[CANCER RESEARCH 52, 5575-5589, October 15, 1992]

Perspectives in Cancer Research

Aspirin and the Potential Role of Prostaglandins in Colon Cancer[1]

Lawrence J. Marnett

A. B. Hancock Jr. Memorial Laboratory for Cancer Research, Departments of Biochemistry and Chemistry, Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

The arachidonic acid cascade generates a family of bioactive lipids that modulate diverse physiological and pathophysiological responses. Although there has been a great deal of interest in the involvement of arachidonic acid metabolites in cancer, there is uncertainty about which metabolites are the most important and how they contribute to specific steps in cell transformation, tumor growth, and metastasis (1-9). The complexity of arachidonic acid metabolism is daunting to neophytes and the multiplicity of responses exerted by a single metabolite can be confusing to the cognoscenti. This has made it difficult to extrapolate results from animal experiments to human beings.

A recent report from Thun *et al.* (10) and previous reports from Kune *et al.* (11) and Rosenberg *et al.* (12) suggest that aspirin intake may reduce colon cancer incidence (11, 12) and mortality (10) in the general population. Other studies from Paganini-Hill *et al.* (13, 14) do not support such an association. Aspirin is a relatively specific inhibitor of one branch of the arachidonic acid cascade and even though there is not universal agreement among these epidemiological investigations, their findings refocus attention on the role of arachidonic acid metabolites in cancer. This Perspective was written to highlight some of the existing literature on the aspirin-inhibitable branch of the arachidonic acid cascade and cancer, to apply that literature to colon cancer where possible, and to identify deficiencies in our knowledge that need to be addressed. Although the major focus is the colon, the mechanistic concepts should be applicable to other organ sites.

The Perspective begins with an overview of arachidonic acid metabolism, proceeds to a consideration of the biochemistry and molecular biology of the enzyme inhibited by aspirin, touches on the signal transduction pathways linked to arachidonic acid metabolism, and then considers mechanisms by which specific arachidonic acid metabolites participate in the carcinogenic process. The review is not meant to be comprehensive and apologies are made in advance to colleagues whose work is not highlighted or was inadvertantly omitted.

Arachidonic Acid Metabolism

Arachidonic acid is an essential fatty acid consumed in the diet or derived from elongation and desaturation of dietary linoleic acid (15). Most polyunsaturated fatty acids in cells are esterified to phospholipids so release of substrate from these intracellular stores is an important control point in eicosanoid biosynthesis (16, 17). Arachidonic acid mobilization is linked to signal transduction pathways that result in the activation of phospholipase C and phospholipase A_2 (18-20). Once arachidonic acid is released from phospholipids it is oxidized by one of three different oxygenases (Fig. 1) (21, 22). Certain cytochromes P-450 insert a single oxygen atom into the double bonds of arachidonic acid to produce epoxy-arachidonic acids (23). Lipoxygenases introduce one molecule of O_2 into the carbon framework of arachidonic acid to produce a series of isomeric hydroperoxy acids (24). These hydroperoxy fatty acids are converted to hydroxy fatty acids, to leukotrienes, and to lipoxins (22). Cyclooxygenase introduces two molecules of O_2 into arachidonic acid to form the hydroperoxy endoperoxide PGG_2,[2] which is reduced by the peroxidase activity to the hydroxy endoperoxide, PGH_2 (Fig. 1) (25-27). PGH_2 is transformed to PGs, TxA_2, PGI_2, and MDA (21).[3] Oxygenation by cytochromes P-450, lipoxygenases, and cyclooxygenase competes with reesterification to phospholipid; therefore eicosanoid biosynthesis is usually pulsatile (28). Eicosanoids are not stored but are synthesized on demand; therefore inhibitors of the various oxygenases and peroxide-metabolizing enzymes have instantaneous effects on steady-state eicosanoid levels (16, 17).

Each prostaglandin has its own range of biological activities; therefore the physiological response to arachidonic acid oxygenation in a given tissue is largely determined by the levels of PGH_2-metabolizing enzymes in the cells making up that tissue (29, 30). For example, platelets divert most of the PGH_2 they biosynthesize to TxA_2, which is a potent proaggregatory and vasoconstrictive agent (27). In contrast, vascular endothelial cells convert PGH_2 mainly to PGI_2, which is an inhibitor of platelet aggregation and a vasodilator (31). Potent inhibitors of TxA_2 synthase have been developed but selective inhibitors of the other enzymes of PGH_2 metabolism are not available (32, 33). Thus, the most common strategy for pharmacological modulation of this branch of the arachidonic acid cascade is inhibition of the cyclooxygenase reaction.

Prostaglandin Endoperoxide Synthase

The protein that oxygenates arachidonic acid to PGG_2 also reduces PGG_2 to PGH_2 (34-36). For this reason, it is called prostaglandin endoperoxide synthase or PGH synthase.[4] PGH synthase is a membrane-bound hemeprotein that is widely distributed in mammalian tissue but not present in all cell types (37). The best substrates for its cyclooxygenase activity are 5,8,11,14-eicosatetraenoic acid (arachidonic acid) and 8,11,14-eicosatrienoic acid (17, 38). Other polyunsaturated fatty acids, *e.g.*, 5,8,11,14,17-eicosapentaenoic acid, are not oxygenated as rapidly and under certain conditions act as competitive inhibitors of the oxidation of arachidonic acid (39). The fatty acid composition of cells is sensitive to diet. Arachidonic acid is

Received 5/7/92; accepted 8/3/92.

[1] Supported by a NIH Research Grant CA47479. This manuscript is dedicated to the memory of Joseph Patrick Marnett, who died of colon cancer at age 34.

[2] The abbreviations used are: PG, prostaglandin; NSAIDs, nonsteroidal antiinflammatory drugs; MDA, malondialdehyde; FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; IGF-1, insulin-like growth factor 1; TPA, tetradecanoylphorbol acetate; RPA, retinoylphorbol acetate; ODC, ornithine decarboxylase; LLC, Lewis lung carcinoma; TxA_2, thromboxane; PGI_2, prostacyclin.

[3] For the sake of brevity, PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and TxA_2 will be collectively referred to as prostaglandins even though TxA_2 does not contain the prostanoic acid nucleus. The term eicosanoids will be used for all arachidonic acid metabolites including those produced via the action of lipoxygenases.

[4] Prostaglandin endoperoxide synthase (EC 1.14.99.1) is also referred to as PGG/H synthase.

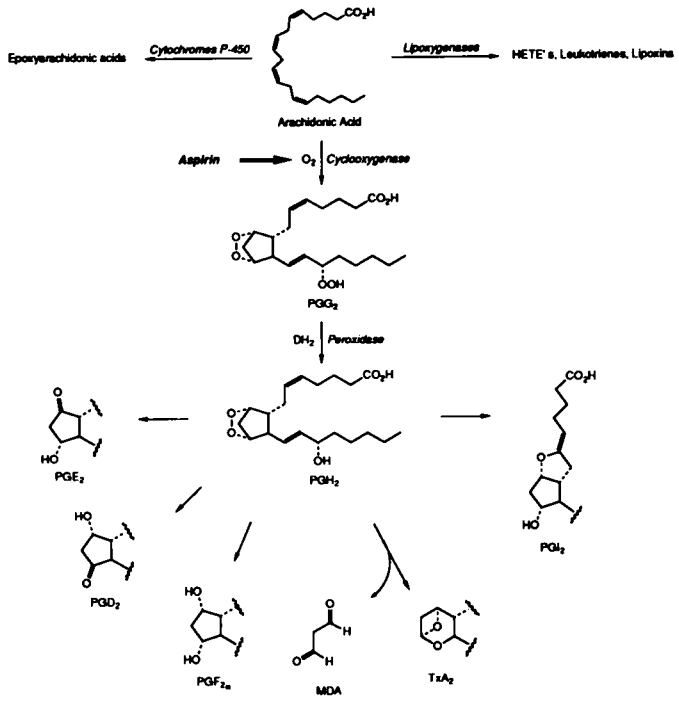


Fig. 1. The arachidonic acid cascade. Metabolite structures are given only for compounds produced via the cyclooxygenase pathway. *HETES*, hydroxy fatty acid. DH_2, reducing substrate.

quantitatively the most important cyclooxygenase substrate in individuals eating a "typical" Western diet. However, eicosapentaenoic acid can become the most abundant cyclooxygenase substrate in individuals who eat large amounts of fish (40).

PGH synthases from ram seminal vesicles, human platelets, and mouse 3T3 cells have been cloned, sequenced, and expressed in heterologous expression systems (41–44). The protein sequences are approximately 90% similar between mice, humans, and sheep. Comparison of sequence homologies and active site labeling experiments have localized the heme binding site and identified potential fifth and sixth ligands to heme iron (44, 45). The mouse and human PGH synthase genes are approximately 25 kilobases long and are composed of 11 exons (Fig. 2) (46, 47). The sizes of the sheep, human, and mouse mRNAs are approximately 2.8–3.0 kilobases (48).

It has been known for some years that prostaglandin synthesis in cells is enhanced by a variety of growth factors, tumor promoters, etc. (49, 50). This was thought to be the result of activation of phospholipases and the attendant release of stored substrate. Although phospholipase activation is an important component of the action of certain agonists, many recent studies reveal significant elevation of PGH synthase protein in response to treatment of cells with various agents (51–67). Enhanced expression results from activation at the transcriptional and translational level but the details of the processes are not yet clear (59, 68, 69).

Very recently, three groups reported the detection of a proliferation-associated gene that is homologous to PGH synthase. Transformation of chicken embryo fibroblasts by Rous sarcoma virus causes the stable expression of a protein with approximately 60% similarity to the mouse PGH synthase protein (now termed PGH synthase 1) (58, 70). Likewise, treatment of mouse fibroblasts with TPA, EGF, interleukin 1β, or serum causes transient expression of an immediate early gene that bears approximately 60% similarity to the sheep PGH synthase 1 sequence (71, 72). Both the *src*- and TPA-stimulated proteins contain many of the key structural elements associated with PGH synthase including the active site residues and the aspirin acetylation site (see below). The complementary DNA for the TPA-stimulated PGH synthase from murine 3T3 cells has been expressed in COS-1 cells and exhibits cyclooxygenase and peroxidase activities (73). This new form of cyclooxygenase from virus-transformed or mitogen-stimulated cells is called PGH synthase 2 and is derived from an 8-kilobase gene composed of 10 exons and 9 introns. The overall exon/intron stucture of the 8-kilobase gene is similar to that of PGH synthase 1 (the 25-kilobase gene) although there are significant differences in the regions corresponding to the NH_2 and COOH termini of the proteins (73). In addition, PGH synthase 2 does not contain a sequence corresponding to the exon coding for the hydrophobic leader sequence of PGH synthase 1 (exon B). The mRNA of PGH synthase 2 is approximately 4.1 kilobases long (48). The greater size of the PGH synthase 2 mRNA is due to a much larger 3′-untranslated region (74).

The detection of a mitogen-inducible PGH synthase gene is a very exciting development and may explain previous reports that viral transformation of cultured fibroblasts dramatically elevates their prostaglandin-biosynthetic capacity (75, 76). At this stage, the generality of these observations and their relation to the control of cellular proliferation is uncertain. For example, a limited survey of different cell lines indicates that treatment with tumor-promoting phorbol esters increased PGH synthase 2 expression in only two cell types (71). Likewise, treatment of *src*-transformed chicken embryo fibroblasts with cyclooxygenase inhibitors does not inhibit growth of the cells (70).

Inhibition of Prostaglandin Biosynthesis

A trademark of PGH synthase is inhibition of its cyclooxygenase activity by a variety of agents known collectively as NSAIDs (77–80). The best known of these are aspirin, indomethacin, ibuprofen, piroxicam, and sulindac (Fig. 3). Aspirin is a competitive inhibitor and also covalently modifies the PGH synthase protein whereas indomethacin, ibuprofen, piroxicam, and sulindac are competitive inhibitors that do not bind covalently to the protein (44, 81–86). Indomethacin has been demonstrated to form a tight 1:1 complex with PGH synthase that dissociates very slowly (87). Covalent modification of PGH synthase by aspirin is the basis for its irreversible inhibition of cyclooxygenase activity.

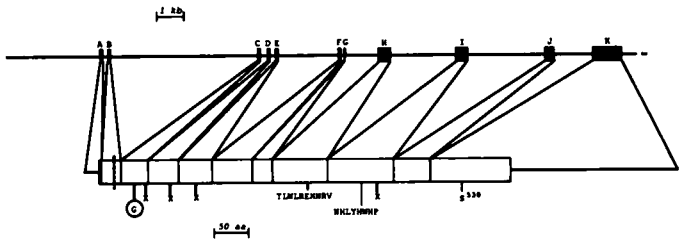


Fig. 2. Gene and protein structure of PGH synthase 1. Exons are identified by *capital letters*. Translated protein is indicated by *open boxes*. Untranslated 5′ and 3′ regions of mRNA are indicated as *lines*. The elements of protein structure are as follows: (*a*) the break in exon B is the cleavage site for removal of the signal sequence; (*b*) the G in exon C represents a region homologous to epidermal growth factor; (*c*) the X's represent consensus glycosylation sequences; (*d*) the polpeptides in exons H and I represent putative heme-binding regions: Ser^{530} is the aspirin acetylation site. *kb*, kilobase. (47).

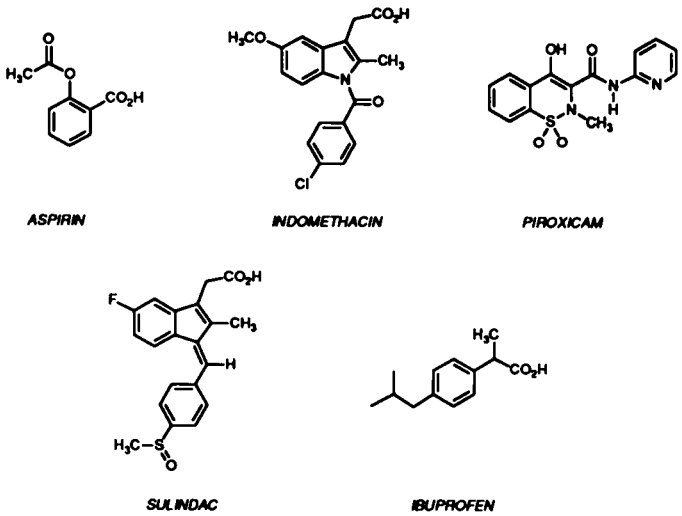
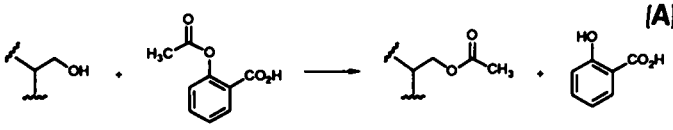


Fig. 3. Structures of some common nonsteroidal antiinflammatory drugs.

Antiinflammatory steroids such as cortisol, dexamethasone, and fluocinolone acetonide also lower prostaglandin production by some cells but they do not inhibit cyclooxygenase activity. Until recently it was believed that steroids inhibited prostaglandin production by inducing the synthesis of a phospholipase inhibitory protein called lipocortin (88). However, this is unlikely to be the major mechanism of inhibition of prostaglandin biosynthesis (89). It has been demonstrated that cortisol and other antiinflammatory steroids are potent inhibitors of interleukin-induced elevation of PGH synthase protein and mechanisms have been proposed at the transcriptional and translational levels to explain this (59, 68, 90, 91). Expression of the PGH synthase 2 gene is particularly sensitive to inhibition by glucocorticoids (48, 92). This may account for the apparent tissue specificity of steroid effects; clinical studies in humans indicate that prostaglandin synthesis in macrophages is particularly sensitive to inhibition by glucocorticoids (93).

Mechanism of Aspirin Inhibition of Cyclooxygenase Activity

Aspirin transfers its acetyl moiety to a single serine hydroxyl group of PGH synthase (Ser^{530}), which blocks the approach of arachidonic acid to the substrate binding site and inhibits cyclooxygenase activity (Equation A) (44, 85)



The acetylated enzyme exhibits normal levels of peroxidase activity (85, 97). Site-directed mutagenesis of the hydroxyl group to a hydrogen atom ($Ser^{530} \rightarrow Ala^{530}$) produces an enzyme with undiminished cyclooxygenase activity but no sensitivity to irreversible aspirin inhibition (44). This suggests Ser^{530} is not essential for cyclooxygenase catalysis and indicates an element of serendipity in the pharmacological activities of aspirin.There are two major determinants of the preferential reactivity of Ser^{530} to aspirin. The first is the heme prosthetic group (95, 96). Although Ser^{530} is well removed in linear sequence from the putative heme-binding residues of PGH synthase, acetylation of its hydroxyl group by aspirin is accelerated at least 100-fold by binding heme to the apoprotein. Considering the short half-life of aspirin in humans, this means that it is essentially a titrant for active holoenzyme (95, 97). The other major determinant of acetylation selectivity is the salicylate moiety of aspirin. Reaction of PGH synthase with other acetyl transferring agents does not selectively acetylate Ser^{530}.[5] This suggests that a specific binding site for the salicylate portion of the molecule exists at or near the substrate binding site of the protein. These selectivity determinants account for the fact that PGH synthase is the principal target of aspirin acetylation in intact cells (82).

Effects of Aspirin on Cardiovascular Disease

Aspirin acetylation of PGH synthase is irreversible (85). Recovery of cellular cyclooxygenase activity following aspirin treatment requires new protein synthesis. In nucleated cells in culture, this usually occurs in a few hours and is dependent on the presence of growth factors in the culture medium (91, 98). *In vivo*, aspirin has a half-life of only 5-10 minutes; therefore it does not persist long enough to inhibit newly synthesized protein (97). Cells with low protein synthetic capacity, such as platelets, are unable to recover cyclooxygenase activity. This differential in cyclooxygenase recovery is an important contributing factor to the "selectivity" of aspirin inhibition of platelet cyclooxygenase activity (78). Another contributing factor is presystemic acetylation of circulating platelets, which precedes first pass metabolism of aspirin in the liver (99). Thus, TxA_2 synthesis is eliminated by aspirin treatment and does not recover until new platelets are produced (a matter of 3-7 days). In contrast, PGH synthase in vascular endothelial cells is less extensively acetylated than the platelet enzyme and recovers within a few hours after aspirin treatment (100). This results in only a transient reduction in PGI_2 levels. Periodic administration to humans of low doses of aspirin selectively depresses TxA_2 synthesis and reduces platelet responsiveness to agonist stimulation (101–104). A single dose of 40–80 mg aspirin dramatically reduces TxA_2 biosynthesis without significantly affecting PGI_2 biosynthesis (105). This selectivity is lost at doses of 80–325 mg (105). The pharmacological effect of aspirin on the cardiovascular system is an example where the axiom "if a little bit works, a lot works better" does not hold.

Low-dose aspirin was efficacious relative to placebo in preventing myocardial infarction in a selected group of healthy physicians (106). However, the generalizability of this finding to other populations is uncertain. The dose of aspirin used in the Physicians Health Study was equivalent to a single adult aspirin every other day but pharmacokinetic studies suggest that lower doses may be just as effective (107). A timed-release formulation of aspirin appears to maximally depress TxA_2 synthesis without affecting PGI_2 synthesis (108).

Effects of NSAIDs on Colon Cancer in Animals

In rat models of colon carcinogenesis, cyclooxygenase inhibitors such as indomethacin, piroxicam, and sulindac exhibit chemopreventive effects as judged by reductions in the number

[5] I. Wells and L. J. Marnett, Biochemistry, in press.

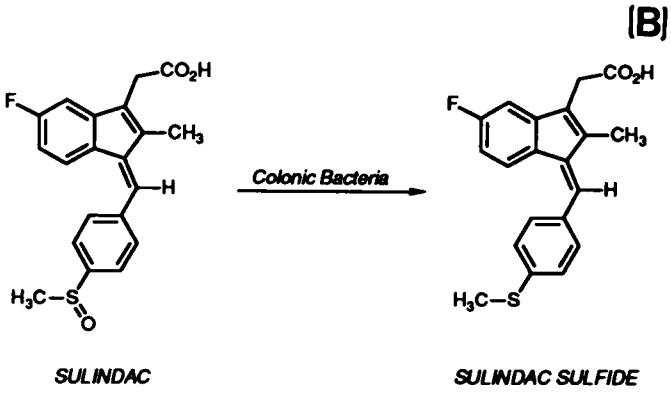
of tumor-bearing animals and the numbers of tumors per animal (109–117). In the case of piroxicam, a dose-response is observed and there is approximately a 70% reduction in tumor incidence and number at 400 ppm administered in the diet (118). This corresponds to 80% of the maximum tolerated dose of piroxicam. Such a dose of piroxicam might indicate that high doses of cyclooxygenase inhibitors are necessary to inhibit colon carcinogenesis in animals and possibly in humans. However, although piroxicam is a cyclooxygenase inhibitor, it is not an irreversible inhibitor and acts by a different mechanism than aspirin (119). Piroxicam and most of the other cyclooxygenase inhibitors in Fig. 3 may not be as effective as aspirin at selective inhibition of prostaglandin synthesis in individual cell types. Alternatively, they may act by mechanisms independent of cyclooxygenase inhibition (120).

Sulindac has been reported to inhibit dimethylhydrazine-induced colon tumorigenesis in mice when administered in the diet throughout carcinogen administration (117). Both the number of mice with tumors and the number of tumors per animal were reduced. However, if dimethylhydrazine treatment was conducted for 17 weeks before the start of sulindac administration no reduction in tumor growth or development was observed.

Sulindac and Polyp Regression in Humans

Waddell and Loughry (121) first reported that sulindac reduces the size and number of rectal polyps in individuals with familial polyposis who have undergone subtotal colectomy and ileoproctostomy. Subsequently, they reported sulindac-induced regression of tumors in affected individuals who had no previous surgical treatment (122). When sulindac administration was discontinued, the tumors recurred and resumption of sulindac treatment caused tumor regression. The reversible nature of sulindac-induced regression was confirmed by Rigau *et al.* (123).

The results of a randomized, placebo-controlled, double-blind crossover study of sulindac efficacy in a small number of familial polyposis patients were recently reported by Labayle *et al.* (124). Administration of sulindac at a dose of 300 mg/day (3 × 100 mg) caused complete polyp regression in 6 of the 9 patients and partial regression in the remaining 3 in less than 4 months. Cessation of sulindac treatment resulted in polyp recurrence. The sulindac effect was significant relative to placebo at $P < 0.01$. Since the original paper by Waddell and Loughry, 22 cases of polyp regression in familial polyposis patients have been reported. Of these patients, 17 had undergone colectomy with ileorectal anastomosis and 5 had not undergone any surgical treatment. These results imply that sulindac is inhibiting an event in the colon associated with a preneoplastic stage of tumor development. Since the reduced form of sulindac is a cyclooxygenase inhibitor, its ability to induce polyp regression may provide support for the hypothesis that cyclooxygenase inhibition is important to the ability of aspirin to reduce colon cancer mortality (125). In fact, Rigau *et al.* (123) have demonstrated reduced prostaglandin biosynthetic capacity in colonic mucosal samples taken from patients on long-term sulindac therapy. Another cyclooxygenase inhibitor, indomethacin, did not induce polyp regression in a small number of patients (126). Although this appears at odds with the results obtained with sulindac, it is more likely a reflection of the unusual pharmacokinetics of sulindac. Sulindac itself is not a cyclooxygenase inhibitor but is reduced *in vivo* to sulindac sulfide which is a powerful cyclooxygenase inhibitor (Equation B) d(125, 127, 128).



A major site of sulindac reduction is in the colon by bacterial microflora (128, 129). Thus, the prodrug sulindac is converted to its active metabolite within the colon and achieves relatively high concentrations. Indeed, sulindac and its metabolites are excreted mainly via the intestine (128). In contrast, a small percentage of a dose of indomethacin reaches the colon; most of the indomethacin metabolites are excreted in urine (129, 130). Consistent with the requirement for intracolonic conversion of sulindac to an active metabolite, sulindac does not induce polyp regression in the stomach, small intestine, or ileum (131).

Aspirin and Human Colon Cancer

In all four of the epidemiological studies mentioned earlier, aspirin intake was estimated by questionnaire and reported as the frequency of ingestion. In the study by Paganini-Hill *et al.*, daily aspirin intake did not alter the risk of colon cancer whereas in the study by Rosenberg *et al.*, an aspirin intake on 4 or more days per week ("regular use") was associated with a halving of colon cancer incidence (12, 13). In the study by Thun *et al.* (10), colon cancer mortality decreased with increasing frequency of aspirin use. A frequency of intake of 1–15 times/month was associated with a lower reduction in risk than a frequency of intake of ≥16 times/month, which suggests that the effect is dose responsive (10). No frequency of aspirin intake was reported in the study by Kune *et al.* (11).

In the 3 case-control studies (11–13), aspirin intake was ascertained at the time of colon cancer diagnosis in cases and prior to interview in comparable controls. The larger studies of Kune *et al.* ($n = 715$) and Rosenberg *et al.* ($n = 1326$) revealed a halving of colon cancer incidence associated with the use of aspirin (unknown frequency) and regular aspirin or other NSAID use (*i.e.*, at least 4 times a week for at least 3 months) compared with nonusers of these drugs. In the smaller study of Paganini-Hill ($n = 181$ cases) there was actually a 50% increased risk of colon cancer among daily aspirin users that was statistically significant. The cohort study of Thun *et al.* was the only one that examined mortality from colon cancer (10). Regular aspirin intake at the time of interview was associated with a halving of colon cancer deaths over the next 6 years. None of these studies examined intensity of screening for colon cancer which could be increased among NSAID users. If aspirin use led to bleeding and early detection of polyps or cancers that were subsequently removed, then colon cancer incidence and

mortality could be decreased (132). Thus, although these studies are very provocative, they do not provide sufficient data to accept, at this point, a causal association between aspirin intake and reduced colon cancer incidence and mortality.

Eicosanoids and Signaling

The diverse range of biological activities effected by low concentrations of eicosanoids suggests they are mediators of different signaling pathways. Their rapid metabolic inactivation during pulmonary circulation implies they are not classical circulating hormones but they are likely to have autocrine or paracrine functions (133). As stated earlier, eicosanoids are synthesized on demand in response to cell stimulation by hormones, tumor promoters, membrane perturbation, etc. The rate-limiting step in biosynthesis appears to be release of arachidonic acid from phospholipid stores. This is catalyzed by phospholipase A_2 or phospholipase C (20, 134). Many agonists trigger rapid eicosanoid production in cells, which appears to be mediated by phospholipase A_2 activation. Arachidonic acid release linked to phospholipase C activation may be a result of hydrolysis of diacylglycerol or the phospholipase C-dependent activation of phospholipase A_2 (20). *ras* transformation of NIH-3T3 cells abolishes prostaglandin synthesis in response to PDGF treatment as a result of reduced phospholipase C activation (135, 136).

Eicosanoid receptors have been reported to be present on many cells including those with eicosanoid biosynthetic capacity (137). Separate receptors have been described for PGE_2, $PGF_{2\alpha}$, PGI_2, PGD_2, and TxA_2 (137). It is likely that each eicosanoid has multiple receptor molecules that are coupled to different intracellular signalling species (20, 138–150). The TxA_2 receptor from platelets has been purified, cloned, and sequenced (151, 152). It appears to be a membrane-spanning, G-protein-linked receptor and there are reports of detection of G-proteins that may be associated with it (142, 148, 153). Likewise, one of the three PGE receptors has recently been cloned and sequenced (154). It also appears to be a G-protein linked receptor.

A good example of eicosanoids acting in an autocrine and paracrine fashion is provided by the regulation of platelet function by TxA_2 and PGI_2 (155, 156). Stimulation of platelets with low concentrations of thrombin leads to release of arachidonic acid, production of TxA_2, and platelet aggregation. TxA_2 synthesis and platelet aggregation are inhibited by aspirin. The effects of TxA_2 are mediated by its receptor which triggers increases in Ca^{2+} influx that lead to aggregation. In contrast to the strong platelet-aggregatory effects of TxA_2, PGI_2 is a potent antiaggregatory agent. PGI_2 is a product of arachidonic acid metabolism in vascular endothelial cells and its antiaggregatory effects are mediated by a platelet receptor that is linked to adenylate cyclase activation. Thus, different families of eicosanoid receptors can be present on the same cell and act through different pathways to control cell function. In the case of platelets, one of the eicosanoid mediators is produced in the target cell whereas the other is not. In fact, even though TxA_2 is produced in platelets, it is released and stimulates other platelets to aggregate.

The principal role of prostaglandins appears to be as kinetic modulators, either amplifiers or attenuators. Again the platelet provides the best example. TxA_2 amplification allows platelets to aggregate at physiological concentrations of thrombin (156). At these low levels of thrombin, aggregation is completely abolished by pretreatment of platelets with aspirin and other cyclooxygenase inhibitors (157). However, cyclooxygenase inhibition does not completely abolish signal transduction pathways leading to aggregation (157). Raising the thrombin concentration overrides the cyclooxygenase block and triggers aggregation by a pathway not sensitive to cyclooxygenase inhibitors. Thus, arachidonic acid metabolism to TxA_2 represents only one signal transduction pathway which exists to amplify platelet responsiveness to low thrombin concentrations. PGI_2 of vascular origin functions as an attenuator of platelet responsiveness to a wide range of agonists, not just TxA_2 (158).

Prostaglandin Synthesis in Human Colon and Colon Cancer

The fact that prostaglandins produced in one cell type can exert an effect on another cell type raises questions about whether the prostaglandins that contribute to tumor development in the colon originate in tumor cells. Numerous studies have demonstrated the ability of human colonic mucosa to synthesize PGE_2, $PGF_{2\alpha}$, PGD_2, and TxB_2 (159–162). In an early study, Bennett *et al.* (159) reported that the basal level of PGE_2 produced by homogenized human colon tumor tissue was higher than that of surrounding normal mucosa. More recently, Narisawa *et al.* (161) demonstrated that venous blood draining from colon tumors contains large amounts of PGE_2 and that the larger the tumor, the higher is the PGE_2 output. The levels of PGE_2 synthesized by the tumors and present in venous blood correlate with the extent of invasion of surrounding tissue but not with the sites or number of distant metastases. The latter correlate better with the levels of PGE_2 in peripheral blood. These observations parallel numerous findings from other laboratories indicating that tumors produce high levels of prostaglandins, most commonly PGE_2 (163).

Some cultured tumor cells have been reported to produce high levels of prostaglandins but this observation is by no means universal. Hubbard *et al.* surveyed 54 different human tumor cell lines for prostaglandin-biosynthetic capacity and found dramatic differences between cell lines from specific anatomical locations (164, 165). The highest levels of prostaglandins were produced by cell lines from non-small cell lung cancers. In nearly all cases, the most abundant products were PGE_2 and $PGF_{2\alpha}$ (165). Prostaglandins were not synthesized by any of five different small cell lung cancer lines. Of interest for the present discussion, only one of six cell lines derived from colorectal adenocarcinomas and one of three from colorectal carcinomas produced detectable prostaglandins. The levels generated by these two cell lines were among the lowest of all the cell lines surveyed. This sampling suggests that human colon cancer cells are not prodigious producers of prostaglandins especially when compared to cells derived from non-small cell lung cancers.

Maxwell *et al.* (162) recently confirmed that human colon cancer tissue produces larger amounts of PGE_2 than surrounding mucosa. There was not a statistically significant difference in the prostaglandin-biosynthetic capacity of epithelial cells derived from tumor tissue compared to epithelial cells derived from uninvolved tissue. However, a significant difference in biosynthetic capacity was observed between tissue-fixed mononuclear cells derived from tumor compared to uninvolved tissue. Interestingly, no difference in PGE_2 synthesis was observed between peripheral mononuclear cells derived from colon cancer patients or normal individuals. The aggregate of these observations suggests that host cells rather than tumor cells may be the major sources of prostaglandins that contribute to colon

tumorigenesis. This should be borne in mind for subsequent discussions of prostaglandin modulation of cell signaling.

Mechanisms of Malignant Transformation and Growth Linked to Prostaglandin Biosynthesis

Mutagenesis

Malondialdehyde Generation. The development of malignant tumors from human colonic epithelial cells is associated with mutations in oncogenes and tumor suppressor genes (166). The accumulation of genetic alterations appears to be more important than a particular sequence of gene inactivation. This suggests that mutations are an important component of malignant transformation. The cyclooxygenase pathway of arachidonic acid metabolism is responsible for generation of a direct-acting mutagen. MDA is produced by enzymatic and nonenzymatic breakdown of PGH_2 (Fig. 1) (167, 168). MDA is mutagenic in bacterial and mammalian test systems and is carcinogenic in rats (169–172). The enzyme thromboxane synthase is particularly active at MDA production; approximately 50% of the PGH_2 that interacts with TxA_2 synthase is converted to MDA (168). Nearly 100% yields are obtained when endoperoxides derived from other fatty acids (*e.g.*, 8,11,14-eicosatrienoic acid) react with TxA_2 synthase (168). Another source of MDA is lipid peroxidation (173, 174). MDA production via the cyclooxygenase pathway is inhibited by aspirin whereas production via lipid peroxidation is not. Thus, MDA is an endogenous mutagen and carcinogen produced in eicosanoid-forming tissues, particularly those with high levels of TxA_2 synthase.

MDA induces frame-shift and base-pair substitution mutations in *Salmonella typhimurium* (169, 175, 176). The frameshifts induced in the *hisD3052* strain of *Salmonella* have been identified by DNA sequence analysis and are primarily additions (177). The spectrum of mutations induced by reaction of MDA with M13 phage DNA and transformation of *Escherichia coli* includes frame-shifts and base pair substitutions.[6] The frameshifts are mainly additions and the base pair substitutions are principally G→T transversions and C→T and A→G transitions.[6] C→T transitions are frequently associated with mutations in the *p53* gene in human colon (178). These are presumed to result from deamination of deoxycytidine or 5-methyldeoxycytidine residues but the rate of spontaneous deamination of deoxycytidine is exceptionally slow at physiological pH and temperature (179). The possibility that C→T transitions are induced by reaction of DNA with an endogenous electrophile derived from polyunsaturated fatty acids is an attractive alternative. MDA forms multiple adducts with deoxyguanosine, deoxycytidine, and deoxyadenosine residues (180–184). The extent to which they form in genomic DNA and their role in mutagenesis are uncertain. Likewise, no information is available on the repair of MDA-DNA adducts.

PGH Synthase-dependent Carcinogen Activation. The peroxidase activity of PGH synthase catalyzes the oxidation of a wide range of xenobiotics, including several classes of chemical carcinogens (2, 185–187). Aromatic amines, heterocyclic amines, and dihydrodiol derivatives of polycyclic hydrocarbons are activated to mutagenic derivatives by PGH synthase (188–190). Aspirin inhibits these oxidations by virtue of its ability to prevent cyclooxygenase-catalyzed generation of the hydroperoxide substrate for the peroxidase.

PGH synthase-dependent xenobiotic metabolism has been demonstrated in several intact cellular systems but there is a paucity of data from intact animal experiments. Aspirin administration to rats inhibits bladder tumorigenesis by FANFT and arachidonic acid-dependent oxidation of FANFT by bladder homogenates and cultured urothelial cells (191, 192). These findings indicate a role for PGH synthase in FANFT bladder carcinogenesis. Cyclooxygenase inhibitors have also been reported to inhibit cyclophosphamide-induced pulmonary toxicity and phenytoin-induced teratogenicity (193, 194).

The current consensus is that PGH synthase does not play a major role in systemic xenobiotic oxidation but may be important in toxicity/carcinogenicity in extrahepatic tissues (187, 195). One such tissue is the colon. A survey of 30 different samples of human colon microsomes revealed detectable PGH synthase-dependent oxidation of benzidine in 9 of them (196). In those samples, the extent of benzidine activation by the PGH synthase-dependent pathway was significantly higher than by a mixed-function oxidase-dependent pathway. It was suggested that the interindividual variation in PGH synthase-dependent activation was due to varying degrees of inflammation which remodeled the metabolic capabilities of colonic mucosa by recruitment of cells with high PGH synthase content (196).

Cell Growth

Numerous observations provide a circumstantial link between prostaglandin synthesis and the control of cell growth. As stated earlier, some transformed cell lines synthesize higher amounts of prostaglandins than their nontransformed counterparts and prostaglandin synthesis is stimulated by treatment of these cells with growth factors. Colon tumors cells do not appear to synthesize prostaglandins (164, 165) but prostaglandins derived from other cells in the colonic mucosa or within the tumor may regulate their growth.

$PGF_{2\alpha}$ stimulates mitogenesis in Swiss 3T3 fibroblasts at concentrations (2 ng/ml) 2–3 orders of magnitude lower than any other prostaglandin (197). $PGF_{2\alpha}$-dependent mitogenesis is synergistically stimulated by insulin (198). Basal and insulin-stimulated $PGF_{2\alpha}$ mitogeneses exhibit parallel sensitivity to alterations in the functional groups in the $PGF_{2\alpha}$ molecule (199). Of paramount importance are the 9- and 15-hydroxyl groups and the 5,6-double bond (199). These structural determinants mirror those required for binding of agonists to the $PGF_{2\alpha}$ receptor of ovine and bovine corpora lutea (200, 201).

EGF stimulates synthesis of prostaglandins by BALB/c 3T3 fibroblasts and EGF-dependent proliferation of these cells is inhibited by indomethacin (202). Indomethacin inhibition is overcome by addition of prostaglandins, and $PGF_{2\alpha}$ is the most potent prostaglandin based on dose response (202). Prostaglandins, including $PGF_{2\alpha}$, are not directly mitogenic in these cells but appear to be permissive for EGF mitogenesis. Interestingly, prostaglandins do not appear to be involved in PDGF-dependent mitogenesis of BALB/c 3T3 fibroblasts (203).

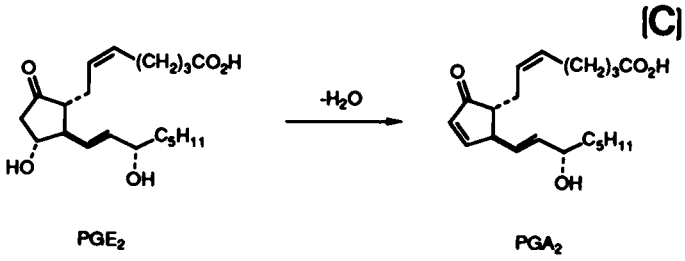
$PGF_{2\alpha}$ also stimulates proliferation of osteoblastic MC3T3-E1 cells (204). The $PGF_{2\alpha}$ effect is inhibited by antibodies to IGF-1, which is an autocrine growth factor secreted by the cells. $PGF_{2\alpha}$ does not enhance secretion of IGF-1 but increases the number of high affinity binding sites for IGF-1 on the cells (204). Thus, the ability of $PGF_{2\alpha}$ to stimulate proliferation of MC3T3-E1 osteoblasts appears to be due to its ability to enhance the synthesis of receptors for an autocrine growth factor.

[6] M. Benamira and L. J. Marnett, submitted for publication.

TxA_2 also stimulates cell growth (205, 206). It has been recently demonstrated that a stable TxA_2 analog[7] stimulates proliferation of a rat mammary epithelial cell line and that proliferation is inhibited by a TxA_2 receptor antagonist (207). Administration of the same TxA_2 receptor antagonist to Wistar rats before and after inoculation of tumor cells completely prevents growth of the tumor *in vivo*. These observations suggest that TxA_2 may be an important stimulus for cell growth and that its effects may be mediated via a receptor. Analysis of the plasma levels of stable metabolites of TxA_2 in breast cancer patients indicates a dramatic decrease following removal of the tumor and an equally dramatic increase coincident with tumor recurrence (208).

A dual role for TxA_2 in regulating human mesangial cell growth in culture has been described (209). Modest stimulation of growth is observed when cells are treated with a stable TxA_2-mimetic agent in the presence of insulin and stimulation is abolished by pretreatment of the cells with a TxA_2 receptor antagonist. However, the same TxA_2 analogue inhibits proliferation of mesangial cells treated with EGF, PDGF, fibroblast growth factor, or fetal bovine serum.

Numerous reports demonstrate that prostaglandins can inhibit proliferation of a wide range of animal and human tumor cells (210–213). Multiple mechanisms of inhibition appear to involved, one receptor mediated, the other receptor independent (214, 215). The receptor-independent mechanism of inhibition is important for PGE_2 and PGD_2, which can undergo spontaneous or protein-catalyzed dehydration to α,β-unsaturated carbonyl derivatives (Equation C) (216).



The latter derivatives bind to fatty acid-binding protein, accumulate in the nucleus, and induce the synthesis of specific proteins, some of which are associated with the heat shock response (217–223). The antiproliferative effect of these α,β-unsaturated prostaglandins is inhibited by reduced glutathione, which adds to the conjugated double bond (224). The extent to which α,β-unsaturated prostaglandins regulate cell growth *in vivo* is uncertain. Although their formation requires nonenzymatic dehydration, a dehydrated derivative of PGD_2 has been reported to be present in normal human urine (225).

Tumor Promotion

Unraveling the role of prostaglandins in tumor promotion is a perplexing task. The usual problem of multiple levels of eicosanoid involvement is compounded by an incomplete understanding of the molecular events responsible for tumor promotion and differences in compound, tissue, species, and strain sensitivity. Nevertheless, there is strong evidence for prostaglandin involvement in tumor promotion in epidermis of NMRI mice by phorbol esters and this will be considered (226).

A necessary but not sufficient condition for tumor promotion by phorbol esters is hyperplasia, which is preceded by induction of ODC and stimulation of DNA synthesis (227–229). Treatment of CD-1 and NMRI mice with the cyclooxygenase inhibitors indomethacin, aspirin, and flufenamic acid inhibits phorbol ester induction of ODC and mitogenesis (230–232). Inhibition can be overcome by coadministration of PGE_1 or PGE_2 but not $PGF_{1\alpha}$ or $PGF_{2\alpha}$. However, neither PGE_1 nor PGE_2 are mitogens when applied topically to skin (230, 231). Administration of tumor-promoting phorbol esters to mouse skin or cultured murine keratinocytes triggers the release of arachidonic acid and the synthesis of a number of eicosanoids including PGE_2 and $PGF_{2\alpha}$ (233, 234). A good correlation exists between the inflammatory and tumor-promoting activities of a series of phorbol esters and their ability to stimulate prostaglandin biosynthesis (235). Fractionation of the epidermal cells obtained from SENCAR mice reveals that the highest concentrations of cyclooxygenase and lipoxygenases are present in the differentiated keratinocytes (236). Thus, epidermal cells possess the ability to oxygenate arachidonic acid to prostaglandins. It should be noted that tumor-promoting phorbol esters are highly irritant and trigger the influx of inflammatory cells within 6 h after treatment (237). Inflammatory cells are prodigious generators of eicosanoids; therefore they may contribute to the multiple waves of prostaglandin synthesis that occur in skin following administration of phorbol esters (see below).

Epidermal tumor promotion by phorbol esters has been subdivided into two stages (238, 239). The complete promoter TPA serves as both a stage I and a stage II promoter whereas the incomplete promoter RPA supports only stage II. Administration of one or two doses of TPA followed by multiple doses of RPA reconstitutes a complete promotion response. Topical administration of TPA or RPA triggers two waves of PGE_2 synthesis at 15 and 90 min posttreatment (240). The increase in PGE_2 synthesis at 90 min is accompanied by an increase in $PGF_{2\alpha}$ synthesis. TPA also induces peaks in $PGF_{2\alpha}$ synthesis at 3.5–4 and 16 h posttreatment whereas RPA does not. Stimulation of $PGF_{2\alpha}$ biosynthesis is one of the few biochemical differences between TPA and RPA, which suggests that it may be important for the first stage of promotion. Administration of indomethacin inhibits TPA-dependent stage I promotion but only if it is applied 3 h *after* TPA, the time at which $PGF_{2\alpha}$ synthesis is maximal. In fact, coadministration of $PGF_{2\alpha}$ with indomethacin 3 h after TPA, overcomes the indomethacin-induced block in promotion. The indomethacin block of stage I promotion is not overcome by coadministration of PGE_2. Curiously, promotion by RPA is inhibited by simultaneous administration of indomethacin and this block is also overcome by coadministration of $PGF_{2\alpha}$ but not PGE_2. Although $PGF_{2\alpha}$ overcomes the indomethacin block of stage I and stage II promotion, it cannot substitute for TPA or RPA as either a first or second stage promoter (240).

Thus, we are faced with a conundrum. TPA-dependent hyperplasia is essential but not sufficient for tumor promotion and is dependent on the synthesis of PGE_2 in mouse epidermis. However, the biochemical events responsible for stage I and stage II of tumor promotion require the synthesis of $PGF_{2\alpha}$. Multiple prostaglandins contribute to promotion; therefore there appears to be a conspiracy of eicosanoids. The highly

[7] TxA_2 has a half-life of 30 s in biological fluids (311). Stable analogues are usually used as surrogates.

probable role of lipoxygenase products in this conspiracy is not considered here but should be noted because lipoxygenase inhibitors abolish phorbol ester tumor promotion in SENCAR and CD-1 mice (241, 242). It is clear that a complex interplay of cell types and mediators contributes to tumor promotion by phorbol esters and non-phorbol ester type compounds. The role of inflammation is not discussed here but should not be ignored because eicosanoids are renowned for their inflammatory, chemotactic, etc., activities which result in the infiltration of a variety of cells that release growth factors, interleukins, etc. Thus, although prostaglandin synthesis is no doubt essential for tumor promotion the precise mechanisms of prostaglandin involvement may be difficult to unravel.

Although most of the studies of prostaglandin participation in tumor promotion have been conducted in mouse skin, some parallel experiments have been conducted in the rat colon using bile acids as promoters. Narisawa *et al.* (243) have found that indomethacin inhibits the increase in ornithine decarboxylase stimulated by deoxycholic acid by approximately 70%. "Low-to-moderate doses" of PGE_2 overcome indomethacin inhibition of ODC induction but do not enhance induction when administered without deoxycholate. Intrarectal installation of deoxycholate into rats stimulates biosynthesis of PGE_2 in the colon and most of the prostaglandin biosynthetic capacity has been localized to the subepithelial layers of the mucosa (244, 245).

Immune Suppression

Another mechanism by which cyclooxygenase inhibitors may reduce tumorigenesis is by modulation of the immune response (246–249). The growth of various tumors is associated with immune suppression in humans and animals (250, 251). One mechanism for immune suppression is dependent on arachidonic acid metabolism. Colony-stimulating factors released by tumor cells activate monocytes and macrophages to synthesize PGE_2 which inhibits blastogenesis of T-cells and the cytotoxic activity of natural killer cells (251–254). This appears to be part of a generalized response of the immune system to the presence of foreign antigens (248). Cyclooxygenase inhibitors such as aspirin, indomethacin, piroxicam, and flurbiprofen abolish PGE_2 synthesis and attentuate the immune suppression (250, 255–264). This may contribute to their ability to reduce tumor growth in several animal models including the colon.

The effects of cyclooxygenase inhibitors on the growth of the LLC in C57/BL mice have been investigated in some detail. The LLC is a spontaneously arising lung tumor that has been used extensively for screening chemotherapeutic and antimetastatic agents. LLC tumor tissue synthesizes large amounts of eicosanoids and the major product is PGE_2 (265). The capacity for PGE_2 synthesis is higher in vegetating tissue than necrotic tissue and increases as the tumor grows *in vivo* (265). The mechanism for increased arachidonic acid oxygenation is unknown but has been suggested to result from increases in PGH synthase activity, induction of phospholipase A_2 activity, or infiltration of inflammatory cells (265). The latter possibility seems unlikely because LLC tumors display rather low levels of macrophage contamination and LLC cells release large amounts of PGE_2 when purified by centrifugal elutriation or when grown in culture (266, 267).

It is not clear if the high level of cyclooxygenase activity of LLC cells represents an essential feature of malignancy or is a reflection of the pulmonary origin of the LLC (see above). Aspirin and indomethacin significantly inhibit the growth of s.c. LLC tumors but do not inhibit proliferation of LLC cells in culture at concentrations that inhibit PGE_2 synthesis (268–271). The ability of indomethacin to inhibit growth of the LLC *in vivo* correlates to its ability to prevent PGE_2-dependent immunosuppression (272–274). Although LLC tumors make considerable amounts of PGE_2, the macrophage appears to be the major source of immunomodulatory PGE_2 and the principal target for indomethacin (272, 274). Variants of the LLC have been described that exhibit altered morphology when grown *in vitro* and differing metastatic potential when injected s.c. or i.v. (272). The metastatic variants produce less PGE_2 than the nonmetastatic ones but are much more potent at triggering macrophage activation *in vivo*. This appears to be due to their ability to release colony-stimulating factors (274). Macrophage activation occurs after about 2 weeks of tumor growth and activated macrophages inhibit T-cell proliferation and natural killer cell-mediated cytotoxicity by mixed spleen cell cultures (274). Both effects are abolished by treatment of the macrophages with indomethacin.

Although cyclooxygenase inhibitors reduce the growth of some transplantable tumors, they do not appear to be effective adjuvant chemotherapeutic agents. For example, at longer times after implantation of LLC into C57/BL mice (4 weeks), macrophages lose their immunosuppressive activity and their ability to make PGE_2 (275). A second phase of immunosuppression ensues that results from enhanced production of bone marrow-derived suppressors that appear to be monocyte-like (275, 276). This second wave of immunosuppresion does not appear to be linked to prostaglandin production because it is not significantly affected by indomethacin treatment of the animals (276). However, its onset is delayed by macrophage PGE_2 synthesis which inhibits hematopoiesis (277).

Indomethacin inhibits the growth of s.c. LLC if it is adminstered several days after the inoculation of tumor cells. However, if further tumor growth occurs before indomethacin adminstration, it is not an effective inhibitor (269). Thus, cyclooxygenase inhibitors do not appear to be good candidates for treatment of humans with large tumor burdens. Nevertheless, they may slow or prevent the growth of developing tumors.

Considering that a major source of prostaglandins in human colon tumors appears to be tissue macrophages and that macrophage-derived PGE_2 appears responsible for immunosuppression, a high research priority is the elucidation of the mechanism of activation of macrophage PGE_2 synthesis. Precedents exist for activation and induction of both PGH synthase and phospholipase A_2 in macrophages and the recent finding of the PGH synthase 2 gene raises questions about its role in macrophage synthesis of PGH synthase protein.

Finally, it should be noted that although prevention of PGE_2-dependent immunosuppression is a plausible mechanism for the antitumor effect of cyclooxygenase inhibitors, indomethacin inhibits the growth of LLC in beige mice and nude mice to the same extent as in normal mice (269). This suggests that T-cells and natural killer cells may not be involved in the antitumor effect of indomethacin against the LLC. Another possibility is that monocytes and macrophages enhance tumor growth by releasing growth factors and that release of these soluble factors is mediated by cyclooxygenase products (269). Alternatively, the indomethacin effect may be independent of cyclooxygenase inhibition (120).

Metastasis

Numerous studies imply a role for eicosanoids in metastasis but there is no consensus on which eicosanoid is responsible or the mechanism(s) by which it acts (278). Indeed, considerable variation is observed in the results of experiments conducted in different model systems. For example, treatment of B16 melanoma cells with indomethacin prior to injection into mice increased the number of metastatic lung colonies in an artificial metastasis model (279, 280). In contrast, treatment of a murine mammary adenocarcinoma with indomethacin prior to injection decreased the number of lung colonies (281). Inhibitory effects of cyclooxygenase inhibitors have also been reported in experiments with several carcinomas and fibrosarcomas (278).

Infusion of pharmacological doses of PGE_1 i.v. inhibits metastasis of LLC cells from a s.c. site (spontaneous metastasis) but enhances metastasis of LLC tumor cells tail vein-injected into mice (artificial metastasis) (282, 283). These conflicting results may reflect differences in the events necessary for lung colonization in the two models and their sensitivity to tumor burden. Given the dependence of the outcome on model system, tumor type, cell number, etc., it is difficult to extract mechanistic information that is useful in a general sense.

The importance of prostaglandins, specifically TxA_2 and PGI_2, in the regulation of platelet function is the basis for a strategy for interrupting the metastatic cascade (284, 285). The formation of tumor cell-platelet aggregates has been proposed to activate tumor cells for attachment to vascular endothelium, assist in the arrest of tumor cells in small capillary beds, or provide an immunological shield for tumor cells (286–288). Furthermore, growth factors released by activated platelets may stimulate growth of the extravasated tumor. On this basis, one might expect antiplatelet agents to inhibit tumor metastasis and a literature documenting this exists (287, 289–293). PGI_2, which is a potent inhibitor of platelet aggregation, inhibits metastasis of melanoma, fibrosarcoma, reticulum carcinoma, and carcinomas of lung, breast, colon, pancreas, and prostate (206, 285, 294). Likewise, PGI_2 analogues and PGI_2 mimetics inhibit metastasis as do agents that inhibit production of TxA_2 from PGH_2 in platelets (206, 295–297). Although there are some isolated reports indicating a lack of an antimetastatic effect of PGI_2, this may be related to differences in dose, route of administration, preparation of the tumor cells, or metastasis model used (298, 299). In fact, not all tumors stimulate platelet aggregation *ex vivo*; therefore antiplatelet agents may not be effective antimetastatic agents in all tumor models (300).

The efficacy of low-dose aspirin as an antiplatelet and antithrombotic agent in humans suggests that inhibition of platelet aggregation should be considered as a mechanism for inhibition of colon cancer mortality. Metastatic disease is frequently the cause of death from colon cancer and inhibition of metastasis would certainly have a significant beneficial effect on mortality from colon cancer. Although antiplatelet agents did not enhance survival from an i.m. implanted tumor in an animal model, the relevance of this observation to humans is not certain (297). As discussed earlier, the dose of aspirin estimated from the study of Thun *et al.* is on the border of low-to-moderate. It is possible that the cell type in which aspirin exerts its preventive effect is highly sensitive to inhibition and slow to recover its cyclooxygenase activity. The cell that best fits this model is the platelet. The mechanistic information that can be inferred from the identification of the cell type inhibited by aspirin reemphasizes the importance of determining the dose response for any protective effects of aspirin on colon cancer mortality.

Conclusion

There is certainly reason to believe that arachidonic acid metabolism contributes to carcinogenesis in humans and that it is possible to modulate its involvement with aspirin or other cyclooxygenase inhibitors. Understanding the mechanisms of action of prostaglandins is complicated by the multistage nature of the carcinogenic process. Considering the available data, it seems to this author that the major contribution of prostaglandins to human colon carcinogenesis is to the growth and possibly spread of the tumor. This opinion is heavily influenced by the reversibility of sulindac inhibition of polyp growth. Of course, for polyps to develop to cancer additional genetic alterations are required; therefore perhaps mutagenesis by MDA or an electrophile produced by PGH synthase-dependent oxidation plays a role in progression; inhibition of arachidonic acid metabolism could reduce the rate of mutation induction. Since the capacity of arachidonic acid metabolism increases as the tumor grows, so might its susceptibility to mutation.

Assuming for discussion that amplification of cell growth is the key, it is not at all clear which prostaglandins are responsible, where they are made, or how they act. Does the tumor make $PGF_{2\alpha}$ that up-regulates synthesis of growth factor receptors? Are similar effects exerted by $PGF_{2\alpha}$ or TxA_2 produced by invading macrophages or platelets? Do growing tumors release colony-stimulating factors that activate macrophages to make PGE_2 which inhibits proliferation of T-cells and the activity of natural killer cells? Did fewer people die of colon cancer because their aspirin intake rendered their platelets less responsive so that they bled more or had fewer liver metastases? There just isn't enough information with which to decide.

Many people are already taking an occasional aspirin for prevention of cardiovascular disease and they will probably keep doing so. Should they start taking more? No, experimental studies are needed to confirm the findings of the epidemiological studies, to establish an optimum dose of aspirin, and to determine the benefits and risks of such therapy. The side effects of chronic aspirin intake do not justify ingestion of amounts in excess of that required to elicit the desired pharmacological effect. Other preventive strategies such as reducing fat intake and increasing consumption of fiber, cruciferous vegetables, and fruits may be more worthwhile and are probably safer. In fact, in the study by Kune *et al.* (11) that first focused attention on aspirin, there was a much more significant protective effect associated with consumption of retinol and vitamin C.

Nevertheless, the possibility that low-dose aspirin might halve colon cancer mortality provides strong impetus for further research in this area. To the extent that the "aspirin effect" reflects cyclooxygenase inhibition, the epidemiological findings reiterate the importance of arachidonic acid metabolism in human cancer and highlight the potential for chemopreventive intervention. Prostaglandins and related compounds are bioactive lipids linked to consumption of dietary fat and they function in intracellular and intercellular signal transduction. Even if they are not obligate intermediates in individual steps in cancer development, they are kinetic modulators of great importance. Actually, the fact that they are not obligate intermediates in signal transduction pathways makes them ideal targets for pharmacological manipulation because inhibition of their

formation should not interrupt basal signal transduction pathways which could prove lethal to normal cells as well as transformed ones. Finally, it is important to reiterate that the pharmaceutical industry has already expended enormous resources on modulating the pathways of arachidonic acid metabolism. Many drugs and drug candidates already exist for which extensive human clinical experience is available. Thus, even if it turns out that aspirin is not the best candidate for an intervention or chemotherapeutic adjuvant trial another cyclooxygenase inhibitor or prostaglandin analogue might be. The opportunity is simply too great to ignore.

This Perspective has concentrated on the involvement in cancer of that branch of the arachidonic acid cascade inhibited by aspirin (the cyclooxygenase branch). It has not considered some of the very exciting recent results implicating the lipoxygenase branch of the cascade in tumor growth and metastasis (301, 302). In fact, there may be a tie between aspirin intake and the lipoxygenase pathway that has not yet been established. The Perspective also did not review studies of dietary fat and colon cancer or animal prevention trials in tissues other than the colon (303–310). All of these topics could and no doubt will be the subject of excellent Perspectives on their own.

Acknowledgments

I am grateful to Celeste Riley for editorial assistance and to the following colleagues for critical readings and helpful suggestions: Thomas Eling, Susan Fischer, Marie Griffin, Brian Henderson, Kenneth Honn, L. Jackson Roberts, Michael Thun, and Michael Wargovich. I would especially like to thank Marie Griffin for editorial assistance on the section, "Aspirin and Human Colon Cancer."

References

1. Powles, T. J., Bockman, R. S., Honn, K. V., and Ramwell, P. (eds.). Prostaglandins and Cancer: First International Conference, Vol. 2. New York: Alan R. Liss, Inc., 1982.
2. Marnett, L. J. (ed.). Arachidonic Acid Metabolism and Tumor Initiation. Boston: Martinus Nijhoff Publishing, 1985.
3. Fischer, S. M. and Slaga, T. J. (eds.). Arachidonic Acid Metabolism and Tumor Promotion. Boston: Martinus Nijhoff Publishing, 1985.
4. Goodwin, J. S. (ed.). Prostaglandins and Immunity. Boston: Martinus Nijhoff, 1985.
5. Thaler-Dao, H., Crastes de Paulet, A., and Paoletti, R. Icosanoids and Cancer. New York: Raven Press, 1984.
6. Honn, K. V., Marnett, L. J., Nigam, S., and Walden, T., Jr. (eds.). Eicosanoids and Other Bioactive Lipids in Cancer and Radiation Injury. Norwell, MA: Kluwer Academic Publishers, 1991.
7. Honn, K. V., Bockman, R. S., and Marnett, L. J. Prostaglandins and cancer: a review of tumor initiation through tumor metastasis. Prostaglandins, *21:* 883–864, 1981.
8. Bockman, R. S. Prostaglandins in cancer: a review. Cancer Invest., *1:* 485–493, 1983.
9. Karmali, R. A. Eicosanoids and cancer. Prog. Clin. Biol. Res., *222:* 687–697, 1986.
10. Thun, M. J., Namboodiri, M. M., and Heath, C. W., Jr. Aspirin use and reduced risk of fatal colon cancer. N. Engl. J. Med., *325:* 1593–1596, 1991.
11. Kune, G. A., Kune, S., and Watson, L. F. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res., *48:* 4399–4404, 1988.
12. Rosenberg, L., Palmer, J. R., Zauber, A. G., Warshauer, M. E., Stolley, P. D., and Shapiro, S. A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of the large-bowel cancer. J. Natl. Cancer Inst., *83:* 355–358, 1991.
13. Paganini-Hill, A., Hsu, G., Ross, R. K., and Henderson, B. E. Aspirin use and incidence of large-bowel cancer in a California retirement community. J. Natl. Cancer Inst., *83:* 1182–1183, 1991.
14. Paganini-Hill, A., Chao, A., Ross, R. K., and Henderson, B. E. Aspirin use and chronic diseases: a cohort study of the elderly. Br. Med. J., *299:* 1247–1250, 1989.
15. Wahle, K. W. Dietary regulation of essential fatty acid metabolism and membrane phospholipid composition. Biochem. Soc. Trans., *18:* 775–778, 1990.
16. Lands, W. E. M. and Samuelsson, B. Phospholipid precursors of prostaglandins. Biochim. Biophys. Acta, *164:* 426–429, 1968.
17. Vonkeman, H., and Van Dorp, D. A. The action of prostaglandin synthetase on 2-arachidonyl-lecithin. Biochim. Biophys. Acta, *164:* 430–432, 1968.
18. Hokin, L. E. Receptors and phosphoinositide-generated second messengers. Annu. Rev. Biochem., *54:* 205–235, 1985.
19. Berridge, M. J. Calcium oscillations. J. Biol. Chem., *265:* 9583–9586, 1990.
20. Parker, J., Daniel, L. W., and Waite, M. Evidence of protein kinase C involvement in phorbol diester-stimulated arachidonic acid release and prostaglandin synthesis. J. Biol. Chem., *262:* 5385–5393, 1987.
21. Needleman, P., Turk, J., Jakschik, B. A., Morrison, A. R., and Lefkowith, J. B. Arachidonic acid metabolism. Annu. Rev. Biochem., *55:* 69–102, 1986.
22. Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A., and Serhan, C. N. Leukotrienes and lipoxins: Structures, biosynthesis, and biological effects. Science (Washington DC), *237:* 1171–1176, 1987.
23. Capdevila, J. H., Falck, J. R., and Estabrook, R. W. Cytochrome P450 and the arachidonate cascade. FASEB J., *6:* 731–736, 1992.
24. Nugteren, D. H. Arachidonate lipoxygenase in blood platelets. Biochim. Biophys. Acta, *380:* 299–307, 1975.
25. Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Natl. Acad. Sci. USA, *71:* 345–349, 1974.
26. Nugteren, D. H., and Hazelhof, E. Isolation and properties of intermediates in prostaglandins biosynthesis. Biochim. Biophys. Acta, *326:* 448–461, 1973.
27. Hamberg, M., and Samuelsson, B. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. Proc. Natl. Acad. Sci. USA, *71:* 3400–3404, 1974.
28. Lands, W. E. M., and Crawford, C. G. Enzymes of membrane phospholipid metabolism in animals. *In:* A. Martonosi (ed.), The Enzymes of Biological Membranes, Vol. 2, pp. 3–85. New York: Plenum Publishing Corp., 1976.
29. Smith, W. L. The eicosanoids and their biochemical mechanisms of action. Biochem. J., *259:* 315–324, 1989.
30. Sun, F. F., Chapman, J. P., and McGuire, J. C. Metabolism of prostaglandin endoperoxide in animal tissues. Prostaglandins, *14:* 1055–1074, 1977.
31. Bunting, S., Gryglewski, R., Moncada, S., and Vane, J. R. Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins, *12:* 897–913, 1976.
32. Patscheke, H. Current concepts for a drug-induced inhibition of formation and action of thromboxane A_2. Blut, *60:* 261–268, 1990.
33. Manley, P. W., Allanson, N. M., Booth, R. F., Buckel, P. E., Kuzniar, E. J., Lad, N., Lai, S. M., Lunt, D. O., and Tuffin, D. P. Structure-activity relationships in an imidazole-based series of thromboxane synthase inhibitors. J. Med. Chem., *30:* 1588–1595, 1987.
34. Hemler, M., Lands, W. E. M., and Smith, W. L. Purification of the cyclooxygenase that forms prostaglandins. Demonstration of two forms of iron in the holoenzyme. J. Biol. Chem., *251:* 5575–5579, 1976.
35. Miyamoto, T., Ogino, N., Yamamoto, S., and Hayaishi, O. Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. J. Biol. Chem., *251:* 2629–2636, 1976.
36. Van Der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H., and Van Dorp, D. A. Purification and characterization of prostaglandin synthetase from sheep vesicular glands. Biochim. Biophys. Acta, *487:* 315–331, 1977.
37. Smith, W. L., and Marnett, L. J. Prostaglandin endoperoxide synthase: structure and catalysis. Biochim. Biophys. Acta Lipids Lipid Metab., *1083:* 1–17, 1991.
38. Struijk, C. B., Beerthuis, R. K., Pabon, H. J. J., and Van Dorp, D. A. Specificity in the enzymic conversion of polyunsaturated fatty acids into prostaglandins. Rec. Trav. Chim., *85:* 1233–1250, 1966.
39. Lands, W. E. M., LeTellier, P. R., Rome, L. H., and Vanderhoek, J. Y. Inhibition of prostaglandin biosynthesis. *In:* S. Bergstrom (ed.), Advances in the Biosciences, pp. 15–28. Elmsford, NY: Pergamon Press, 1973.
40. Higgs, E. A., Moncada, S., and Vane, J. R. Prostaglandins and thromboxanes from fatty acids. Progr. Lipid Res., *25:* 5–11, 1986.
41. DeWitt, D. L., and Smith, W. L. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc. Natl. Acad. Sci. USA, *85:* 1412–1416, 1988.
42. Merlie, J. P., Fagan, D., Mudd, J., and Needleman, P. Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J. Biol. Chem., *263:* 3550–3553, 1988.
43. Yokoyama, C., Takai, T., and Tanabe, T. Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. FEBS Lett., *231:* 347–351, 1988.
44. DeWitt, D. L., El-Harith, E. A., Kraemer, S. A., Andrews, M. J., Yao, E. F., Armstrong, R. L., and Smith, W. L. The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases. J. Biol. Chem., *265:* 5192–5198, 1990.
45. Shimokawa, T., and Smith, W. L. Essential histidines of prostaglandin endoperoxide synthase. J. Biol. Chem., *266:* 6168–6173, 1991.
46. Yokoyama, C., and Tanabe, T. Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. Biochem. Biophys. Res. Commun., *165:* 888–894, 1989.
47. Kraemer, S. A., Meade, E. A., and DeWitt, D. L. Prostaglandin endoperoxide synthase gene structure: identification of the transcriptional start site and 5′-flanking regulatory sequences. Arch. Biochem. Biophys., *293:* 391–400, 1992.

48. O'Banion, M. K., Sadowski, H. B., Winn, V., and Young, D. A. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. J. Biol. Chem., *266:* 23261–23267, 1991.
49. Levine, L., and Hassid, A. Epidermal growth factor stimulates prostaglandin biosynthesis by canine kidney (MDCK) cells. Biochem. Biophys. Res. Commun., *76:* 1181–1187, 1977.
50. DeWitt, D. L. Prostaglandin endoperoxide synthase: regulation of enzyme expression. Biochim. Biophys. Acta Lipids Lipid Metab., *1083:* 121–134, 1991.
51. Raz, A., Wyche, A., Siegel, N., and Needleman, P. Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. J. Biol. Chem., *263:* 3022–3028, 1988.
52. Maier, J. A. M., Hla, T., and Maciag, T. Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. J. Biol. Chem., *265:* 10805–10808, 1990.
53. Pfeilschifter, J., Pignat, W., Leighton, J., Märki, F., Vosbeck, K., and Alkan, S. Transforming growth factor β_2 differentially modulates interleukin-1β- and tumour-necrosis-factor-α-stimulated phospholipase A_2 and prostaglandin E_2 synthesis in rat renal mesangial cells. Biochem. J., *270:* 269–271, 1990.
54. Nothnick, W. B., and Pate, J. L. Interleukin-1β is a potent stimulator of prostaglandin synthesis in bovine luteal cells. Biol. Reprod., *43:* 898–903, 1990.
55. Tahara, K., Grollman, E. F., Saji, M., and Kohn, L. D. Regulation of prostaglandin synthesis by thyrotropin, insulin or insulin-like growth factor-I, and serum in FRTL-5 rat thyroid cells. J. Biol. Chem., *266:* 440–448, 1991.
56. Herrmann, F., Lindemann, A., Gauss, J., and Mertelsmann, R. Cytokine-stimulation of prostaglandin synthesis from endogenous and exogenous arachidonic acids in polymorphonuclear leukocytes involving activation and new synthesis of cyclooxygenase. Eur. J. Immunol., *20:* 2513–2516, 1990.
57. Simonson, M. S., Wolfe, J. A., Konieczkowski, M., Sedor, J. R., and Dunn, M. J. Regulation of prostaglandin endoperoxide synthase gene expression in cultured rat mesangial cells: induction by serum via a protein kinase-C-dependent mechanism. Mol. Endocrinol., *5:* 441–451, 1991.
58. Xie, W., Chipman, J. G., Robertson, D. L., Erikson, R. L., and Simmons, D. L. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc. Natl. Acad. Sci. USA, *88:* 2692–2696, 1991.
59. Bailey, J. M. New mechanisms for effects of anti-inflammatory glucocorticoids. BioFactors, *3:* 97–102, 1991.
60. Sumitani, K., Kawata, T., Yoshimoto, T., Yamamoto, S., and Kumegawa, M. Fatty acid cyclooxygenase activity stimulated by transforming growth factor-β in mouse osteoblastic cell (MC3T3-E1). Arch. Biochem. Biophys., *270:* 588–595, 1989.
61. Pfeilschifter, J., Pignat, W., Vosbeck, K., and Märki, F. Interleukin 1 and tumor necrosis factor synergistically stimulate prostaglandin synthesis and phospholipase A_2 release from rat renal mesangial cells. Biochem. Biophys. Res. Commun., *159:* 385–394, 1989.
62. Casey, M. L., Korte, K., and MacDonald, P. C. Epidermal growth factor stimulation of prostaglandin E_2 biosynthesis in amnion cells. Induction of prostaglandin H_2 synthase. J. Biol. Chem., *263:* 7846–7854, 1988.
63. Wu, K. K., Hatzakis, H., Lo, S. S., Seong, D. C., Sanduja, S. K., and Tai, H. H. Stimulation of *de novo* synthesis of prostaglandin G/H synthase in human endothelial cells by phorbol ester. J. Biol. Chem., *263:* 19043–19047, 1988.
64. Han, J., Sadowski, H., Young, D. A., and Macara, I. G. Persistent induction of cyclooxygenase in $p60^{v\text{-}src}$-transformed 3T3 fibroblasts. Proc. Natl. Acad. Sci. USA, *87:* 3373–3377, 1990.
65. Duniec, Z. M., Nettesheim, P., and Eling, T. E. Stimulation of prostaglandin H synthase mRNA levels and prostaglandin biosynthesis by phorbol ester: Mediation by protein kinase C. Mol. Pharmacol., *39:* 164–170, 1991.
66. Salamonsen, L. A., Hampton, A. L., Clements, J. A., and Findlay, J. K. Regulation of gene expression and cellular localization of prostaglandin synthase by oestrogen and progesterone in the ovine uterus. J. Reprod. Fertil., *92:* 393–406, 1991.
67. Oshima, T., Yoshimoto, T., Yamamoto, S., Kumegawa, M., Yokoyama, C., and Tanabe, T. cAMP-dependent induction of fatty acid cyclooxygenase mRNA in mouse osteoblastic cells (MC3T3-E1). J. Biol. Chem., *266:* 13621–13626, 1991.
68. Raz, A., Wyche, A., and Needleman, P. Temporal and pharmacological division of fibroblast cyclooxygenase expression into transcriptional and translational phases. Proc. Natl. Acad. Sci. USA, *86:* 1657–1661, 1989.
69. Fu, J-Y., Masferrer, J. L., Seibert, K., Raz, A., and Needleman, P. The induction and suppression of prostaglandin H_2 synthase (cyclooxygenase) in human monocytes. J. Biol. Chem., *265:* 16737–16740, 1990.
70. Xie, W., Chipman, J., Robertson, D. L., and Simmons, D. L. Cloning and characterization of a pp60v-src-inducible prostaglandin synthase. FASEB J., *5:* 602, 1991.
71. Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W., and Herschman, H. R. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J. Biol. Chem., *266:* 12866–12872, 1991.
72. O'Banion, M. K., Winn, V. D., and Young, D. A. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc. Natl. Acad. Sci. USA, *89:* 4888–4892, 1992.
73. Fletcher, B. S., Kujubu, D. A., Perrin, D. M., and Herschman, H. R. Structure of the mitogen-inducible TIS10 gene and demonstration that the TIS10-encoded protein is a functional prostaglandin G/H synthase. J. Biol. Chem., *267:* 4338–4344, 1992.
74. Xie, W., Robertson, D. L., and Simmons, D. L. Mitogen-inducible prostaglandin G/H synthase: a new target for nonsteroidal antiinflammatory drugs. Drug Dev. Res., *25:* 249–265, 1992.
75. Hammarstrom, S., Samuelsson, B., and Bjursell, G. Prostaglandin levels in normal and transformed baby hamster kidney fibroblasts. Nat. New Biol., *243:* 50–52, 1973.
76. Hammarstrom, S. Prostaglandin production by normal and transformed 3T3 fibroblasts in cell culture. Eur. J. Biochem., *74:* 7–12, 1977.
77. Vane, J. R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat. New Biol., *231:* 232–235, 1971.
78. Vane, J., and Botting, R. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J., *2:* 89–96, 1988.
79. Robinson, H. J., and Vane, J. R. Prostaglandin synthetase inhibitors—their effects on physiological functions and pathological states. New York: Raven Press, 1974.
80. Flower, R. J. Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev., *26:* 33–67, 1974.
81. Lands, W. E. M., and Hanel, A. M. Inhibitors and activators of prostaglandin biosynthesis. *In:* C. Pace-Asciak and E. Granstrom (eds.), Prostaglandins and Related Substances, pp. 203–223. Amsterdam: Elsevier/North-Holland Biomedical Press, 1983.
82. Roth, G. J., and Majerus, P. W. The mechanism of the effect of aspirin on human platelets I. Acetylation of a particulate fraction protein. J. Clin. Invest., *56:* 624–632, 1975.
83. Roth, G. J., Stanford, N., and Majerus, P. W. Acetylation of prostaglandin synthase by aspirin. Proc. Natl. Acad. Sci. USA, *72:* 3073–3076, 1975.
84. Rome, L. H., Lands, W. E. M., Roth, G. J., and Majerus, P. W. Aspirin as a quantitative acetylating reagent for the fatty acid oxygenase that forms prostaglandins. Prostaglandins, *11:* 23–30, 1976.
85. Van Der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H., and Van Dorp, D. A. Acetylation of prostaglandin endoperoxide synthetase with acetylsalicylic acid. Eur. J. Biochem., *109:* 1–8, 1980.
86. Rome, L. H., and Lands, W. E. M. Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. Proc. Natl. Acad. Sci. USA, *72:* 4863–4865, 1975.
87. Kulmacz, R. J., and Lands, W. E. M. Stoichiometry and kinetics of the interaction of prostaglandin H synthase with antiinflammatory agents. J. Biol. Chem., *260:* 12572–12578, 1985.
88. Flower, R. J. Eleventh Gaddum Memorial Lecture—lipocortin and the mechanism of action of glucocorticoids. Br. J. Pharmacol., *94:* 987–1015, 1988.
89. Davidson, F. F., Dennis, E. A., Powell, M., and Glenney, J. R., Jr. Inhibition of phospholipase A_2 by "lipocortins" and calpactins. An effect of binding to substrate phospholipids. J. Biol. Chem., *262:* 1698–1705, 1987.
90. Bailey, J. M., Makheja, A. N., Pash, J., and Verma, M. Corticosteroids suppress cyclooxygenase messenger RNA levels and prostanoid synthesis in cultured vascular cells. Biochem. Biophys. Res. Commun., *157:* 1159–1163, 1988.
91. Pash, J. M., and Bailey, J. M. Inhibition by corticosteroids of epidermal growth factor-induced recovery of cyclooxygenase after aspirin inactivation. FASEB J., *2:* 2613–2618, 1988.
92. Masferrer, J. L., Seibert, K., Zweifel, B., and Needleman, P. Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. Proc. Natl. Acad. Sci. USA, *89:* 3917–3921, 1992.
93. Sebaldt, R. J., Sheller, J. R., Oates, J. A., Roberts, L. J., II, and FitzGerald, G. A. Inhibition of eicosanoid biosynthesis by glucocorticoids in humans. Proc. Natl. Acad. Sci. USA, *87:* 6974–6978, 1990.
94. Roth, G. J., Machuga, E. T., and Ozols, J. Isolation and covalent structure of the aspirin modified, active site region of prostaglandin synthetase. Biochemistry, *22:* 4672–4675, 1983.
95. Chen, Y-N. P., and Marnett, L. J. Heme prosthetic group required for acetylation of prostaglandin H synthase by aspirin. FASEB J., *3:* 2294–2297, 1989.
96. Kulmacz, R. J. Topography of prostaglandin H synthase. Antiinflammatory agents and the protease-sensitive arginine 253 region. J. Biol. Chem., *264:* 14136–14144, 1989.
97. Higgs, G. A., Salmon, J. A., Henderson, B., and Vane, J. R. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Proc. Natl. Acad. Sci. USA, *84:* 1417–1420, 1987.
98. Bailey, J. M., Muza, B., Hla, T., and Salata, K. Restoration of prostacyclin synthase in vascular smooth muscle cells after aspirin treatment: regulation by epidermal growth factor. J. Lipid Res., *26:* 54–61, 1985.
99. Pedersen, A. K., and FitzGerald, G. A. Dose related pharmacokinetics of aspirin: presystemic acetylation of platelet cyclooxygenase in man. N. Engl. J. Med., *311:* 1206–1211, 1984.
100. Jaffe, E. A., and Weksler, B. B. Recovery of endothelial cell prostacyclin production after inhibition by low doses of aspirin. J. Clin. Invest., *63:* 532–535, 1979.
101. Patrignani, P., Filabozzi, P., and Patrono, C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J. Clin. Invest., *69:* 1366–1372, 1982.

102. Hanley, S. P., Bevan, J., Cockbill, S. R., and Heptinstall, S. A regimen for low-dose aspirin? Br. Med. J., *285:* 1299–1302, 1982.
103. Hanley, S. P., Bevan, J., Cockbill, S. R., and Heptinstall, S. Differential inhibition by low dose aspirin of human venous prostacyclin synthesis and platelet thromboxane synthesis. Lancet, *1:* 969–971, 1981.
104. Preston, F. E., Whipps, S., Jackson, C. A., French, A. J., Wyld, P. J., and Stoddard, C. J. Inhibition of prostacyclin and platelet thromboxane A_2 after low-dose aspirin. N. Engl. J. Med., *304:* 76–79, 1981.
105. Weksler, B. B., Pett, S. B., Alonso, D., Richter, R. C., Stelzer, P., Subramanian, V., Tack-Goldman, K., and Gay, W. A., Jr. Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. N. Engl. J. Med., *308:* 800–805, 1983.
106. Final report on the aspirin component of the ongoing physicians' health study. N. Engl. J. Med., *321:* 129–135, 1989.
107. De Caterina, R., Giannessi, D., Boem, A., Bernini, W., Battaglia, D., Michelassi, C., Dell'Amico, F., L'Abbate, A., Patrignani, P., and Patrono, C. Equal antiplatelet effects of aspirin 50 or 324 mg/day in patients after acute myocardial infarction. Thromb. Haemost., *54:* 528–532, 1985.
108. FitzGerald, G. A., Lupinetti, M., Charman, S. A., and Charman, W. N. Presystemic acetylation of platelets by aspirin: Reduction in rate of drug delivery to improve biochemical selectivity for thromboxane A_2. J. Pharmacol. Exp. Ther., *259:* 1043–1049, 1991.
109. Kudo, T., Narisawa, T., and Abo, S. Antitumor activity of indomethacin on methylazoxymethanol-induced large bowel tumors in rats. Gann, *71:* 260–264, 1980.
110. Narisawa, T., Sato, M., Tani, M., Kudo, T., Takahashi, T., and Goto, A. Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin. Cancer Res., *41:* 1954–1957, 1981.
111. Pollard, M., and Luckert, P. H. Prolonged antitumor effect of indomethacin on autochthonous intestinal tumors in rats. J. Natl. Cancer Inst., *70:* 1103–1105, 1983.
112. Pollard, M., and Luckert, P. H. Effect of piroxicam on primary intestinal tumors induced by rats by *N*-methylnitorsourea. Cancer Lett., *25:* 117–121, 1984.
113. Pollard, M., Luckert, P. H., and Schmidt, M. A. The suppressive effect of piroxicam on autochthonous intestinal tumors in the rat. Cancer Lett., *21:* 57–61, 1983.
114. Nigro, N. D., Bull, A. W., and Boyd, M. E. Inhibition of intestinal carcinogenesis in rats: effect of difluoromethylornithine with piroxicam or fish oil. J. Natl. Cancer Inst., *77:* 1309–1313, 1986.
115. Reddy, B. S., Nayini, J., Tokumo, K., Rigotty, J., Zang, E., and Kelloff, G. Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with DL-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, in diet. Cancer Res., *50:* 2562–2568, 1990.
116. Rao, C. V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., and Reddy, B. S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer Res., *51:* 4528–4534, 1991.
117. Moorghen, M., Ince, P., Finney, K. J., Sunter, J. P., Appleton, D. R., and Watson, A. J. A protective effect of sulindac against chemically-induced primary colonic tumours in mice. J. Pathol., *156:* 341–347, 1988.
118. Reddy, B. S., Maruyama, H., and Kelloff, G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. Cancer Res., *47:* 5340–5346, 1987.
119. Lombardino, J. G., and Wiseman, E. H. Piroxicam and other anti-inflammatory oxicams. Med. Res. Rev., *2:* 127–152, 1982.
120. Carter, C. A., Ip, M., and Ip, C. A comparison of the effects of the prostaglandin synthesis inhibitors indomethacin and carprofen on 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis in rats fed different amounts of essential fatty acid. Carcinogenesis (Lond.), *10:* 1369–1374, 1989.
121. Waddell, W. R., and Loughry, R. W. Sulindac for polyposis of the colon. J. Surg. Oncol., *24:* 83–87, 1983.
122. Waddell, W. R., Gasner, G. F., Cerise, E. J., and Loughry, R. W. Sulindac for polyposis of colon. Am. J. Surg., *157:* 175–178, 1989.
123. Rigau, J., Pique, J. M., Rubio, E., Planas, R., Tarrech, J. M., and Bordas, J. M. Effects of long-term sulindac therapy on colonic polyposis. Ann. Intern. Med., *115:* 952–954, 1991.
124. Labayle, D., Fischer, D., Vielh, P., Drouhin, F., Pariente, A., Bories, C., Duhamel, O., Trousset, M., and Attali, P. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology, *101:* 635–639, 1991.
125. Duggan, D. E., Hooke, K. F., Risley, E. A., Shen, T. Y., and Van Arman, C. G. Identification of the biologically active form of sulindac. J. Pharmacol. Exp. Ther., *201:* 8–13, 1977.
126. Klein, W. A., Miller, H. H., Anderson, M., and DeCosse, J. J. The use of indomethacin, sulindac, and tamoxifen for the treatment of desmoid tumors associated with familial polyposis. Cancer (Phila.), *60:* 2863–2868, 1987.
127. Duggan, D. E., Hare, L. E., Ditzler, C. A., Lei, B. W., and Kwan, K. C. The disposition of sulindac. Clin. Pharmacol. Ther., *21:* 326–335, 1977.
128. Hucker, H. B., Stauffer, S. C., White, S. D., Rhodes, R. E., Arison, B. H., Umberhauer, E. R., Bower, R. J., and McMahon, F. G. Physiologic disposition and metabolic fate of a new anti-inflammatory agent, *cis*-5-fluoro-2-methyl-1[*p*-(methylsulfinyl)-benzylidenyl]-idene-3-acetic acid in the rat, dog, rhesus monkey and man. Drug Metab. Dispos., *1:* 721–736, 1973.
129. Shen, T. Y., and Winter, C. A. Chemical and biological studies on indomethacin, sulindac and their analogs. Adv. Drug Res., *12:* 89–245, 1977.
130. Hucker, H. B., Zacchei, A. G., Cox, S. V., Brodie, D. A., and Cantwell, N. H. R. Studies on the absorption, distribution and excretion of indomethacin in various species. J. Pharmacol. Exp. Ther., *153:* 237–249, 1966.
131. Waddell, W. R., Gerner, R. E., and Reich, M. P. Nonsteroid antiinflammatory drugs and tamoxifen for desmoid tumors and carcinoma of the stomach. J. Surg. Oncol., *22:* 197–211, 1983.
132. Buring, J. E., and Hennekens, C. H. Overt gastrointestinal bleeding in the course of chronic low-dose aspirin administration for secondary prevention of arterial occlusive disease. Am. J. Gastroenterol., *86:* 1279–1280, 1991.
133. Oliw, E., Granstrom, E., and Anggard, E. The prostaglandins and essential fatty acids. *In:* C. Pace-Asciak and E. Granstrom (eds.), Prostaglandins and Related Substances, pp. 1–44. Amsterdam: Elsevier/North-Holland Biomedical Press, 1983.
134. Irvine, R. F. How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J., *204:* 3–16, 1982.
135. Benjamin, C. W., Tarpley, W. G., and Gorman, R. R. Loss of platelet-derived growth factor-stimulated phospholipase activity in NIH-3T3 cells expressing the EJ-*ras* oncogene. Proc. Natl. Acad. Sci. USA, *84:* 546–550, 1987.
136. Benjamin, C. W., Tarpley, W. G., and Gorman, R. R. The lack of PDGF-stimulated PGE_2 release from *ras*-transformed NIH-3T3 cells results from reduced phospholipase C but not phospholipase A_2 activity. Biochem. Biophys. Res. Commun., *145:* 1254–1259, 1987.
137. Halushka, P. V., Mais, D. E., Mayeux, P. R., and Morinelli, T. A. Thromboxane, prostaglandin and leukotriene receptors. Annu. Rev. Pharmacol. Toxicol., *29:* 213–239, 1989.
138. Dorn, G. W. II. Distinct platelet thromboxane A_2/prostaglandin H_2 receptor subtypes. A radioligand binding study of human platelets. J. Clin. Invest., *84:* 1883–1891, 1989.
139. Morinelli, T. A., Okwu, A. K., Mais, D. E., Halushka, P. V., John, V., Chen, C-K., and Fried, J. Difluorothromboxane A_2 and stereoisomers: stable derivatives of thromboxane A_2 with differential effects on platelets and blood vessels. Proc. Natl. Acad. Sci. USA, *86:* 5600–5604, 1989.
140. Armstrong, R. A., Lawrence, R. A., Jones, R. L., Wilson, N. H., and Collier, A. Functional and ligand binding studies suggest heterogeneity of platelet prostacyclin receptors. Br. J. Pharmacol., *997:* 657–668, 1989.
141. Masuda, A., Mais, D. E., Oatis, J. E., Jr., and Halushka, P. V. Platelet and vascular thromboxane A_2/prostaglandin H_2 receptors. Evidence for different subclasses in the rat. Biochem. Pharmacol., *42:* 537–544, 1991.
142. Furci, L., Fitzgerald, D. J., and FitzGerald, G. A. Heterogeneity of prostaglandin H_2/thromboxane A_2 receptors: Distinct subtypes mediate vascular smooth muscle contraction and platelet aggregation. J. Pharmacol. Exp. Ther., *258:* 74–81, 1991.
143. Takahara, K., Murray, R., FitzGerald, G. A., and Fitzgerald, D. J. The response to thromboxane A_2 analogues in human platelets. Discrimination of two binding sites linked to distinct effector systems. J. Biol. Chem., *265:* 6836–6844, 1990.
144. Garrity, M. J., Reed, M. M., and Brass, E. P. Coupling of hepatic prostaglandin receptors to adenylate cyclase through a pertussis toxin sensitive guanine nucleotide regulatory protein. J. Pharmacol. Exp. Ther., *248:* 979–983, 1989.
145. Negishi, M., Ito, S., and Hayaishi, O. Prostaglandin E receptors in bovine adrenal medulla are coupled to adenylate cyclase via G_i and to phosphoinositide metabolism in a pertussis toxin-insensitive manner. J. Biol. Chem., *264:* 3916–3923, 1989.
146. Sonnenburg, W. K., Zhu, J., and Smith, W. L. A prostaglandin E receptor coupled to a pertussis toxin-sensitive guanine nucleotide regulatory protein in rabbit cortical collecting tubule cells. J. Biol. Chem., *265:* 8479–8483, 1990.
147. Muallem, S., Merritt, B. S., Green, J., Kleeman, C. R., and Yamaguchi, D. T. Classification of prostaglandin receptors based on coupling to signal transduction systems. Biochem. J., *263:* 769–774, 1989.
148. Shenker, A., Goldsmith, P., Unson, C. G., and Spiegel, A. M. The G protein coupled to the thromboxane A_2 receptor in human platelets is a member of the novel G_q family. J. Biol. Chem., *266:* 9309–9313, 1991.
149. Gusovsky, F. Prostaglandin receptors in NIH 3T3 cells: coupling of one receptor to adenylate cyclase and of a second receptor to phospholipase C. Mol. Pharmacol., *40:* 633–638, 1991.
150. Kitanaka, J-I., Onoe, H., and Baba, A. Astrocytes possess prostaglandin $F_{2\alpha}$ receptors coupled to phospholipase C. Biochem. Biophys. Res. Commun., *178:* 946–952, 1991.
151. Ushikubi, F., Nakajima, M., Hirata, M., Okuma, M., Fujiwara, M., and Narumiya, S. Purification of the thromboxane A_2/prostaglandin H_2 receptor from human blood platelets. J. Biol. Chem., *264:* 16496–16501, 1989.
152. Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y., Kageyama, R., Nakanishi, S., and Narumiya, S. Cloning and expression of cDNA for a human thromboxane A_2 receptor. Nature (Lond.), *349:* 617–620, 1991.
153. Nakahata, N., Matsuoka, I., Ono, T., and Nakanishi, H. Thromboxane A_2 activates phospholipase C in astrocytoma cells via pertussis toxin-insensitive G-protein. Eur. J. Pharmacol., *162:* 407–417, 1989.
154. Sugimoto, Y., Namba, T., Honda, A., Hayashi, Y., Negishi, M., Ichikawa,

A., and Narumiya, S. Cloning and expression of a cDNA for mouse prostaglandin E receptor EP_3 subtype. J. Biol. Chem., *267:* 6463–6466, 1992.
155. Colman, R. W. Platelet receptors. Hematol. Oncol. Clin. North Am. , *4:* 27–42, 1990.
156. Arita, H., Nakano, T., and Hanasaki, K. Thromboxane A_2: its generation and role in platelet activation. Prog. Lipid Res., *28:* 273–301, 1989.
157. Ruggiero, M., and Lapetina, E. G. Protease and cyclooxygenase inhibitors synergistically prevent activation of human platelets. Proc. Natl. Acad. Sci. USA, *83:* 3456–3459, 1986.
158. Kerins, D. M., Murray, R., and FitzGerald, G. A. Prostacyclin and prostaglandin E_1: molecular mechanisms and therapeutic utility. Prog. Hemost. Thromb., *10:* 307–337, 1991.
159. Bennett, A., Del Tacca, M., Stamford, I. F., and Zebro, T. Prostaglandins from tumours of human large bowel. Br. J. Cancer, *35:* 881–884, 1977.
160. Boughton-Smith, N. K., and Whittle, B. J. R. Laboratory methods for studying the role of eicosanoids in inflammatory bowel disease. *In:* K. Hillier (ed.), Eicosanoids and the Gastrointestinal Tract, pp. 12–45. Boston: Lancaster, 1992.
161. Narisawa, T., Kusaka, H., Yamazaki, Y., Takahashi, M., Koyama, H., Koyama, K., Fukaura, Y., and Wakizaka, A. Relationship between blood plasma prostaglandin E_2 and liver and lung metastases in colorectal cancer. Dis. Colon Rectum, *33:* 840–845, 1990.
162. Maxwell, W. J., Kelleher, D., Keating, J. J., Hogan, F. P., Bloomfield, F. J., MacDonald, G. S., and Keeling, P. W. N. Enhanced secretion of prostaglandin E_2 by tissue-fixed macrophages in colonic carcinoma. Digestion, *47:* 160–166, 1990.
163. Karim, S. M. M., and Rao, B. Prostaglandins and tumors. *In:* S. M. M. Karim (ed.), Advances in Prostaglandin Research, Vol. 2, pp. 303–325. Boston: MTP Press, 1976.
164. Hubbard, W. C., Alley, M. C., McLemore, T. L., and Boyd, M. R. Profiles of prostaglandin biosynthesis in sixteen established cell lines derived from human lung, colon, prostate, and ovarian tumors. Cancer Res., *48:* 4770–4775, 1988.
165. Hubbard, W. C., Alley, M. C., McLemore, T. L., and Boyd, M. R. Fatty acid cyclooxygenase metabolism of arachidonic acid in human tumor cells. *In:* K. V. Honn, L. J. Marnett, S. Nigam, and T. Walden Jr. (eds.), Eicosanoids and Other Bioactive Lipids in Cancer and Radiation Injury, pp. 27–32. Norwell, MA: Kluwer Academic Publishers, 1991.
166. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.
167. Hamberg, M., and Samuelsson, B. On the Mechanism of the biosynthesis of prostaglandins E_1 and F_{1a}. J. Biol. Chem., *242:* 5336–5343, 1967.
168. Diczfalusy, U., Falardeau, P., and Hammarstrom, S. Conversion of prostaglandin endoperoxides to C_{17}-hydroxyacids by human platelet thromboxane synthase. FEBS Lett., *84:* 271–274, 1977.
169. Mukai, F. H., and Goldstein, B. D. Mutagenicity of malondialdehyde, a decomposition product of peroxidized polyunsaturated fatty acids. Science (Washington DC), *191:* 868–869, 1976.
170. Basu, A. K., and Marnett, L. J. Unequivocal demonstration that malondialdehyde is a mutagen. Carcinogenesis (Lond.), *4:* 331–333, 1983.
171. Spalding, J. W. Toxicology and carcinogenesis studies of malondialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F344/N rats and $B6C3F_1$ mice. NTP Tech. Rep., *331:* 5–13, 1988.
172. Yau, T. M. Mutagenicity and cytotoxicity of malondialdehyde in mammalian cells. Mech. Ageing Dev., *11:* 137–144, 1979.
173. Bernheim, F., Bernheim, M. L. C., and Wilbur, K. M. The reaction between thiobarbituric acid and the oxidation products of certain lipids. J. Biol. Chem., *174:* 257–264, 1948.
174. Janero, D. R. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biol. Med., *9:* 515–540, 1990.
175. Basu, A. K., Marnett, L. J., and Romano, L. J. Dissociation of malondialdehyde mutagenicity in *Salmonella typhimurium* from its ability to induce interstrand DNA cross-links. Mutat. Res., *129:* 39–46, 1984.
176. Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H., and Ames, B. N. Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutat. Res., *148:* 25–34, 1985.
177. O'Hara, S. M., and Marnett, L. J. DNA sequence analysis of spontaneous and β-methoxy-acrolein-induced mutations in *Salmonella typhimurium hisD3052*. Mutat. Res. Fundam. Mol. Mech. Mutagen., *247:* 45–56, 1991.
178. Hollstein, M., Sidransky, D., Vogelestein, B., and Harris, C. C. *p53* mutations in human cancer. Science (Washington DC), *253:* 49–53, 1991.
179. Frederico, L. A., Kunkel, T. A., and Shaw, B. R. A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and the activation energy. Biochemistry, *29:* 2532–2537, 1990.
180. Seto, H., Okuda, T., Takesue, T., and Ikemura, T. Reaction of malondialdehyde with nucleic acid. I. Formation of fluorescent pyrimido[1,2-*a*]purin-10(3*H*)-one nucleosides. Bull. Chem. Soc. Jpn., *56:* 1799–1802, 1983.
181. Seto, H., Seto, T., Takesue, T., and Ikemura, T. Reaction of malonaldehyde with nucleic acid. III. Studies of the fluorescent substances released by enzymatic digestion of nucleic acids modified with malonaldehyde. Chem. Pharm. Bull., *34:* 5079–5085, 1986.
182. Basu, A. K., O'Hara, S. M., Valladier, P., Stone, K., Mols, O., and Marnett, L. J. Identification of adducts formed by reaction of guanine nucleosides with malondialdehyde and structurally related aldehydes. Chem. Res. Toxicol., *1:* 53–59, 1988.
183. Stone, K., Ksebati, M., and Marnett, L. J. Investigation of the adducts formed by reaction of malondialdehyde with adenosine. Chem. Res. Toxicol., *3:* 33–38, 1990.
184. Stone, K., Uzieblo, A., and Marnett, L. J. Studies of the reaction of malondialdehyde with cytosine nucleosides. Chem. Res. Toxicol., *3:* 467–472, 1990.
185. Marnett, L. J. Polycyclic aromatic hydrocarbon oxidation during prostaglandin biosynthesis. Life Sci., *29:* 531–546, 1981.
186. Marnett, L. J., and Eling, T. E. Cooxidation during prostaglandin biosynthesis: a pathway for the metabolic activation of xenobiotics. *In:* E. Hodgson, J. R. Bend, and R. M. Philpot (eds.), Reviews in Biochemical Toxicology, pp. 135–172. New York: Elsevier/North Holland, 1983.
187. Smith, B. J., Curtis, J. F., and Eling, T. E. Bioactivation of xenobiotics by prostaglandin H synthase. Chem.-Biol. Interact., *79:* 245–264, 1991.
188. Marnett, L. J., Reed, G. A., and Dennison, D. J. Prostaglandin synthetase dependent activation or 7,8-dihydro-7,8-dihydroxy-benzo(*a*)pyrene to mutagenic derivatives. Biochem. Biophys. Res. Commun., *82:* 210–216, 1978.
189. Robertson, I. G. C., Sivarajah, K., Eling, T. E., and Zeiger, E. Activation of some aromatic amines to mutagenic products by prostaglandin endoperoxide synthetase. Cancer Res., *43:* 476–480, 1983.
190. Sarkar, F. H., Radcliff, G., Callewaert, D. M., Marnett, L. J., Eling, T., Daston, D. S., and Caspary, W. J. Mutagenic response of mouse lymphoma cells after activation of benzidine and 2-aminofluorene with purified prostaglandin H synthase. Mutat. Res. Genet. Toxicol. Testing, *242:* 319–328, 1990.
191. Murasaki, G., Zenser, T. V., Davis, B. B., and Cohen, S. M. Inhibition by aspirin of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide-induced bladder carcinogenesis and enhancement of fore-stomach carcinogenesis. Carcinogenesis (Lond.), *5:* 53–55, 1984.
192. Zenser, T. V., Palmier, M. O., Mattammal, M. B., Bolla, R. I., and Davis, B. B. Comparative effects of prostaglandin H synthase-catalyzed binding of two 5-nitrofuran urinary bladder carcinogens. J. Pharmacol. Exp. Ther., *227:* 139–143, 1983.
193. Smith, R. D., and Kehrer, J. P. Cooxidation of cyclophosphamide as an alternative pathway for its bioactivation and lung toxicity. Cancer Res., *51:* 542–548, 1991.
194. Kubow, S., and Wells, P. G. *In vitro* bioactivation of phenytoin to a reactive free radical intermediate by prostaglandin synthetase, horseradish peroxidase, and thyroid peroxidase. Mol. Pharmacol., *35:* 504–511, 1989.
195. Rice, J. R., Zenser, T. V., and Davis, B. B. Prostaglandin synthase-dependent cooxidation and aromatic amine carcinogenesis. *In:* L. J. Marnett (ed.), Arachidonic Acid Metabolism and Tumor Initiation, pp. 125–169. Boston: Martinus Nijhoff Publishing, 1985.
196. Flammang, T. J., Yamazoe, Y., Benson, R. W., Roberts, D. W., Potter, D. W., Chu, D. Z. J., Lang, N. P., and Kadlubar, F. F. Arachidonic acid-dependent peroxidative activation of carcinogenic arylamines by extrahepatic human tissue microsomes. Cancer Res., *49:* 1977–1982, 1989.
197. Jimenez de Asua, L., Clingan, D., and Rudland, P. S. Initiation of cell proliferation in cultured mouse fibroblasts by prostaglandin F_{2a}. Proc. Natl. Acad. Sci. USA, *72:* 2724–2728, 1975.
198. Jimenez de Asua, L., Otto, A. M., Ulrich, M-O., Martin-Perez, J., and Thomas, G. The regulation of DNA replication in animal cells by prostaglandin F_{2a}. *In:* T. J. Powles, R. S. Bockman, K. V. Honn, and P. Ramwell (eds.), Prostaglandins and Cancer: First International Conference, pp. 309–331. New York: Alan R. Liss, Inc., 1982.
199. Jimenez de Asua, L., and Otto, A. M. The stimulation of the initiation of DNA synthesis and cell division in Swiss mouse 3T3 cells by prostaglandin F_{2a} requires specific functional groups in the molecule. J. Biol. Chem., *258:* 8774–8780, 1983.
200. Powell, W. S., Hammarstrom, S., and Samuelsson, B. Prostaglandin F_{2a} receptor in ovine corpora lutea. Eur. J. Biochem., *41:* 103–107, 1974.
201. Powell, W. S., Hammarstrom, S., and Samuelsson, B. Occurrence and properties of a prostaglandin F_{2a} receptor in bovine corpora lutea. Eur. J. Biochem., *56:* 73–77, 1975.
202. Nolan, R. D., Danilowicz, R. M., and Eling, T. E. Role of arachidonic acid metabolism in the mitogenic response of BALB/c 3T3 fibroblasts to epidermal growth factor. Mol. Pharmacol., *33:* 650–656, 1988.
203. Handler, J. A., Danilowicz, R. M., and Eling, T. E. Mitogenic signaling by epidermal growth factor (EGF), but not platelet-derived growth factor, requires arachidonic acid metabolism in BALB/c 3T3 cells. Modulation of EGF-dependent *c-myc* expression by prostaglandins. J. Biol. Chem., *265:* 3669–3673, 1990.
204. Hakeda, Y., Harada, S., Matsumoto, T., Tezuka, K., Higashino, K., Kodama, H., Hashimoto-Goto, T., Ogata, E., and Kumegawa, M. Prostaglandin F_{2a} stimulates proliferation of clonal osteoblastic MC3T3-E1 cells by up-regulation of insulin-like growth factor I receptors. J. Biol. Chem., *266:* 21044–21050, 1991.
205. Honn, K. V., and Meyer, J. Thromboxanes and prostacyclin: positive and negative modulators of tumor growth. Biochem. Biophys. Res. Commun., *102:* 1122–1129, 1981.
206. Honn, K. V., Meyer, J., Neagos, G., Henderson, T., Westley, C., and Ratanatharathorn, V. Control of tumor growth and metastasis with prostacyclin and thromboxane synthetase inhibitors: evidence for a new antitumor and antimetastatic agent (Bay g 6575). *In:* G. A. Jamieson (ed.), Interaction of Platelets and Tumor Cells, pp. 295–331. New York: Alan R. Liss, Inc., 1982.

207. Nigam, S., and Zakrzewicz, A. Tumour cell proliferation by thromboxane A_2: A receptor-mediated event. *In:* B. Samuelsson, P. W. Ramwell, R. Paoletti, G. Folco, and E. Granström (eds.), Advances in Prostaglandin, Thromboxane, and Leukotriene Research, Vol. 21, pp. 925–928. New York: Raven Press, 1990.
208. Nigam, S., Zakrezewicz, A., Lubbert, H., and Benedetto, C. Prognostic importance of thromboxane in breast cancer. *In:* K. V. Honn, L. J. Marnett, S. Nigam, and T. L. Walden, Jr. (eds.), Eicosanoids and Other Bioactive Lipids in Cancer and Radiation Injury, pp. 489–496. Norwell, MA: Kluwer Academic Publishers, 1991.
209. Mene, P., Abboud, H. E., and Dunn, M. J. Regulation of human mesangial cell growth in culture by thromboxane A_2 and prostacyclin. Kidney Int., *38:* 232–239, 1990.
210. Santoro, M. G., Philpott, G. W., and Jaffe, B. M. Inhibition of tumor growth *in vivo* and *in vitro* by prostaglandin E. Nature (Lond.), *263:* 777–779, 1976.
211. Fukushima, M., Kato, T., Ueda, R., Ota, K., Narumiya, S., and Hayaishi, O. Prostaglandin D_2, a potential antineoplastic agent. Biochem. Biophys. Res. Commun., *105:* 956–964, 1982.
212. Kato, T., Fukushima, M., Kurozumi, S., and Noyori, R. Antitumor activity of Δ^7-prostaglandin A_1 and Δ^{12}-prostaglandin J_2 *in vitro* and *in vivo*. Cancer Res., *46:* 3538–3542, 1986.
213. Santoro, M. G., Benedetto, A., Carruba, G., Garaci, E., and Jaffe, B. M. Prostaglandin A compounds as antiviral agents. Science (Washington DC), *209:* 1032–1034, 1980.
214. Ito, S., Narumiya, S., and Hayaishi, O. Prostaglandin D_2: a biochemical perspective. Prostaglandins Leukotrienes Essen. Fatty Acids, *37:* 219–234, 1989.
215. Fukushima, M. Prostaglandin J_2—anti-tumour and anti-viral activities and the mechanisms involved. Eicosanoids, *3:* 189–199, 1990.
216. Honn, K. V., and Marnett, L. J. Requirement of a reactive α,β-unsaturated carbonyl for inhibition of tumor growth and induction of differentation by "A" series prostaglandins. Biochem. Biophys. Res. Commun., *129:* 34–40, 1985.
217. Khan, S. H., and Sorof, S. Preferential binding of growth inhibitory prostaglandins by the target protein of a carcinogen. Proc. Natl. Acad. Sci. USA, *87:* 9401–9405, 1990.
218. Narumiya, S., and Fukushima, M. Site and mechanism of growth inhibition by prostaglandins. I. Active transport and intracellular accumulation of cyclopenteneone prostaglandins, a reaction leading to growth inhibition. J. Pharmacol. Exp. Ther., *239:* 500–505, 1986.
219. Narumiya, S., Ohno, K., Fujiwara, M., and Fukushima, M. Site and mechanism of growth inhibition by prostaglandins. II. Temperature-dependent transfer of a cyclopentenone prostaglandin to nuclei. J. Pharmacol. Exp. Ther., *239:* 506–511, 1986.
220. Narumiya, S., Ohno, K., Fukushima, M., and Fujiwara, M. Site and mechanism of growth inhibition by prostaglandins. III. Distribution and binding of prostaglandin A_2 and Δ^{12}-prostaglandin J_2 in nuclei. J. Pharmacol. Exp. Ther., *242:* 306–311, 1987.
221. Ohno, K., Sakai, T., Fukushima, M., Narumiya, S., and Fujiwara, M. Site and mechanism of growth inhibition by prostaglandins. IV. Effect of cyclopentenone prostaglandins on cell cycle progression of G_1-enriched HeLa cells. J. Pharmacol. Exp. Ther., *245:* 294–298, 1988.
222. Santoro, M. G., Garaci, E., and Amici, C. Prostaglandins with antiproliferative activity induce the synthesis of a heat shock protein in human cells. Proc. Natl. Acad. Sci. USA, *86:* 8407–8411, 1989.
223. Santoro, M. G., Jaffe, B. M., Elia, G., and Benedetto, A. Prostaglandin A1 induces the synthesis of a new protein in cultured AGMK cells. Biochem. Biophys. Res. Commun., *107:* 1179–1184, 1982.
224. Atsmon, J., Freeman, M. L., Meredith, M. J., Sweetman, B. J., and Roberts, L. J., II Conjugation of 9-deoxy-$\Delta^9,\Delta^{12}(E)$-prostaglandin D_2 with intracellular glutathione and enhancement of its antiproliferative activity by glutathione-depletion. Cancer Res., *50:* 1879–1885, 1990.
225. Hirata, Y., Hayashi, H., Ito, S., Kikawa, Y., Ishashi, M., Sudo, M., Miyazaki, H., Fukushima, M., Narumiya, S., and Hayaishi, O. Occurrence of 9-deoxy-Δ^9,Δ^{12}-13,14-dihydroprostaglandin D_2 in human urine. J. Biol. Chem., *263:* 16619–16625, 1988.
226. Furstenberger, G. Role of eicosanoids in mammalian skin epidermis. RBC, *24:* 1–111, 1990.
227. Baird, W. M., Sedgwick, J. A., and Boutwell, R. K. Tumor-promoting activity of phorbol and four diesters of phorbol in mouse skin. Cancer Res., *31:* 1434–1439, 1971.
228. Boutwell, R. K. Biochemical mechanism of tumor promotion. *In:* T. J. Slaga, A. Sivak, and R. K. Boutwell (eds.), Carcinogenesis, Vol. 2, pp. 49–58. New York: Raven Press, 1978.
229. O'Brien, T. G., Simsiman, R. C., and Boutwell, R. K. Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor-promoting agents. Cancer Res., *35:* 1662–1670, 1975.
230. Verma, A. K., Rice, H. M., and Boutwell, R. K. Prostaglandins and skin tumor promotion: inhibition of tumor promoter-induced ornithine decarboxylase activity in epidermis by inhibitors of prostaglandin synthesis. Biochem. Biophys. Res. Commun., *79:* 1160–1166, 1977.
231. Furstenberger, G., and Marks, F. Indomethacin inhibition of cell proliferation induced by the phorbol ester TPA is reversed by prostaglandin E_2 in mouse epidermis *in vivo*. Biochem. Biophys. Res. Commun., *84:* 1103–1111, 1978.
232. Verma, A. K., Ashendel, C. L., and Boutwell, R. K. Inhibition by prostaglandin synthesis inhibitors of the induction of epidermal ornithine decarboxylase activity, the accumulation of prostaglandin and tumor promotion caused by TPA. Cancer Res., *40:* 708–715, 1980.
233. Bresnick, E., Meunier, P., and Lamden, M. Epidermal prostaglandins after topical application of a tumor promoter. Cancer Lett., *7:* 121–125, 1979.
234. Furstenberger, G., Richter, H., Fusenig, N. E., and Marks, F. Arachidonic acid and prostaglandin E_2 release and enhanced cell proliferation induced by the phorbol ester TPA in a murine epidermal cell line. Cancer Lett., *11:* 191–198, 1981.
235. Brune, K., Kalin, H., Schmidt, R., and Hecker, E. Inflammatory, tumor initiating and promoting activities of polycyclic aromatic hydrocarbons and diterpene esters in mouse skin as compared with their prostaglandin releasing potency *in vitro*. Cancer Lett., *4:* 333–342, 1978.
236. Cameron, G. S., Baldwin, J. K., Jasheway, D. W., Patrick, K. E., and Fischer, S. M. Arachidonic acid metabolism varies with the state of differentiation in density gradient-separated mouse epidermal cells. J. Invest. Dermatol., *94:* 292–296, 1990.
237. Trush, M. A., and Kensler, T. W. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Radic. Biol. Med., *10:* 201–209, 1991.
238. Slaga, T. J., Fischer, S. M., Nelson, K., and Gleason, G. L. Studies on the mechanism of skin tumor promotion: evidence for several stages in promotion. Proc. Natl. Acad. Sci. USA, *77:* 3659–3663, 1980.
239. Furstenberger, G., Berry, D. L., Sorg, B., and Marks, F. Skin tumor promotion by phorbol esters is a two-stage process. Proc. Natl. Acad. Sci. USA, *78:* 7722–7726, 1981.
240. Furstenberger, G., Gross, M., and Marks, F. Eicosanoids and multistage carcinogenesis in NMRI mouse skin: role of prostaglandins E and F in conversion (first stage of tumor promotion) and promotion (second stage of tumor promotion). Carcinogenesis (Lond.), *10:* 91–96, 1989.
241. Fischer, S. M., Gleason, G. D., Mills, G. D., and Slaga, T. J. Indomethacin enhancement of TPA tumor promotion in mice. Cancer Lett., *10:* 343–350, 1980.
242. Fischer, S. M., Furstenberger, G., Marks, F., and Slaga, T. J. Events associated with mouse skin tumor promotion with respect to arachidonic acid metabolism. A comparison between Sencar and NMRI mice. Cancer Res., *47:* 3174–3179, 1987.
243. Narisawa, T., Takahashi, M., Niwa, M., Fukaura, Y., and Wakizaka, A. Involvement of prostaglandin E_2 in bile acid-caused promotion of colon carcinogenesis and anti-promotion by the cyclooxygenase inhibitor indomethacin. Jpn. J. Cancer Res., *78:* 791–798, 1987.
244. Craven, P. A., and DeRubertis, F. R. Profiles of eicosanoid production by superficial and proliferative colonic epithelial cells and sub-epithelial colonic tissue. Prostaglandins, *32:* 387–399, 1986.
245. Craven, P. A., and DeRubertis, F. R. Patterns of prostaglandin synthesis and degradation in isolated superficial and proliferative colonic epithelial cells compared to residual colon. Prostaglandins, *26:* 583–604, 1983.
246. Plescia, O. J. Does prostaglandin synthesis effect *in vivo* tumour growth by altering tumour/host balance? *In:* T. J. Powles, R. S. Bockman, K. V. Honn, and P. Ramwell (eds.), Prostaglandins and Cancer: First International Conference, pp. 619–631. New York: Alan R. Liss, Inc., 1982.
247. Salmon, S. E. Interrelationship of endogenous macrophages, prostaglandin synthesis and tumor cell clonogenicity in human tumor biopsies. *In:* T. J. Powles, R. S. Bockman, K. V. Honn, and P. Ramwell (eds.), Prostaglandins and Cancer: First International Conference, pp. 633–649. New York: Alan R. Liss, Inc., 1982.
248. Goodwin, J. S., and Ceuppens, J. L. Prostaglandins, cellular immunity and cancer. *In:* J. S. Goodwin (ed.), Prostaglandins and Immunity, pp. 1–34. Boston: Martinus Nijhoff Publishing, 1985.
249. Hwang, D. Essential fatty acids and immune response. FASEB J., *3:* 2052–2061, 1989.
250. Plescia, O. J., Smith, A. H., and Grinwich, K. Subversion of immune system by tumor cells and role of prostaglandins. Proc. Natl. Acad. Sci. USA, *72:* 1848–1854, 1975.
251. Goodwin, J. S., Bankhurst, A. D., and Messner, R. P. Suppression of human T-cell mitogenesis by prostaglandin; existence of a prostaglandin-producing suppressor cell. J. Exp. Med., *146:* 1719–1734, 1977.
252. Goodwin, J. S., Messner, R. P., and Peake, G. T. Prostaglandin suppression of mitogen-stimulated lymphocytes *in vitro*. J. Clin. Invest., *62:* 753–760, 1978.
253. Bockman, R. S. PGE inhibition of T-lymphocyte colony formation. J. Clin. Invest., *64:* 812–821, 1979.
254. Pelus, L. M. Modulation of myelopoiesis by prostaglandin E_2: demonstration of a novel mechanism of action *in vivo*. Immunol. Res., *8:* 176–184, 1989.
255. Baich, C. M., Doghert, P. A., Cloud, G. A., and Tilden, A. B. Prostaglandin E_2-mediated suppression of cellular immunity in colon cancer patients. Surgery, *95:* 71–77, 1984.
256. Balch, C. M., Dougherty, P. A., and Tilden, A. B. Excessive prostaglandin E_2 production by suppressor monocytes in head and neck cancer patients. Ann. Surg., *196:* 645–650, 1982.
257. Han, T., and Takita, H. Indomethacin-mediated enhancement of lymphocyte response to mitogens in healthy subjects and lung cancer patients. Cancer (Phila.), *46:* 2416–2420, 1980.

258. Tilden, A. B., and Balch, C. M. Indomethacin enhancement of immunocompetence in melanoma patients. Surgery, *90:* 77–84, 1981.
259. Grinwich, K. D., and Plescia, O. J. Tumor-mediated immunosuppression: prevention by inhibitors of prostaglandin synthesis. Prostaglandins, *14:* 1175–1183, 1977.
260. Brunda, M. J., Herberman, R. B., and Holden, H. T. Inhibition of murine natural killer cell activity by prostaglandins. J. Immunol., *124:* 2682–2688, 1980.
261. Hial, V., Horakova, Z., Shaff, F. E., and Beaven, M. A. Alteration of tumor growth by aspirin and indomethacin: studies with two transplantable tumors in mouse. Eur. J. Pharmacol., *37:* 367–376, 1976.
262. Lynch, N. R., Casatles, M., Astonin, M., and Salomon, J. C. Mechanism of inhibition of tumor growth by aspirin and indomethacin. Br. J. Cancer, *38:* 503–509, 1978.
263. Humes, J. L., Cupo, J., and Strausser, H. R. Effects of indomethacin on Moloney sarcoma virus-induced tumors. Prostaglandins, *6:* 463–469, 1974.
264. Bennett, A., Houghton, J., Leaper, D. J., and Stamford, I. F. Cancer growth, response to treatment and survival tme in mice: beneficial effect of the prostaglandin synthesis inhibitor flurbiprofen. Prostaglandins, *17:* 179–191, 1979.
265. Chiabrando, C., Broggini, M., Castagnoli, M. N., Donelli, M. G., Noseda, A., Visintainer, M., Garattini, S., and Fanelli, R. Prostaglandin and thromboxane synthesis by Lewis lung carcinoma during growth. Cancer Res., *45:* 3605–3608, 1985.
266. Polentarutti, N., Acero, R., Bottazzi, B., Taraboletti, G., and Mantovani, A. The macrophage content of tumors is unrelated to levels of NK cell-mediated resistance. J. Leukocyte Biol., *39:* 113–119, 1986.
267. Dunn, J. R. Ph.D. Dissertation, Wayne State University, 1987.
268. Young, M. R., and Knies, S. Prostaglandin E production by Lewis lung carcinoma: Mechanism for tumor establishment *in vivo*. J. Natl. Cancer Inst., *72:* 919–922, 1984.
269. Maca, R. D. Inhibition of the growth of Lewis lung carcinoma by indomethacin in conventional, nude, and beige mice. J. Biol. Response Modif., *7:* 568–580, 1988.
270. Alino, S. F., Unda, F. J., Perez-Yarza, G., and Canavate, M. L. Are laminin binding sites on tumor cell survace involved in the indomethacin-induced sensitivity to natural cytotoxic cells? Biol. Cell, *66:* 255–261, 1989.
271. Alino, S. F., Unda, F. J., and Perez-Yarza, G. Laminin surface binding sites and metastatic potential of 3LL tumor cells, increased by indomethacin. Biochem. Biophys. Res. Commun., *167:* 731–738, 1990.
272. Young, M. R., and Newby, M. Differential induction of suppressor macrophages by cloned Lewis lung carcinoma variants in mice. J. Natl. Cancer Inst., *77:* 1255–1260, 1986.
273. Young, M. R., and Hoover, C. S. Inhibition of spleen cell cytotoxic capacity toward tumor by elevated prostaglandin E_2 levels in mice bearing Lewis lung carcinoma. J. Natl. Cancer Inst., *77:* 425–429, 1986.
274. Young, M. R., Wheeler, E., and Newby, M. Macrophage-mediated suppression of natural killer cell activity in mice bearing Lewis lung carcinoma. J. Natl. Cancer Inst., *76:* 745–749, 1986.
275. Young, M. R. I., Young, M. E., and Kim, K. Regulation of tumor-induced myelopoiesis and the associated immune suppressor cells in mice bearing metastatic Lewis lung carcinoma by prostaglandin E_2. Cancer Res., *48:* 6826–6831, 1988.
276. Young, M. R., Duffie, G. P., Lozano, Y., Young, M. E., and Wright, M. A. Association of a functional prostaglandin E_2-protein kinase A coupling with responsiveness of metastatic Lewis lung carcinoma variants to prostaglandin E_2 and to prostaglandin E_2-producing nonmetastatic Lewis lung carcinoma variants. Cancer Res., *50:* 2973–2978, 1990.
277. Young, M. R., Newby, M., and Wepsic, H. T. Hematopoiesis and suppressor bone marrow cells in mice bearing large metastatic Lewis lung carcinoma tumors. Cancer Res., *47:* 100–105, 1987.
278. Fulton, A. M. The role of eicosanoids in tumor metastasis. Prostaglandins Leukotrienes Essen. Fatty Acids, *34:* 229–237, 1988.
279. Fitzpatrick, F. A., and Stringfellow, D. A. Prostaglandin D_2 formation by malignant melanoma cells correlates inversely with cellular metastatic potential. Proc. Natl. Acad. Sci. USA, *76:* 1765–1769, 1979.
280. Stringfellow, D. A., and Fitzpatrick, F. A. Prostaglandin D_2 controls pulmonary metastasis of malignant melanoma cells. Nature (Lond.), *282:* 76–78, 1979.
281. Fulton, A. M. Interactions of natural effector cells and prostaglandins in the control of metastasis. J. Natl. Cancer Inst., *78:* 735–741, 1987.
282. Ellis, L. M., Copeland, E. M., III, Bland, K. I., and Sitren, H. S. Inhibition of tumor growth and metastasis by chronic intravenous infusion of prostaglandin E_1. Ann. Surg., *212:* 45–50, 1990.
283. Ellis, L. M., Copeland, E. M., III, Bland, K. I., and Sitren, H. S. Differential role of prostaglandin E_1 on tumor metastasis. J. Surg. Res., *48:* 333–336, 1990.
284. Honn, K. V., Onoda, J. M., Menter, D. G., Cavanaugh, P. G., Crissman, J. D., Taylor, J. D., and Sloane, B. F. Platelet-tumor cell interactions as a target for antimetastatic therapy. *In:* K. V. Honn, W. E. Powers, and B. F. Sloane (eds.), Mechanisms of Cancer Metastasis. Potential Therapeutic Implications, pp. 117–144. Boston: Martinus Nijhoff Publishing, 1986.
285. Honn, K. V., Tang, D. G., and Chen, Y. Q. Platelets and cancer metastasis: more than an epiphenomenon. Semin. Thomb. Hemostas., *18:* 390–413, 1992.
286. Gasic, G. J. Role of plasma, platelets, and endothelial cells in tumor metastasis. Cancer Metastasis Rev., *3:* 99–116, 1984.
287. Al-Mondhiry, H. Tumor interaction with hemostasis: the rationale for the use of platelet inhibitors and anticoagulants in the treatment of cancer. Am. J. Hematol., *16:* 193–202, 1984.
288. Honn, K. V., Busse, W. D., and Sloane, B. F. Prostacyclin and thromboxanes: implications for their role in tumor cell metastasis. Biochem. Pharmacol., *32:* 1–11, 1983.
289. Maat, B., and Hilgard, P. Critical analysis of experimental models for the antimetastatic effects of anticoagulants. *In:* K. Hellmann, P. Hilgard, and S. Eccles (eds.), Metastasis. Clinical and Experimental Aspects, pp. 109–115. The Hague: Martinus Nijhoff, Inc., 1980.
290. Temple, W. J., and Ketcham, A. S. Current clinical trials with anticoagulant therapy in the management of cancer patients. *In:* K. V. Honn, and B. F. Sloane (eds.), Hemostatic Mechanisms and Metastasis, pp. 409–425. Boston: Martinus Nijhoff Publishing, 1984.
291. Maniglia, C. A., Tudor, G., Gomez, J., and Sartorelli, A. C. The effect of 2,6-bis(diethanolamino)-4-piperidinopyrimido[5,4-*d*]pyrimidine (RA233) on growth, metastasis and lung colony formation of B16 melanoma. Cancer Lett., *16:* 253–260, 1982.
292. Donati, M. B., Rotillio, D., Delaini, F., Giavazzi, R., Mantovani, A., and Poggi, A. Animal models for the study of platelet-tumor cell interactions. *In:* G. A. Jamieson (ed.), Interaction of Platelets and Tumor Cells, pp. 159–176. New York: Alan R. Liss, Inc., 1982.
293. Maat, B., and Hilgard, P. Anticoagulants and experimental metastasis—evaluation of antimetastatic effects in different systems. J.Cancer Res. Clin. Oncol., *101:* 275–283, 1981.
294. Honn, K. V., Cicone, B., and Skoff, A. Prostacyclin: a potent antimetastatic agent. Science (Washington DC), *212:* 1270–1272, 1981.
295. Schirner, M., and Schneider, M.R. Cicaprost inhibits metastases of animal tumors. Prostaglandins, *42:* 451–461, 1991.
296. Ambrus, J. L., Ambrus, C. M., Gastpar, H., and Williams, P. Study of platelet aggregation *in vivo*. IX. Effect of nafazatrom on *in vivo* platelet aggregation and spontaneous tumor metastasis. J. Med., *13:* 35–47, 1982.
297. Merriman, R. L., Shackelford, K. A., Tanzer, L. R., Campbell, J. B., Bemis, K. G., and Matsumoto, K. Drug treatments for metastasis of the Lewis lung carcinoma: lack of correlation between inhibition of lung metastasis and survival. Cancer Res., *49:* 4509–4516, 1989.
298. Karpatkin, S., Ambrogio, C., and Pearlstein, E. Lack of effect of *in vivo* prostacyclin on the development of pulmonary metastasis in mice following intravenous injection of CT26 colon carcinoma, Lewis lung carcinoma, or B16 amelanotic melanoma cells. Cancer Res., *44:* 3880–3883, 1984.
299. Haas, J. H., Corbett, T. H., and Haas, C. D. Nafazatrom: lack of antimetastatic effect. Proc. Am. Assoc. Cancer Res. , *26:* 51, 1985.
300. Estrada, J., and Nicolson, G. L. Tumor-cell platelet aggregation does not correlate with metastatic potential of rat 13762NF mammary adenocarcinoma tumor cell clones. Int. J. Cancer, *34:* 101–105, 1984.
301. Glasgow, W. C., Afshari, C. A., Barrett, J. C., and Eling, T. E. Modulation of the epidermal growth factor mitogenic response by metabolites of linoleic acid and arachidonic acid in Syrian hamster embryo fibroblasts. Differential effects in tumor suppressor gene (+) and (−) phenotypes. J. Biol. Chem., *267:* 10771–10779, 1992.
302. Grossi, I. M., Fitzgerald, L. A., Umbarger, L. A., Nelson, K. K., Diglio, C. A., Taylor, J. D., and Honn, K. V. Bidirectional control of membrane expression and/or activation of the tumor cell IRGpIIb/IIIa receptor and tumor cell adhesion by lipoxygenase products of arachidonic acid and linoleic acid. Cancer Res., *49:* 1029–1037, 1989.
303. Abraham, S. (ed.). Carcinogenesis and Dietary Fat. Norwell, MA: Kluwer Academic Publishers, 1989.
304. Reddy, B. S., Burill, C., and Rigotty, J. Effect of diets high in ω-3 and ω-6 fatty acids on initiation and postinitiation stages of colon carcinogenesis. Cancer Res., *51:* 487–491, 1991.
305. Fischer, S. M., Conti, C. J., Locniskar, M., Belury, M. A., Maldve, R. E., Lee, M. L., Leyton, J., Slaga, T. J., and Bechtel, D. H. The effect of dietary fat on the rapid development of mammary tumors induced by 7,12-dimethylbenz(a)anthracene in SENCAR mice. Cancer Res., *52:* 662–666, 1992.
306. Welsch, C. W. Enhancement of mammary tumorigenesis by dietary fat: review of potential mechanisms. Am. J. Clin. Nutr., *45:* 192–202, 1987.
307. Ip, C., Carter, C. A., and Ip, M. M. Requirement of essential fatty acid for mammary tumorigenesis in the rat. Cancer Res., *45:* 1997–2001, 1985.
308. Mehta, R. G., Steele, V., Kelloff, G. J., and Moon, R. C. Influence of thiols and inhibitors of prostaglandin biosynthesis on the carcinogen-induced development of mammary lesions *in vitro*. Anticancer Res., *11:* 587–592, 1991.
309. Noguchi, M., Taniya, T., Koyasaki, N., Kumaki, T., Miyazaki, I., and Mizukami, Y. Effects of the prostaglandin synthetase inhibitor indomethacin on tumorigenesis, tumor proliferation, cell kinetics, and receptor contents of 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in Sprague-Dawley rats fed a high- or low-fat diet. Cancer Res., *51:* 2683–2689, 1991.
310. Pariza, M. W. Dietary fat and cancer risk: evidence and research needs. Annu. Rev. Nutr., *8:* 167–183, 1988.
311. Granstrom, E., Diczfalusy, U., and Hamberg, M. The thromboxanes. *In:* C. Pace-Asciak, and E. Granstrom (eds.), Prostaglandins and Related Substances, pp. 45–94. Amsterdam: Elsevier/North-Holland Biomedical Press, 1983.