce responses in addition to inhibition of prostaglandin biosynthesis. For example, macrophages are essential to chronic inflammation and release both prostaglandins and tissue-destructive lysosomal enzymes. In studies with cultured cells, indomethacin completely blocked formation of PGE_2 and PGI_2 but had no effect on lysosomal enzyme release; dexamethasone inhibited both (*2, 33*). Nevertheless, despite their potential therapeutic advantages, adverse side effects of steroids such as bone degeneration and suppression of the immune response limit their use as anti-inflammatory agents.

Increase in Endogenous Prostaglandin During Inflammation

Studies of the relative concentrations of cyclooxygenase-derived products in numerous inflammatory lesions [for review, see (*34*)] have led to the conclusion that PGE_2 and $PGF_{2\alpha}$ concentrations increase in a number of inflammatory conditions. In addition, both PGI_2 and TXA_2 have been identified in inflammatory exudates (*35*).

The origin of cyclooxygenase-derived products in inflamed joints has not been established. The PMN's, phagocytic cells essential for the initiation of acute inflammation, are relatively poor producers of prostaglandins (*36*). Another phagocytic cell, the monocyte, is somewhat more productive, and the macrophage derived therefrom releases large amounts of PGE_2 and PGI_2 in response to inflammatory stimuli (*2*). TXA_2 production by this cell has also been reported (*37*). The low capacity of PMN's to produce prostaglandins and the occurrence of acute inflammation in the absence of macrophages, suggest that cyclooxygenase-derived compounds are produced locally during the initial stage of inflammation. For example, interstitial and synovial cells have the capacity to produce copious quantities of prostaglandins (*38*). The macrophage subsequently contributes to chronic inflammation. However, irrespective of the source, it is clear that there are high concentrations of cyclooxygenase-derived products in the inflamed joint and that reduction of these concentrations is associated with reduced inflammation.

Cyclooxygenase-Derived Products Mimic Symptoms of Inflammation

Although there may be some overlap among the causes of the symptoms of acute inflammation, it is convenient to divide these symptoms into three categories: erythema, edema, and pain. At a time when only PGE's and PGF's were identified as major biologically active prostaglandins, subdermally administered PGE's were shown to cause itch and flare responses in the human forearm whereas PGF's were inactive (*39*). Additional studies in animals have confirmed the efficacy of PGE's as vasodilatory agents capable of inducing erythema (*40, 41*). However, neither PGE's nor PGF's fully induce edema or pain. Nevertheless, the edematous and pain response caused by histamine or bradykinin could be enhanced dramatically by PGE_1, leading to the suggestion that PGE's are modulators of inflammation (Fig. 3) (*41*). Although this interpretation fitted most facts at that time, it did not explain the capability of NSAID's, acting at the cyclooxygenase level, to suppress all three symptoms of inflammation. With our current realization that other biologically active products are derived from the cyclooxygenase and that bradykinin releases arachidonic acid from cell membranes, it is now possible to suggest that PGE_2 acts in conjunction with other products of this biosynthetic pathway and, thereby, accounts for the efficacy of NSAID's.

Recently, PGI_2 has been shown to potentiate the effect of histamine and bradykinin in carrageenan-induced rat paw edema (*42*). In a comparative study of the effects of PGE_2, PGI_2, PGD_2, and $PGF_{2\alpha}$ on plasma exudation and blood flow, both PGE_2 and PGI_2 potentiated the action of bradykinin. The effects of PGD_2 and $PGF_{2\alpha}$, however, were too small to have physiological significance. Although PGI_2 was five to ten times less

potent than PGE_2 (*43*), PGI_2 proved considerably more effective than PGE_2 in inducing pain (*44*). The ability of PGI_2 to cause hyperalgesia seems to relate to its capacity to stimulate adenosine 3′,5′-monophosphate (cyclic AMP) (*61*), a property characteristic of the E-type prostaglandins (*45*). Thus, these limited studies support a role for PGI_2 in inflammatory conditions.

Considering the extreme lability of TXA_2, it would be very difficult to establish its ability to trigger inflammatory responses. However, studies in our laboratory have shown that OKY-1555, an extremely potent inhibitor of TXA_2 synthesis with no appreciable cyclooxygenase inhibition (*17*), has no effect on mouse ear edema (*46*).

Implications of the Actions of NSAID's

Despite the convincing evidence that, as a class, NSAID's suppress inflammation by acting at the level of the cyclooxygenase, these compounds have also been shown to inhibit a number of other enzymes. Indomethacin, because of its wide use and availability, has been particularly well studied and is known to inhibit prostaglandin 15-dehydrogenase, phosphodiesterase, dopa decarboxylase, histidine decarboxylase, a platelet peroxidase, diglyceride lipase, and a protein kinase (*47*). However, with the possible exception of the kinase, the concentration required to inhibit these enzymes is 100- to 1000-fold greater than that necessary to inhibit the synthesis of prostaglandins.

Recent work shows that NSAID's are capable of interacting with two sites on the cyclooxygenase (*48*). Occupation of the secondary site does not necessarily inhibit catalytic activity, although occupancy appears to be essential for cyclooxygenase inhibition. This supplementary site may be simply a lipophilic pocket in the enzyme that exhibits a high affinity for the corresponding nonpolar moiety of the inhibitor, a concept consistent with the structure of NSAID's (*48*). The high affinity of NSAID's for albumin and the remarkable correlation of this property with the efficacy of these compounds as anti-inflammatory drugs indicate that this lipophilic region is common to many proteins and enzymes (*49*). Hence, the wide range of activities of high concentrations of NSAID's may simply be a reflection of an interaction with this commonly occurring lipophilic site and have little bearing on the catalytic component of action of NSAID's.

Since NSAID's almost fully prevent the formation of prostaglandins in vivo, as reflected by urinary metabolites (*50*), and only occasionally have deleterious effects, prostaglandins appear on the one hand to be involved primarily in pathological responses. On the other hand, prostaglandins can mimic hormone action by triggering a specific membrane receptor to increase intracellular cyclic AMP formation, favoring a regulatory as well as a pathological role (*51*). Indeed, the fraction of the prostaglandins involved in cellular regulation may be small compared to the total prostaglandin output; those detected as urinary metabolites would be formed primarily in tissues with unusually high biosynthetic capacity such as the kidney (*52*).

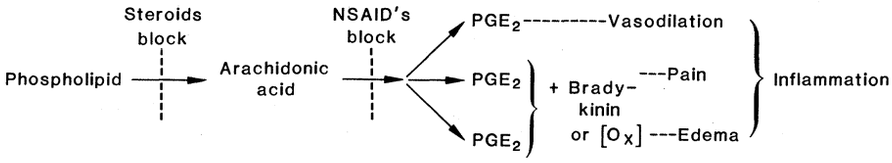

Fig. 3. Relationship of prostaglandins to inflammation.

Furthermore, anti-inflammatory action depends on accumulation of a drug at the inflamed site as well as on its innate activity, and NSAID's concentrate in the stomach, kidneys, and inflamed joint (*30*). Since prostaglandins are known as cytoprotective agents in the stomach wall (*53*) and as mediators of renal blood flow, salt and water loss, and angiotensin release (*54*), this accumulation explains the side effects of NSAID's often observed in these particular organs. Thus, in most tissues where prostaglandins are essential modulators, because of pharmacokinetic considerations their synthesis may not normally be depressed sufficiently by NSAID's to interfere with their regulatory role. In addition, some prostaglandins are antagonistic, for example, PGI_2 prevents platelet aggregation whereas TXA_2 promotes it. In view of this relationship, depressing both compounds by inhibiting the cyclooxygenase (Fig. 2) would not be as consequential as decreasing only one. Therefore, although prostaglandins may increase and be intimately involved in certain pathological states, they are also important under normal physiological conditions.

Involvement of Lipoxygenase Products in Acute Inflammation

The involvement of the products of the lipoxygenase enzymes in inflammation is based on rather tenuous evidence. The finding that 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) is chemotactic to PMN's drew attention to this phenomenon since the influx of these cells is essential for initiation of inflammatory responses (*55*). Other products of the lipoxygenase pathways, including the hydroperoxy acids (HPETE's) and the corresponding HETE, are also chemotactic to PMN's, the order of activity in the latter case being 5-HETE > 8-HETE ≈ 9-HETE > 12-HETE > 15-HETE ≈ 0 (*20*). However, the recent report that leukotriene B (5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid) is more chemotactic by several orders of magnitude than the other lipoxygenase products suggests this to be the truly relevant lipoxygenase product in inflammation (*56, 57*).

Although the chemotactic property of lipoxygenase products seems very secure, attempts to relate this property to inflammation by employing enzyme inhibitors are difficult to evaluate because of the absence of inhibitors that are specific for the lipoxygenase pathway. Unlike NSAID's, which block cyclooxygenase at concentrations that have no effect on lipoxygenases, all effective lipoxygenase inhibitors known to us also inhibit cyclooxygenase in varying degrees. Extensive studies with benoxaprofen and 3-amino-*o*-[*m*-(trifluoromethyl)phenyl]-2-pyrazolone (BW755C) (*58*), compounds with mixed activities against both enzymes but with a preference for lipoxygenase inhibition, have not provided convincing evidence in support of the concept that the lipoxygenase pathway is important in acute inflammation. A more definitive statement regarding the involvement of this pathway in inflammation awaits the discovery of more selective inhibitors.

Oxygen-Centered Radicals in Inflammation

Superoxide. Polymorphonuclear leukocytes and macrophages, in the course of phagocytosing bacteria and other foreign particles, consume a large amount of molecular oxygen which is transformed by membranous NADPH oxidase to superoxide ($O_2\cdot^-$) and subsequently to other oxidizing species (*59*). These phagocytic cells also respond to particulate and nonparticulate inflamma-

tory stimuli in a similar manner, and a substantial portion of $O_2 \cdot^-$ is made available to the external medium (*60*). Superoxide anion is yet further implicated in inflammation by the actions of superoxide dismutase (SOD), an enzyme that dismutes $O_2 \cdot^-$ to H_2O_2 and O_2 and is anti-inflammatory in animal models (*61*). Nevertheless, the low oxidizing potential of $O_2 \cdot^-$, coupled with its capacity to form a hydroxyl radical (·OH) via the iron-catalyzed Haber-Weiss reaction, suggested that the ·OH that reacts with virtually any organic compound was the actual inflammatory species (*62*). This radical would be depressed by either SOD or catalase.

Long-lasting derivatives of SOD and catalase have been prepared by coupling with polyethylene glycol and other polymers (*63*). If ·OH, produced by the Haber-Weiss reaction, were causal in inflammation, either enzyme, SOD by its conversion of $O_2 \cdot^-$ to hydrogen peroxide or catalase by its conversion of H_2O_2 to O_2, would prevent the formation of ·OH by removing one of the essential components of the Haber-Weiss reaction. Since the SOD but not the catalase derivatives showed anti-inflammatory activity in animal models of inflammation, an effect associated with a decrease in leukocyte infiltration in the inflamed site, superoxide itself, and not ·OH derived from it, was reasoned to participate in this inflammatory response (*63*). In a more recent study, treatment of serum with a superoxide-generating system produced a potent chemotactic factor, suggesting that a product of the reaction of the serum with $O_2 \cdot^-$ is the inflammogen (*64*). Subsequent studies revealed the active product to be lipid-soluble, possibly an oxygenated fatty acid (*64*). Others have also shown that reaction of arachidonic acid with a superoxide-generating system yields an extremely potent chemotactic agent (*65*). These findings raise the interesting possibility that substances similar to those derived from the lipoxygenase pathway initiate the inflammatory response, but that they originate from the reaction of $O_2 \cdot^-$ with arachidonic acid rather than from the lipoxygenase enzymes.

Oxidizing radicals derived from the prostaglandin biosynthetic pathway. During the metabolism of arachidonic acid by the microsomal prostaglandin synthetase complex, cooxygenations of reducing agents such as epinephrine and luminol have been identified (*66*). A study of the enzyme that converts PGG_2 to PGH_2, prostaglandin hydroperoxidase, showed that the oxidant (see $[O_x]$ in Fig. 4) released from the hydroperoxy group during the peroxidatic reduction is

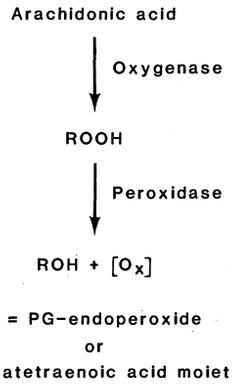

Fig. 4. Prostaglandin cyclooxygenase-peroxidase catalyzed reactions.

responsible for this cooxygenation phenomenon (*67*). In addition, this oxidizing moiety was found to be capable of deactivating certain enzymes of the prostaglandin biosynthetic pathway. In fact, compounds with reducing groups susceptible to this oxidizing species stimulated the metabolism of arachidonic acid to prostaglandins by countering its destructive action on the cyclooxygenase (*67, 68*). Thus, the reason for the obligatory requirement of epinephrine, phenol, and hydroquinone for maintaining full cyclooxygenase activity in reactions in vitro is now explained on the basis of the ability of these reducing cofactors to scavenge the oxidant released from PGG_2.

A characteristic of this peroxidase is the ability of the oxidant released to act on a wide variety of organic compounds even including proteins. Thus this enzyme has generalized destructive effects on cells, whereas the function of other peroxidases such as glutathione peroxidase or catalase is clearly to reduce hydroperoxides to less potentially toxic materials. Hence, it was suggested that prostaglandin hydroperoxidase is responsible for some of the cellular damage that characterizes inflammatory lesions (*69*). In support of this concept is the finding that scavengers exhibit anti-inflammatory activity in animal models of inflammation (*70*). In the mouse ear edema assay, this could account for the effects of structures as diverse as phenol, lipoic acid, and sodium iodide, all of which also can scavenge the oxidant (*69, 70*).

Low concentrations of hydroperoxides are required for the cyclooxygenase reaction to start (*71*). Hence, the degree of prostaglandin synthesis can depend on the oxidative state of the cell. As noted previously, low concentrations of reducing agents stimulate the cyclooxygenase, probably by protecting against oxidative deactivation. Conversely, higher concentrations of the same materials inhibit, probably by lowering hydroperoxide levels below those required for initiation of cyclooxygenase activity (*72*). Extending this concept to the cellular level, the sum of endogenous and exogenous reducing agents could regulate prostaglandin synthesis, an effect which would vary among cell types depending on the endogenous concentration. Indeed, both stimulation (*73*) and inhibition (*74*) have been observed to occur with reducing agents at the cellular level.

Although enzymes of the prostaglandin biosynthetic pathway are susceptible to oxidative deactivation by the peroxidase-derived oxidant to varying degrees (*67–70*), the anti-inflammatory activity of scavengers must be explained on another basis. However, the finding that hydroperoxides of arachidonic and other unsaturated acids react with prostaglandin hydroperoxidase to generate the same oxidizing species as does PGG_2 (Fig. 4) may have some bearing on circulatory diseases. Prostacyclin synthetase is particularly sensitive to the oxidant generated by the reaction of 15-HPETE and other hydroperoxy acids with this peroxidase (*75*). This finding contrasts with the report that 15-HPETE itself is a potent inhibitor of this enzyme (*76*). Since TXA_2 synthetase is resistant to such oxidative deactivation, reactions of lipid peroxide with prostaglandin hydroperoxidase have the potential for altering the PGI_2/TXA_2 ratio in favor of the latter, a change that would be expected to favor formation of atherogenic plaques (*75*). This may explain the adverse effects ascribed to lipid peroxides in circulatory diseases.

To explain the anti-inflammatory action of scavengers of the peroxidase-generated oxidant, targets other than enzymes of the prostaglandin biosynthetic pathway must be explored. The finding that the ability of 15-HPETE to contract the guinea pig ileum is blocked by either NSAID's or oxidant scavengers implicated the oxidant in the release of arachidonic acid from phospholipid pools (*77*). Depending on the sensitivity of this site compared to enzymes of the prostaglandin biosynthetic pathway, such an action would trigger both the cyclooxygenase and lipoxygenase pathways, with obvious implications in inflammatory diseases. In support of this premise, 13-hydroperoxylinoleic acid triggers the release of leukotriene C from the perfused guinea pig lung (*78*). The ability of high concentrations of reducing agents to prevent cyclooxygenase initiation may also be relevant in vivo. Clearly, this area requires further study.

Beneficial Role of Prostaglandins in Chronic Inflammation

Although suppression of the synthesis of prostaglandins by NSAID's does inhibit acute inflammation and suppress the overt symptoms in chronic inflammation and arthritis, in arthritis the destructive nature of the disease continues unabated. In adjuvant arthritis, an animal model of chronic inflammation, high pharmacological doses of PGE_2 were found to suppress the inflammatory response (*79*). Since these initial findings, the question of a beneficial role of prostaglandins in chronic inflammation has remained a controversial issue. There is substantial evidence that E-type prostaglandins inhibit some lymphocyte functions including mitogen responsiveness, antibody response, T-lymphocyte cytotoxicity, lymphokine secretion, and antibody-dependent, cell-mediated cytotoxicity (*80*). These actions are believed to be secondary to those of prostaglandins in increasing intracellular concentrations of cyclic AMP (*81*). Consequently, glucocorticoids, by inhibiting the release of arachidonic acid and thus suppressing release of prostaglandins, might be expected to lower cyclic AMP concentrations and enhance release of lymphocytic pro-inflammatory products. The fact that they do not, emphasizes the complex nature of steroid action. If one bears in mind that in the macrophage, release of destructive lysosomal enzymes is divorced from PGE_2 synthesis, it is evident that immunosuppressive action of glucocorticoids depends on a different mechanism. Clearly, the immune aspect of chronic inflammation is a matter of prime importance, and a full understanding of this process is essential for the development of useful drugs in this area.

Conclusion

It is evident that the initial oxygenation of arachidonic acid by cyclooxygenase leads to a far greater variety of active metabolites than envisioned almost a decade ago when indomethacin and aspirin were shown to inhibit the enzyme. Although there is no reason to question the role of PGE_2 in inflammatory responses, PGI_2 and an oxidizing moiety, a by-product of the prostaglandin biosynthetic pathway, also appear to be important inflammogens, subject to control by NSAID's. The recent discovery that the slow-reacting substance of anaphylaxis is an eicosanoid (leukotriene D), adds yet another group of arachidonic acid oxygenation products that seem likely to contribute to inflammatory processes.

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Science and the University

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Summary. The federal government-university relationship in scientific research has been eroded by excessive, unthinking regulations for the purposes of accountability. The Office of Management and Budget's Circular A-21 continues to jeopardize the quality of government-sponsored research in universities by demanding wasteful, meaningless work-load documentation. These regulatory demands must be revised to reflect the realistic obligations of accountability by a leadership capable of transcending special interests. Mutual respect between government and universities must be restored to achieve a partnership that helps better the national life while also protecting the integrity of the scientific faculty and its mission.

I have chosen to speak on the difficulties and challenges of doing basic research in science in a research university. Basic research is not, of course, confined to the activity of scientists. Basic research, that is, investigation that seeks new knowledge and understanding rather than solutions to immediate problems, is the essential nature of research on the part of all scholars. It obviously includes but is not restricted to basic research in the biological, medical, physical, and many social sciences. In the sciences, however, there is a particular style to the enterprise. Teaching in these areas, done in laboratories, in groups or teams, through colloquia, on field trips, with undergraduates and graduate and postdoctoral students, with assistants and associates in research, is intimately and inextricably connected to research. In science, teaching and research not only go hand in hand, they are often the same hand, the pedagogical act an act of investigation, the investigatory act shared with students and associates who are also colleagues, the whole a splendid instance of intellectual and human collaboration. Of course, scientists also work alone. Not all that is done is the result of a group effort. And not everything that is done occurs in a unified act that is both pedagogical and investigatory. But the distinctive style of scientific investigation is collaborative, and the distinctive process is such that is it impossible finally to distinguish research from teaching, seeking from sharing.

Federal Support of Basic Research

The dollars involved in supporting and furthering this kind of basic research are immense. They are largely federal dollars, which is to say taxpayers' dollars. In constant 1972 dollars, the government spent $2.8 billion on basic research in 1978, up $1.8 billion since 1960, when the reaction to Sputnik was in full flight. In 1958, 32 percent of all basic research in America was done in universities; by 1978, 52 percent was being done in universities. And in those universities, in 1978, 72 percent of the money for basic research came from the federal government. The result of this federal support to university-based science has been tremendous improvements in the life of America's citizens. In health care, in the production of food, in the handling of information—in the quality of our life—our government has brought about massive benefits by encouraging science and scientific research in universities.

The federal money that comes to universities brings with it money for the support of the administration of these complex projects; it brings reimbursements for "indirect costs." Indirect costs, or overhead, provide reimbursement for expenses which cannot be accurately assessed for each research project. They include, therefore, reimbursements for part of the cost of heating, cooling, and maintaining research laboratories, as well as part of the cost of essential supporting services (such as accounting and purchasing). Finally, these reimbursements bear part of the price of meeting federal requirements in certain areas (affirmative action, biosafety, the protection of human subjects). In 1960, Yale received some $24 million in federal funds, $3 million of which was indirect cost money; in 1980, Yale received $68 million in federal money, $21 million of which was in indirect costs. Thus about 30 percent of the total operating budget of the university—a great deal of money, but not a particularly high percentage compared to that at other universities—comes from the government.

It was not difficult for the government in the last 20 years partially to turn universities into installations for federally sponsored basic research in space, cancer, agriculture, energy, and a thousand other areas. Scientists were delighted to have their work supported and appreci-

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