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POTENTIAL THERAPEUTIC APPLICATIONS OF ASPIRIN AND OTHER CYCLO-OXYGENASE INHIBITORS

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1 The ubiquitous actions of the cyclo-oxygenase inhibitors are described.

2 These include the inhibitory effect on prostaglandin synthesis and the direct effect of aspirin on lymphocytes and their ability to produce lymphokines.

3 Aspirin reduces some types of platelet aggregation possibly involving inhibition of the precursors of thromboxane A_2 and prostacyclin.

4 The therapeutic implications in relation to transient ischaemic attacks, coronary artery disease and reno-allograft rejection are discussed.

5 The beneficial and adverse effects on the gastro-intestinal tract are described.

6 The effects of aspirin-like drugs on the genito-urinary tract are described with particular reference to their adverse effects on labour and their therapeutic effect on dysmenorrhoea.

Introduction

Aspirin was first introduced as an analgesic and anti-inflammatory drug in 1899 by Dreser and has remained for years one of the mainstays for the therapy of pain and inflammation. The mechanism of action of the salicylates has been studied extensively (Smith, 1966; Collier, 1969). Vane (1971) and his colleagues (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Blackwell, Flower & Vane, 1975) have discovered the inhibitory effects of aspirin on prostaglandin synthesis (for a review, see Vane & Ferreira, 1978; Moncada & Vane, 1979*a*). This effect on prostaglandin synthesis in different organs explains many of the ubiquitous pharmacological and therapeutic effects of aspirin.

Figures 1*a* and *b* show the formation of prostaglandins and the leukotrienes (see below) from arachidonic acid (AA). The formation of the prostaglandin precursor AA from membrane phospholipids by the action of phospholipase A is a rate-limiting step, as prostaglandins are not stored in cells. AA is transformed by a cyclo-oxygenase to cyclic peroxides, which are the intermediates for thromboxane, prostacyclin and other prostaglandins ($F_{2\alpha}$, E_2 and D_2). Aspirin and aspirin-like drugs inhibit cyclo-oxygenase and thus block the formation of the prostaglandins.

There is a second metabolic pathway for AA which is insensitive to aspirin. In platelets, lung tissue and white blood cells, a lipoxygenase enzyme and oxygen form leukotriene A from AA which, in the presence of the amino acid cysteine, is then converted to the biologically important leukotriene C or slow reacting substance of anaphylaxis (SRSA) (Figure 1*b*). This is a powerful constrictor of smooth muscle and is probably released from cells following an antigen-antibody reaction on their surface; it may therefore play an important role in inflammation and certain types of asthma and other sensitivity reactions.

The presence of the cyclo-oxygenase and prostaglandins in a variety of organs explains the therapeutic actions of aspirin in inflammation, pain and fever, as well as its other actions on the gastric mucosa, platelets, kidney, uterus and other organs. Thus, it might be expected that aspirin would have so many side-actions that its therapeutic usefulness would be seriously limited; but this is contrary to clinical experience. The reason for this is possibly the differences in sensitivity of the various organs to the inhibitory effects of aspirin (Vane, 1971; Flower, 1974; Bhattacherjee & Eakins, 1974; Debmińska-Kiéc, Grodzinska & Piotrowicz, 1974; Debmińska-Kiéc, Żmuda & Krupińska, 1976). Table 1 gives some data on the sensitivity of various prostaglandin-generating systems to aspirin; differences in sensitivity to aspirin of several orders of magnitude have been found.

In most instances these data have been obtained from intact organ systems; a number of factors could be operating to explain these differences. The inhibition of cyclo-oxygenase will depend on

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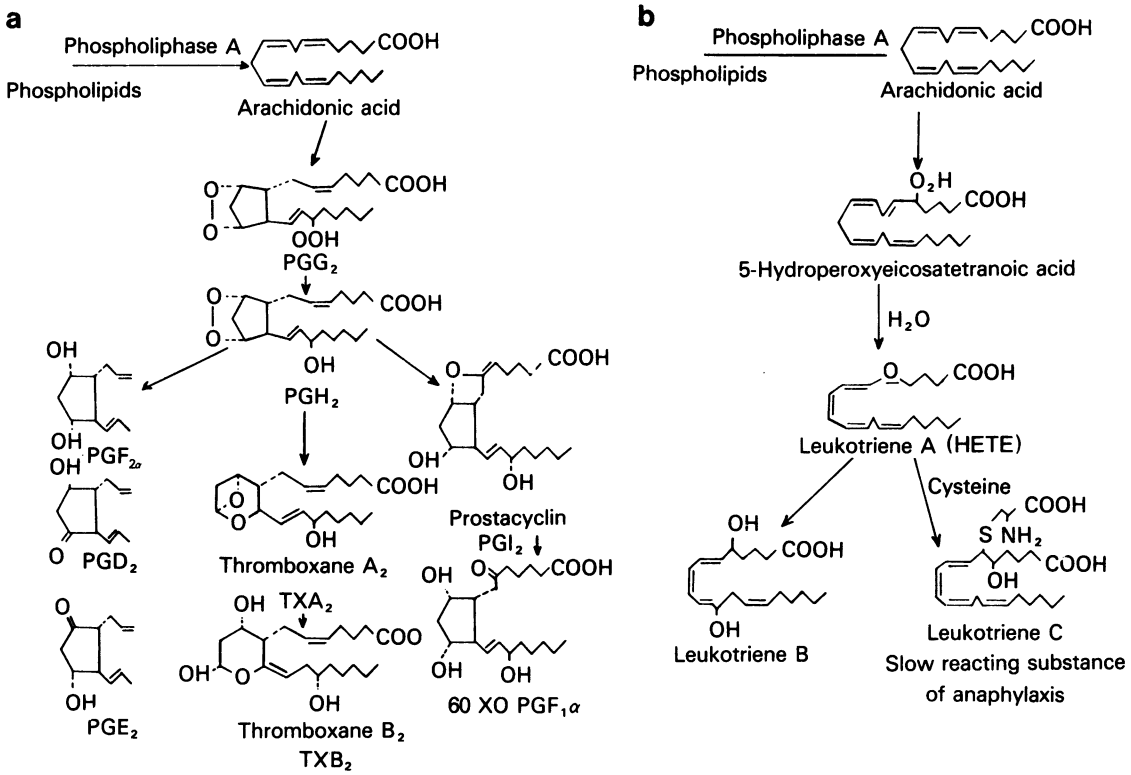

Figure 1 Production of prostaglandins, thromboxanes and leukotrienes from arachidonic acid. *a*, Cyclo-oxygenase path; *b*, lipoxygenase path.

the ability of the aspirin to reach the organ and penetrate the cell membranes. This inhibition of the enzyme by aspirin is substrate-dependent (Flower, Cheung & Cushman, 1973) and thus the concentration of AA and the activity of the phospholipase could play a role in determining the sensitivity of the enzyme to aspirin. Another possibility is that the cyclo-oxygenase of various organs may be a complex of several isoenzymes and thus have differences in affinity for substrate and aspirin (Bhattacherjee & Eakins, 1974; Dembińska-Kiéc *et al.*, 1976).

The evidence marshalled by Vane & Ferreira (1978) indicates that prostaglandin synthesis and prostaglandin effects play an important role in the inflammatory response. The ability of aspirin to block prostaglandin synthesis could account for the anti-inflammatory effects of the drug. However, the discovery of the lipoxygenase pathway and the production of leukotrienes from unsaturated fatty acids reported by B. Samuelsson (International Prostaglandin Conference, Washington DC, 27–31 May, 1979) may force us to reevaluate our conceptions concerning inflammation, as these leukotreines may play an important role in the pathology of inflammation.

Substances that are weak prostaglandin synthetase inhibitors are still effective anti-inflammatory agents (Cashin, Dawson & Kitchen, 1977). Bonta, Bult & Vincent (1977) have shown that animals on a diet deficient in polyunsaturated fatty acids show a deficiency in prostaglandin production but still show an unimpaired response to aspirin. It is also well known that thymus lymphocytes (T-cells) are abundant in the inflammatory tissue of patients suffering from rheumatoid arthritis (Van Boxel & Paget, 1975). Lymphokines are produced by activated T-cells and these produce increased vascular permeability and white blood cell chemotaxis, activate macrophages, and stimulate lymphocyte DNA synthesis (Morley, 1976; 1977; 1978). Macrophages are capable of producing prostaglandins (Morley *et al.*, 1979), which are enhanced by antigens or lymphokines and inhibited by anti-inflammatory drugs (Bray & Gordon, 1976;

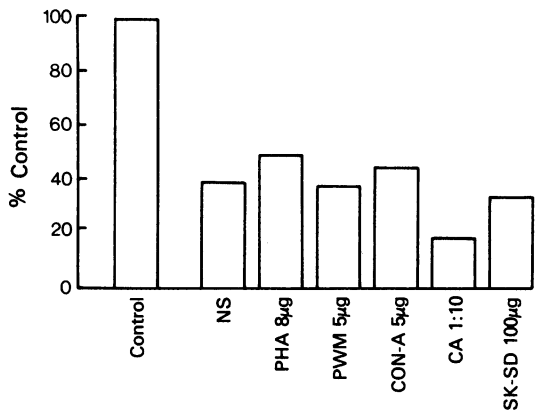

Figure 2 Effect of oral ingestion of aspirin 3.6 g daily on *in vitro* blastogenesis of peripheral blood lymphocytes isolated from normal individuals (modified from Panush & Anthony, 1976). At day of assay, serum salicylate = 23 mg per dl. NS, Not stimulated; PHA, Bact-phytohaemagglutinin; CON-A, Concavalin A; PWM, Pokeweed Mitogen; CA, Extract of Candida Albicans; SK-SD, Streptokinase-Streptodornase.

Morley, Bray, Jones, Nugteren & Van Dorp, 1979). Morley (1976; 1977; 1978) has suggested that aspirin is a good inhibitor of lymphocyte activation, in spite of the fact that it is a relatively weak inhibitor of prostaglandin synthesis. Thus, the effects of aspirin on the lymphocyte-macrophage axis involve not only inhibition of prostaglandin synthesis but also a direct effect on lymphocytes and their ability to produce lymphokines. Panush & Anthony (1976) and Crout, Hepburn & Ritts (1975) have observed that therapeutic doses of aspirin reduce the reactivity of lymphocytes to antigen or mitogen stimulation (see Figure 2). This effect of aspirin is consistent with Morley's suggestion that aspirin may have a direct effect on lymphocyte activation and lymphokine production. Thus, Morley (1978) has proposed that in normal conditions or during a self-limiting inflammatory episode the production of lymphokines from activated T-cells induces the release of lysosomal enzymes as well as prostaglandins (possibly of the E type) from macrophages. The prostaglandins produce their vascular and platelet effects and are also potent inhibitors of lymphocyte activation. Thus, the prostaglandins, besides causing many manifestations of inflammation, act also as a potent negative feedback mechanism by inhibiting lymphokine production (Morley, 1978; Morley *et al.*, 1975; 1979).

This interesting concept has led to the hypothesis that in certain chronic inflammatory reactions, the lymphocyte is relatively unreactive to inhibition by prostaglandin, which then would result in the overproduction of the various mediators of the inflammatory process. This hypothesis is further strengthened by the observation that a variety of viral bacterial, endocrinological and pharmacological mechanisms can reduce the reactivity of lymph cells to this negative feedback mechanism (Morley *et al.*, 1979; Morley, 1978). This hypothesis would predict that in acute inflammations non-steroidal anti-inflammatory agents would remove the negative feedback mechanisms. This is supported by the observation that indomethacin enhances the production of lymphokines in man and guinea-pig (Bray, Gordon & Morley, 1973, quoted from Morley, 1978) and the use of such a drug would have contradictory effects on the overall inflammatory process. In contrast, aspirin in therapeutic doses has marked effects on lymphocyte transformation and may therefore reduce lymphokine production in a normal system and one in which the normal negative feedback mechanism has been blunted by the disease-state. Aspirin and related drugs suppress the mitogen activation of the normal lymphocytes. However, spleen cells contain a type of lymphocyte which adheres to glass wool. This cell produces prostaglandins which suppress the mitogen activation of glass wool non-adherent cells and this suppression is removed by the addition of a prostaglandin synthetase inhibitor (Webb & Jamieson, 1976). Furthermore, Pelus & Strausser (1976) have shown that indomethacin enhances the mitogen response in spleen cells of tumour-bearing mice but not of normal mice. In a somewhat similar manner, lymphocytes obtained from patients suffering from Hodgkin's disease show an increased responsiveness to phytohaemagglutinin activation following the addition of indomethacin. This phenomenon is probably due to the presence of suppressor lymphocytes that produce large amounts of prostaglandins which in turn suppress the response of the lymphocyte to activation (Goodwin, Messner, Bankhurst, Peake, Saiki & Williams, 1977). Monocytes, on the other hand, are helper cells and tend to increase the response to lymphocyte activation (Potter & Moore, 1977). Thus, there seems to be a fine balance between helper and suppressor activity which is modified by disease. The effects of aspirin and related substances will thus vary with the physical state and the interaction of the suppressor and helper lymphocyte activity.

Aspirin and platelets: their relation to haemostasis and thrombosis

One of the early events following injury to small blood vessels is the adhesion of platelets to the injury

site. These platelets then spread over the injury site and other platelets stick to these transformed platelets to form an aggregate which is then further reinforced by the formation of a fibrin clot (see Willis, 1978; Baumgartner, Tschopp & Weiss, 1977). The adhesion of platelets to the subendothelial surfaces is not substantially diminished by aspirin (Baumgartner, Tschopp & Weiss, 1977); however, the final process of platelet aggregation is highly sensitive to this drug (for reviews, see Mustard & Packham, 1970; Willis, 1978). Platelets play a role in tissue injury by their ability to aggregate and physically block blood flow and by the release of a variety of agents which enhance inflammation.

Large platelet aggregates may be harmful to the system by blocking blood vessels and curtailing blood flow, thus producing infarction of various tissues and further injury produced by the agents released from platelets. The use of drugs that prevent aggregation of platelets intravascularly may be important therapy for a variety of conditions in which intravascular platelet aggregation plays a role.

It is possible to demonstrate platelet aggregation *in vitro* by utilizing platelet-rich citrated plasma. The addition of agents that cause aggregation (collagen, adrenaline 5-hydroxytryptamine, adenosine diphosphate (ADP), thrombin, thromboxane A_2) produces changes in light transmission of a suspension of platelets which can be followed in an aggregometer first devised by Born (1962) and O'Brien (1962). This *in vitro* method has been extensively used for the study of the pharmacology of platelets. *In vivo* methods for the study of platelet aggregation have also been devised (Baumgartner, 1974; Gordon, 1973; Murphy, Roswell, Downie, Robinson & Mustard, 1962; Rosenberg, Phillips & Druzba, 1974).

In the *in vitro* system ADP, collagen, adrenaline, thrombin and thromboxane are the most commonly used aggregating agents. With ADP there is first a decrease in light transmission followed by the first phase of aggregation (light transmission increased), which is not sensitive to aspirin, and the second phase of aggregation which is accompanied by a release of ADP, endoperoxides and thromboxanes from the platelets. It is this second phase of aggregation caused by prostaglandins and the ADP release that can be blocked by aspirin and aspirin-like drugs. Platelet aggregation induced by a low concentration of collagen causes a release of ADP and this can be prevented by pretreatment with aspirin. However, the aggregation produced by a high concentration of collagen is not blocked by aspirin. In a similar manner, the aggregation and ADP release produced by a low thrombin concentration is partially blocked, whereas that produced by a high concentration of thrombin is not blocked by pretreatment with aspirin. It is thus clear that not all types of platelet aggregation are blocked by aspirin or aspirin-like drugs (Willis, 1978).

In conditions in which normal platelet function is inhibited due to congenital conditions such as ADP storage pool disease, or Von Willebrand's disease by by the oral intake of aspirin or aspirin-like drugs, one does not usually observe serious bleeding disorders unless other haemostatic mechanisms are also inhibited (by anticoagulants, or in haemophilia). It is thus likely that aspirin-induced inhibition of platelet aggregation is only one of a number of haemostatic mechanisms. Other platelet mechanisms such as aggregation produced by thrombin or high concentrations of collagen could be operating; or even haemostatic mechanisms other than those involving platelet aggregation could come into play. It is thus reasonable to postulate that a combination therapy of a substance of the aspirin series and one that can inhibit the aggregation produced by thrombin and high concentrations of collagen would in theory prove superior to either substance alone.

In addition to the inhibition of the cyclo-oxygenase system, aspirin and aspirin-like drugs will inhibit a variety of other enzymes (Smith & Dawkins, 1971; Paulus & Whitehouse, 1973; Piper & Vane, 1979; Vargaftig, 1978; Morley, 1978). However, the evidence indicates that many of the effects of this class of drugs on platelet function can be explained by their ability to block prostaglandin synthesis and thus block the second phase of platelet aggregation observed in the *in vitro* system (Smith & Willis, 1971). It has been demonstrated that aspirin inhibits platelet aggregation both *in vitro* and *in vivo* (Weiss, Aledort & Kochwa, 1968; O'Brien, 1962; Zucker & Peterson, 1968; Rosenberg, Gimber-Phillips, Groblewski, Davison, Phillips, Goralnick & Cahill, 1971) in a variety of species including man. Aspirin inhibits platelet aggregation in concentrations that are several orders of magnitude lower than those that are required by salicylic acid (Rosenberg *et al.*, 1971).

In the intact animal the effects of a single dose of aspirin can be demonstrated for days after its ingestion (O'Brien, 1962). Zucker & Peterson (1968) have suggested that the platelets may be irreversibly acetylated by aspirin. This idea was further strengthened by the observation that the acetyl group of aspirin is readily and irreversibly transferred to platelets and that the degree of acetylation correlates with the inhibition of aggregation produced by aspirin (Rosenberg *et al.*, 1971). The acetylation of prostaglandin synthetase of seminal vesicles by aspirin has also been demonstrated by Roth, Stanford & Majerus (1975). They have shown that AA, AA analogues and indomethacin inhibit this acetylation by aspirin. It is thus likely that aspirin acetylates the active site of the cyclo-oxygenase, and this may explain some of the biological effects of aspirin.

The recent discoveries of the thromboxane A_2 by Hamberg, Svensson & Samuelsson (1975) and prostacyclin by Moncada, Greglewski, Bunting & Vane (1976), Greglewski, Bunting, Moncada, Flower & Vane (1976) and Johnson, Morton, Kinner, Gorman, McGuire & Sun (1976) have produced a better understanding of the involvement of platelets and the effects of aspirin on haemostasis.

The precursors prostaglandin G_2 and prostaglandin H_2, as well as thromboxane A_2, are powerful platelet aggregators and vasoconstrictors. It is now generally believed that the major platelet-aggregating material produced from the endoperoxides is thromboxane A_2. Prostacyclin is produced by vascular endothelial cells and is also derived from the endoperoxides by the activity of a prostacyclin synthetase. It is one of the most powerfully acting platelet deaggregators and vasodilators, and probably produces its effect by modulating the cyclic adenosine monophosphate (cyclic AMP) content of the platelets (Gorman, Bunting & Miller, 1977; Tateson, Moncada & Vane, 1977). If the intima of a blood vessel is injured or removed, a major source of prostacyclin is eliminated; and simultaneously the subintimal layers have the ability to produce thromboxane from the endoperoxides supplied by the platelets and the subintimal region. This will cause the aggregation of platelets on the denuded surfaces and the formation of a platelet plug. In normal conditions platelet aggregation on the intimal endothelial surface is prevented, and existing small aggregates are deaggregated by prostacyclin, thus keeping the vasculature patent. Aspirin, which acetylates the cyclo-oxygenase, will inhibit the formation of endoperoxides which are the precursors of both prostacyclin and thromboxane A_2. Thus, aspirin interference with the haemostatic processes would depend on the ratio of thromboxane to prostacyclin. It has been claimed that aspirin in man prolongs bleeding time only when given in small doses, and its effects on bleeding time are reduced when large doses are given (Grady & Moncada, 1978; Amezcua, Parsons & Moncada, 1978). However, the vascular endothelial cell cyclo-oxygenase seems to be less sensitive to aspirin inhibition than that of platelets (see Table 1), and its effects on prostacyclin synthesis are shorter lasting than on platelet thromboxane synthesis (see Gordon & Pearson, 1978; Vargaftig & Lefort, 1977). Thus, it is likely that therapeutic doses of aspirin will produce a lesser inhibition of the vascular prostacyclin system than of the platelet thromboxane-forming mechanism.

The effects of aspirin given orally on platelets have been extensively studied and reviewed (see Moncada & Vane, 1979; Ferreira & Vane, 1979; Shen, 1978). Aspirin, in contrast to salicylic acid, acetylates platelets irreversibly (Rosenberg *et al.*, 1971) and thus inhibits aggregation for the lifespan of the platelet. This inhibition can be demonstrated in man with single oral doses of aspirin of 300 to 1200 mg.

The possible relationship of platelet aggregation to a number of thrombotic diseases has been suggested (for a review, see Evans, Mustard & Packham, 1971). A most interesting finding is that in a relatively high percentage of patients suffering from cerebrovascular disease, *in vitro* spontaneous platelet aggregation could be demonstrated, whereas an equivalent group of normal subjects did not show this spontaneous aggregation (Table 2). Platelets from these patients also showed an increased tendency to aggregate due to ADP. This spontaneous aggregation and ADP sensitivity could be reversed by the daily

Table 1 Inhibition of various prostaglandin synthetase systems by aspirin

	μm	*Inhibition (%)*	
Human platlets	0.17	>50	Smith & Willis (1971)
Rabbit platelets	5	50	Rosenberg *et al.* (1971)
Dog spleen	7.5	>50	Ferreira *et al.* (1971)
Guinea-pig lung	5.5–28	100	Palmer *et al.* (1973)
Bull seminal vesicles	2000	50	Flower *et al.* (1973)
Rabbit kidney microsomes	660	50	Ku *et al.* (1976)
Rabbit brain	15	50	Flower & Vane (1972)
Vascular endothelial cells	100	100	Gordon & Pearson (1978)
Rabbit Iris	270	50	Ku *et al.* (1976)
Rabbit conjunctiva	655	50	Ku *et al.* (1976)
Sheep seminal vesicles	5500	50	Ku *et al.* (1976)

Table 2 Positive gross and microscopic spontaneous platelet aggregation (SPA) in patients with arterial insufficiency (from Wu & Hoak, 1976)

	Number of patients	*Positive SPA gross*	*Microscopic*	*Total (%)*
Transient ischaemic attacks	66	6	30	36 (55)
Angina pectoris	32	4	2	6 (19)
Myocardial infarction	10	2	5	7 (70)
Acute PAI	14	9	2	11 (80)
Chronic PAI	8	0	0	0 (0)
Normal subjects	150	0	0	0 (0)
Patient control	22	0	0	0 (0)

PAI, peripheral arterial insufficiency.

administration of aspirin 500 mg (Ten Cate, Vos, Oosterhuis, Prenger & Jenkins, 1978). In a similar manner, spontaneous platelet aggregation was observed in patients suffering from essential thrombocythemia and peripheral gangrene, and these platelets also showed an abnormally high sensitivity to collagen-induced aggregation. Oral aspirin therapy produced a clinical improvement which could be correlated with a reversal of the platelet aggregation abnormalities (Preston, Emmanuel, Winfield & Malia, 1974). Studies have shown increased second-phase platelet aggregation in latent and frank diabetics which is not changed by the usual therapy of insulin and the oral antidiabetic agents, and could not be correlated with glucose and free fatty acid plasma levels (Kwaan, Colwell, Cruz, Suwanwela & Dobbie, 1972; Colwell, Sagel, Pennington, Meeks, Scarpatto & Laimins, 1973; O'Mally, Ward, Timperley, Porter & Preston, 1975; Heath, Brigden, Canever, Pollock, Hunter, Kelsey & Bloom, 1971). Kwaan *et al.* (1972) have shown a correlation between diabetic retinopathy, nephropathy and enhanced platelet aggregation; and Halushka, Weiser, Chambers & Colwell (1976) have shown a decrease in synthesis of a prostaglandin E-like material when platelets from diabetics were compared with those obtained from normal patients. Colwell *et al.* (1973) have shown that this abnormality of platelets obtained from diabetics could be reversed by the oral administration of small doses of aspirin. Similar abnormalities in platelet aggregation were described by Wu & Hoak (1976) in over 50% of patients diagnosed as suffering from transient ischemic heart disease, acute myocardial infarction and peripheral artery disease. In the latter disease the combined administration of dipyridamole and aspirin produced a reversal of the spontaneous platelet aggregation and improvement in the clinical picture. All these observations suggest that platelet disorders may play an important role in a variety of thrombotic diseases and that aspirin and aspirin-like drugs may be beneficial in the therapy of these conditions.

Transient ischaemic attack (TIA)

This "is a temporary and focal episode of neurological dysfunction of presumed vascular origin, typically lasting 2–15 min but occasionally as long as 24 hours. These episodes clear without residue. They can be classified either as carotid or vertebral-basilar depending on the vascular territory of the presumed ischemia." Amaurosis fugax is included as part of the definition of carotid TIA; but ". . . certain symptoms as lightheadedness, syncope, confusion, vertigo, dysarthria or diplopia appearing in isolation are not to be included in the definition of TIA." (Sandok, Furlan, Whisnant & Sundt, 1978).

The usefulness of aspirin in the therapy of TIA and stroke has been suggested by a number of investigators; however, two definitive studies have been published recently (Fields, Lemak, Frankowski, *et al.*, 1977; The Canadian Cooperative Study, Barnett, 1978) which show that daily administration of aspirin 1300 mg significantly reduces the recurrence of TIA, stroke and death when compared with patients receiving placebo (Table 3). Aspirin is effective in decreasing TIA, stroke and death in this group of patients, especially in cases in which there are multiple attacks (Table 3). A second group of patients that had undergone reconstructive surgery of the carotid artery before being allocated to an aspirin or placebo group showed results that were consistent with those observed for the medical group of patients. In the other study known as The of patients. In the Canadian Cooperative Study (1978), 585 patients with threatened stroke were followed in a clinical trial of aspirin or sulphinpyrazone singly or in combination. Aspirin significantly reduced the risk for stroke or death by 30%, but this effect was sex-dependent in that female patients did not respond significantly. When only males were analyzed, the risk reduction was 58%; however, such a sex difference was not apparent in the study of Fields *et al.* (1977). Sulphinpyrazone did not show a significant risk reduction in this group of patients. The sex difference in response to aspirin has been confirmed by Kelton, Hirsh, Carter & Buchanan (1978) in an experimental procedure in rabbits in which thrombus size was measured. However, both males and females showed an equal reduction in prostaglandin synthesis. The reason for this sex difference is not known.

Coronary artery disease

Patients on long-term aspirin therapy may have a relatively low incidence of coronary thrombosis (for a

Table 3 Clinical outcome in six months following randomization by treatment group (Fields *et al.*, 1977)

	Aspirin	*Placebo*
Unfavourable outcome:		
Death: cerebral infarction	0	2
Death: cardiovascular	1	4
Death: intracerebral haemorrhage	1	0
Non-fatal cerebral infarction	4	8
Retinal infarction	1	0
Excessive ratio of TIA's	8	20
Total unfavourable outcomes	15	34
Total favourable outcomes	63	43
Total number of patients*	78	77

*23 Patients are not included in this table because their follow-up periods were less than 6 months.

review, see Didisheim & Fuster, 1978). These uncontrolled studies have prompted large scale controlled studies in patients who have previously suffered a cardiac infarction. One of these studies has been carried out in British hospitals, using double-blind randomized techniques in 1239 patients. The administration of aspirin 300 mg daily produced suggestive results (placebo group 25% deaths; aspirin group 12% deaths), although this difference was not statistically significant (Elwood *et al.*, 1974). Two other studies, one by the Boston Collaborative Drug Surveillance Group (1974) and the German-Austrian Multicenter two-year prospective study (Breddin, Uberla & Walter, 1977) have come to a similar conclusion. Other controlled studies are still in progress. Pick, Chediac & Glick (1979) have reported studies with monkeys on a high fat and cholesterol diet that have received aspirin. The coronary blood vessel involvement and the ratio of the lesion to the media was significantly reduced in the aspirin group compared with the control high lipid group.

It would not be surprising if all these studies should show only borderline drug effects, as this class of patients already has a substantial reduction in coronary blood flow and the severe damage to the vessels is probably not reversible. Better results with drug therapy may be obtained with prophylaxis in a patient population that has a high risk of infarction; some diabetics and patients suffering from angina and coronary disease show a high incidence of platelet disorders (spontaneous aggregation and sensitivity to ADP aggregation). A group of patients showing these platelet disorders before they have suffered a coronary thrombosis or cerebral accident may be a useful class of patients for a double-blind aspirin study.
high incidence of platelet disorders (spontaneous aggregation and sensitivity to ADP aggregation). A group of patients showing these platelet disorders before they have suffered a coronary thrombosis or cerebral accident may be a useful class of patients for a double-blind aspirin study.

The contact between blood and artificial surfaces is associated with platelet adhesion, the release of platelet contents, and thrombus formation. These phenomena have been observed in a number of clinical situations. Thus, arteriovenous shunts have a tendency to clog up because of thrombus formation; this can be reduced by administration of aspirin and aspirin-like drugs (Andreasy, Ritz, Schoeffner, Hahn & Walter, 1971; Harker & Slichter, 1970; Kaegi, Pineo, Shimizu, Trivedi, Hirsh & Gent, 1974).

A similar situation arises with artificial cardiac valves. There is a high incidence of embolic complications in these patients and this is probably related to atrial fibrillation as well as the presence of the artificial valves. Reduced platelet survival can be correlated with thromboembolic episodes (Genton, Gent, Hirsh & Harker, 1975). Altman, Boullon, Rouvier *et al.* (1976) have shown that small doses of aspirin significantly reduce thromboembolism in these patients. Similar observations have been made with aspirin combined with dipyridamole (Harker & Slichter, 1970).

The use of extracorporeal circulation and oxygenators exposes the blood to foreign surfaces, and then there is a marked reduction in blood platelets, the release of platelet contents and the formation of thromboxanes (Addonizio *et al.*, 1978; 1979*a*; Beurling-Horbury & Galvan, 1978). The administration of aspirin reduces the release phenomenon and thromboxane formation but does not significantly change the reduction in platelet counts (Addonizio *et al.*, 1979*b*). These findings reinforce the view that platelet aggregation can be caused by mechanisms other than those sensitive to aspirin and aspirin-like drugs.

Other vascular disease

This may be related to platelet aggregation in the blood vessels of the transplanted kidney. The use of anti-aggregating agents has been tried, but with inconclusive results (Anderson *et al.*, 1974; Kincaid-Smith, 1975).

Aspirin and related compounds have been used in the treatment of thrombotic thrombocytopenic purpura. It has been postulated that there is a substance in the blood of these patients that causes platelet adhesion, aggregation and destruction. The use of antiplatelet agents would thus be indicated. This disease is rare and no definitive data on therapy are available. From clinical observations the use of steroid therapy, splenectomy and aspirin administration either singly or in combination may have a beneficial therapeutic effect (Peterson, Amare, Henry & Bone, 1979; Zeluff, Natelson & Jackson, 1978).

Other studies have attempted to define the usefulness of aspirin in preventing postoperative venous thromboembolism. Both positive and negative results have been reported; the use of aspirin and other inhibitors of platelet aggregation for the prevention of postoperative venous thrombosis must await further definitive studies (for a review, see Didisheim & Fuster, 1978).

Effects of aspirin and aspirin-like drugs on the reproductive cycle

In animals there exists a pattern of prostaglandin synthesis in the hypothalamus, the ovaries, placenta and uterus which varies with the period of pregnancy and the ovulatory cycle.

Prostaglandins possibly cause the release of the 'releasing factors' from the hypothalamus. Thus, luteinizing hormone is released from the anterior

pituitary and its plasma concentration increases dramatically when prostaglandin $F_{2\alpha}$ is infused in sheep (Roberts, Carlson & McCracken, 1976). The synthesis of prostaglandin in the hypothalamus can be inhibited by aspirin and aspirin-like drugs which then interfere with the release of luteinizing hormone from the pituitary and inhibit ovulation (Behrman, Orczyk & Greep, 1972; Orczyk & Behrman, 1972; Roberts *et al.*, 1976). Evidence exists that the inhibition of ovulation produced by aspirin is due to the inhibition of hypothalamic prostaglandin synthesis, as luteinizing hormone and luteinizing hormone-releasing factor are still effective in instituting ovulation in rats that have received aspirin in a dose which blocks normal ovulation (Orczyk & Behrman, 1972). This block of ovulation has been applied to fertility control in man; however, the results have been negative (Leader *et al.*, 1976; Behrman and Anderson, 1974; Chaudhuri & Elder, 1976; Greenway & Swerdloff, 1978).

In some species, prostaglandin $F_{2\alpha}$ produces luteolysis. It has been postulated that prostaglandins are produced by the uterus and that its close presence to the ovary allows the direct transfer of prostaglandin from the uterine tissue to the ovary, thus causing involution of the corpus luteum. The luteolytic effect of prostaglandins results in a reduction in progesterone production (Pharris & Wyngarden, 1969; Leader, Bygdeman, Eneroth *et al.*, 1976) and cyclo-oxygenase inhibitors prolong the oestrous cycle in rats (Horton & Poyser, 1973; Poyser, 1976). However, no significant luteolytic effect could be demonstrated in man (Wentz & Jones, 1973). Csapo (1976) claims that local application of prostaglandin for abortion lowers the plasma concentration of progesterone in pregnant women. From his data, it is not clear whether the abortion or the prostaglandin application lowers the progesterone levels.

A third effect of prostaglandins is the stimulation of uterine contractions, especially of the pregnant uterus. The production of uterine prostaglandin is markedly stimulated, especially towards the late stages of pregnancy, and it is likely that prostaglandins are a major factor in the initiation of labour (Fuchs, 1974; Fuchs, Smitasiri & Chantharaksri, 1976; Vane & Williams, 1973; Karim, 1975; Zuckerman, Reiss, Atad, Lampert, Ben Ezra & Sklan, 1977; Garrioch, 1978; Kirton, Kimball & Porteus, 1976). Prostacylin or some other prostaglandin markedly increases the contractility of isolated uterine horns from pregnant animals. The spontaneous uterine contractions can be inhibited by aspirin and other anti-inflammatory drugs (Vane & Williams, 1973; Aiken, 1972; Williams, El-Tahir & Marcinkiewicz, 1979). The powerful stimulant effects of prostaglandins on uterine contractions have been utilized for induction of labour at term as well as the induction of premature labour (Roux, Mofid, Moss & Dmytrus, 1977; Karim, 1975; Mackenzie, Hillier & Embrey, 1976; Bygdeman *et al.*, 1976).

The administration of cyclo-oxygenase inhibitors prolongs labour in pregnant animals and women (Chester *et al.*, 1972; Fuchs *et al.*, 1976; Reiss, Atad, Rubenstein & Zuckerman, 1976; Waltman, Tricomi & Palav, 1972; Aiken, 1972). The inhibition of prostaglandin synthesis by aspirin and aspirin-like drugs has been utilized successfully for the treatment of premature labour; however, aspirin was much less effective than the other compounds tested (Zuckerman *et al.*, 1976; Waltman *et al.*, 1972). A potential danger involved in this type of therapy with potent cyclo-oxygenase inhibitors is the possible damage to the foetus, which may be caused by the premature closure of the ductus arteriosis, and pulmonary hypertension induced by the non-steroidal anti-inflammatory agents (Heymann & Rudolph, 1976; Levin, Mills, Parkey, Garriott & Campbell, 1979). Although aspirin is a weak inhibitor of vascular cyclo-oxygenase, it should not be used without good reason during pregnancy.

Pickles, Hall, Best *et al.* (1965), Chan & Hill (1978) and Pulkkinen, Henzl & Csapo (1978) have reported that in women suffering from dysmenorrhoea high prostaglandin $F_{2\alpha}$ levels can be found in the menstrual blood. The presence of these high concentrations of prostaglandins may be causally related. Thus, the treatment of this condition with anti-inflammatory agents has been tried with success (Lundström, 1978; Budoff, 1979; Anderson, Fraser, Haynes & Turnbull, 1978; Kapadia & Elder, 1978; Ylikorhala, Kauppila & Puolakka, 1979; Pulkkinen *et al.*, 1978; Pulkkinen & Csapo, 1978; Marx, 1979; Janbu, Løkken & Nesheim, 1978; Smith, Temple & Schearman, 1975).

The treatment of dysmenorrhea with cyclo-oxygenase inhibitors is an important new therapy for a common and disabling disease condition. It is of interest that not all inhibitors are of equal potency, and this may be due to differences in the sensitivity of the endometrial cyclo-oxygenase to the group of drugs.

It is clear that the interactions of the prostaglandins with the various processes relating to conception are complex and of opposing directions. This may explain the lack of efficacy of the prostaglandins and the cyclo-oxygenase inhibitors in the control of conception.

The gastrointestinal tract

Among the many actions of the non-steroidal anti-inflammatory agents, the effects on the gastro-intestinal tract are probably the major side actions.

Prostaglandins of the F and A type can inhibit gastric secretion, have anti-ulcerogenic properties, and are tissue-protective but produce cramps and diarrhoea in a variety of species, including man. Thus, the inhibition of prostaglandin synthesis should counteract some of these effects. Cyclo-oxygenase inhibitors can produce ulcers and increase gastric secretions, and they produce gastric and intestinal discomfort and bleeding.

Diarrhoea occurring with medullary carcinoma of the thyroid, tumours of the gastrointestinal tract, irradiation of the bowel, bacterial endotoxins, ulcerative colitis and pancreatitis have been related to prostaglandin overproduction. However, the data available are incomplete and many questions remain unanswered. Thus, diarrhoea caused by cholera has been treated with cyclo-oxygenase inhibitors with equivocal results (De, Sircar, Sasmal *et al.*, 1974) and aspirin has been found useful in the treatment of X-ray-induced diarrhoea (Mennie & Dalley, 1973; Mennie *et al.*, quoted in Bennett, 1976).

The diarrhoea associated with medullary carcinoma of the thyroid has not responded well to aspirin and indomethacin but it has responded to an extract of nutmeg, which is supposedly a prostaglandin synthetase inhibitor (Barrowman, Bennett, Hillenbrand, Rolles, Pollock & Wright, 1975; Bennett, Gradidge & Stamford, 1975).

There are clues that certain types of diarrhoea may have a causal relation to an overproduction of prostaglandins and that therapy with cyclo-oxygenase inhibitors with some specificity for the intestinal prostaglandin system could become useful in the treatment of a very common gastro-intestinal disturbance.

Interaction of prostaglandins in the kidney

Prostaglandins are involved in the excretory functions and act as local hormones for the regulation of vascular reactivity and the release of renin from the kidney. Vasoconstrictor effects caused by a variety of factors such as angiotensin, bradykinin, adrenaline, sympathetic stimulation or haemorrhage are blunted by the release of a prostaglandin which reduces the vasoconstrictor effects on the renal circulation (Aiken & Vane, 1973).

There is an inverse relationship between salt intake and the concentration of plasma prostaglandin A, which is a vasodilator. This relationship may explain the blood pressure-lowering properties of a low sodium diet. Thus, the volume reduction produced by a diuretic reduces blood pressure in hypertensive man and this effect can be inhibited by a relatively large dose of indomethacin, which also blunts the frusemide natriuresis (Lee, 1976). Thus, strong cyclo-oxygenase inhibitors will reduce renal prostaglandin formation, blunt renin release (Ånggård, Larsson & Weber, 1976; Romero, Dunlap & Strong, 1976), and increase the renal constrictor effects of renal ischaemia, sympathetic stimulation, antiogensin II and adrenaline (Solez, Fox, Miller & Heptinstall, 1974; Kirschenbaum *et al.*, 1974). This increased vasoconstrictor effect may be accompanied by a shift in blood flow from the medullary to cortical portion of the kidney and this may explain the papillary damage observed with high doses of strong cyclo-oxygenase inhibitors such as indomethacin.

There are certain pathological conditions in which renal prostaglandin overproduction plays an important role such as Bartter's syndrome. This is a rare syndrome characterized by weakness, loss of body weight, hyperplasia of the juxtaglomerular region of the kidney, low serum potassium concentration, high renin and aldosterone production, alkalosis, and insensitivity of the blood vessels to the vasoconstrictor effect of angiotensin II (Bartter, Pronove, Gill & MacCardle, 1962; Solomon & Brown, 1975; Trygstad, Mangos, Bloodworth & Lobeck, 1969). These patients did not suffer from thirst or hypertension, and the administration of spirinolactone or potassium salts produced only a slight improvement in the hypokalaemia. The familial nature of this condition has been suggested and Bartter, Gill & Frolich (1977) and Gardner, Simopoulos, Lapey & Shibolet (1972) have noted elevated sodium and reduced potassium concentration in striated muscle biopsies and red blood cells obtained from cases of this syndrome. This suggested to Bartter *et al.* that the main defect was one of electrolyte transport in the blood vessels, which then produced the changes in renin and aldosterone output.

It was Fichman, Teffer, Zia, Speckhart, Golub & Rude (1976) and Verberkmoes, Van Damme, Clement, Amery & Michielsen (1976) who simultaneously reported high plasma prostaglandin concentrations and hyperplasia of the renal medullary interstitial cells, which produce prostaglandins. These investigators have shown that the cyclo-oxygenase inhibitor indomethacin reduces the prostaglandin, renin and aldosterone concentrations, corrects the hypopotassaemia, and produces an improvement in the clinical picture. Aspirin inhibits renal prostaglandin synthesis (Romero *et al.*, 1976), and Norby, Flamenbaum, Lentz & Ramwell (1976) have demonstrated the usefulness of aspirin therapy in a case of Bartter's disease (see Table 4). It is not clear whether this inhibition of prostaglandin synthesis completely reverses the pathology; however, it is clear that such therapy with prostaglandin synthetase inhibitors is an important new therapeutic approach that prolongs the life and improves the comfort of these patients. It is

necessary to use high concentrations of these inhibitors, and renal complications due to indomethacin have been observed, especially in the very young patients.

Tumour cell growth, osteolysis, prostaglandins and aspirin

Certain tumours of animals and man contain prostaglandin-like material (Jaffe, 1974; Sykes & Maddox, 1972; Bennett, McDonald, Simpson & Stamford, 1975). Normal breast tissue has low levels of prostaglandins, whereas breast cancer tissue contains relatively high levels. The prostaglandin content is high in the bone-metastatic foci of breast, bronchial and prostatic cancer and may be related to the osteoclastic properties of these tumours (Klein & Raisz, 1970; Powles, Clark, Easty *et al.*, 1973). Certain transplantable tumours in animals will cause bone resorption *in vitro* (Tashjian, Voelkel, Goldhaber & Levine, 1974; Galasko & Bennett, 1976) and the osteolytic material produced by these cells has been identified as prostaglandins by chemical and immunological methods. Prostaglandin E_2 has been most commonly implicated as the active material (Klein & Raisz, 1970). The hypercalcaemia which occurs in these animals is inhibited by cyclo-oxygenase inhibitors (Powles *et al.*, 1973); Galasko & Bennett (1976) have shown that indomethacin reduced the osteolysis caused by these metastatic bone tumours.

The clinical implications of these findings could be most important since Powles *et al.* (1973) have shown that certain human breast cancers have osteolytic properties which are suppressed by aspirin. Breast cancer with osteolytic properties has a greater tendency to produce bone tumours than those breast tumours that did not have osteolytic properties.

Dental cysts produce prostaglandins, especially if inflamed, and the cells from these cysts have osteolytic properties (Harris, Jenkins, Bennett & Wills, 1973; Robinson, Tashjian & Levine, 1975). The possible use of aspirin and other cyclo-oxygenase inhibitors in this common dental condition are suggested by these findings. However, other osteolytic factors besides prostaglandins may be active in all these conditions (Horton, Raisz, Simmons, Oppenheim & Mergenhagen, 1972; Harris, Jenkins, Bennett, Hird & Wills, 1974; Mundy, Luben, Raisz, Oppenheim & Buell, 1974), thus explaining some of the contradictory results.

Platelets can attach themselves to various tumour cells; however, this reaction shows wide variations depending on the type of tumour cell. The ability of the tumour cells to aggregate platelets may be related to their ability to produce metastases (for reviews, see Gasic *et al.*, 1973; 1978; Ambrus, Ambrus, Gastpar, Spavente, Weber & Thurber, 1976; Gastpar, 1970).

Thrombocytopenia is associated with a decrease in the dissemination of a lung tumour in mice (Gasic, Gasic & Stewart, 1968). Hilgard (1978) has shown that the intravenous injection of a suspension of Walker 256 tumour cells cause a rapid reduction in the number of circulating platelets. These platelets accumulated mainly in the lungs in conjunction with the tumour cells to form an embolus consisting of cancer cells, platelets and fibrin. The use of aspirin as a deaggregating substance reduced these lung emboli (Gastpar, 1970); however, other investigators (Hilgard, 1978) have not confirmed these findings. Bennett, Houghton, Leaper & Stamford (1979) have suggested that the combined use of a cyclo-oxygenase inhibitor in conjunction with X-ray or chemotherapy produces a highly significant weight reduction and a reduction in the prostaglandin-like material in the transplanted tumour.

The data on the effect of aspirin in cancer cell growth and dissemination are quite contradictory, possibly because different investigators have utilized different species and different types of tumour cells to carry out their experiments. A second factor may be that thrombin-induced aggregation of platelets may play a role in certain types of tumour cell metastasis; and with this type of aggregation, cyclo-oxygenase inhibitors are not very effective.

Prostaglandins, aspirin and the pulmonary vasculature

Embolization of the lung causes a release of prostaglandin (Lindsey & Wyllie, 1970; Vaage & Piper, 1975), which in turn causes the increase in pulmonary arterial and intratracheal pressures, hypoventilation and hypoxaemia. All these

Table 4 Bartter's syndrome. *Effect of aspirin (100 mg/kg daily) (data modified from Norby* et al. *1976)*

	Serum K (nmol/l)	*Urine renin activity (ng/ml/m)*	*Urine aldosterone (ng daily)*	*Serum salicylate (mg per dl)*	*Urine PGE (ng daily)*	*Plasma PGE (pg/ml)*	*Urine PGF (ng daily)*	*Plasma PFG (pg/ml)*
Control	2.9	83	14	0	225	293	252	218
Aspirin 100 mg/kg daily	3.6	20	6	15.8	25	23	90	110
Control	2.6	85	12	0	225	Slight increase	Over 200	Over 200
Aspirin	3.5	—	—	—	—	—	—	—

manifestations have been reversed by the administration of aspirin and other cyclo-oxygenase inhibitors (Rådegran & McAslan, 1972; Tucker, Weir, Reeves & Grover, 1976; Rosoff & Salzman, 1971). It is likely that embolism of the lung releases prostaglandin-like materials from the lung tissue, platelets and other cells; and the use of aspirin and aspirin-like drugs may have beneficial therapeutic effects in this condition.

The foetus *in utero* has a direct connection between the pulmonary artery and aorta through the ductus arteriosus. In some infants, especially premature ones, this foetal ductus remains patent, causing a severe respiratory impairment and heart failure. In the past this condition has been treated surgically by tying the patent ductus arteriosus. It is believed that *in utero* the ductus arteriosus is kept patent by the formation of a dilator prostaglandin (Heymann & Rudolph, 1976) and on birth ductus closure occurs as a result of the high oxygen tension (Barcroft & Mason, 1938; Born, Dawes, Mott & Rennick, 1956). Administration of a cyclo-oxygenase inhibitor to the mother will cause the closure of the ductus arteriosus in a variety of species (see Weir & Grover, 1978; Levin *et al.*, 1979), and possibly in man (Elliott, Starling & Neutze, 1975). This effect on the ductus arteriosus by cyclo-oxygenase inhibitors has been utilized to produce a medically induced closure of a patent ductus in infants. Heymann, Rudolph & Silverman (1976) and Friedman, Hirschklau, Printz, Pitlick & Kirkpatrick (1976) have shown that aspirin and indomethacin will cause this closure. The results with indomethacin were superior to those with aspirin, and Friedman (1979) has shown that small doses of indomethacin (0.2 mg/kg) are effective in producing a closure of a patent ductus arteriosus in infants (see Table 5).

Inhibition of erythema and hyperthermia of the skin

Solar radiation which reaches the earth's surface ranges in wavelengths from 290–1000 nM. Of this radiant energy the ultraviolet band, ranging from 250–320 nM is responsible for sunburn, and the maximum effects are produced by the ultraviolet B irradiation (280–320 nM) (Hausser & Vahle, 1969). Ultraviolet A light (300–400 nM) is weakly erythemogenic but has been implicated in light-induced melanogenesis and photosensitivity to drugs (Ying, Parrish & Pathak, 1974).

In general, the ultraviolet-B range of light is used in experimental studies and the induced cell injury produced by ultraviolet light occurs in the superficial strata of the skin and erythema is caused by the diffusion of the released vasoactive agents to the dermal layer and its blood vessels, thus causing a delayed erythema (Breit & Kligman, 1969). The implicated mediators of this hyperaemia have been hydrolytic enzymes released from lysosomes, histamine kinins, prostaglandins and unidentified substances (Swingle, Trancik & Kvam, 1979).

Erythema is assessed either by subjective observation, by measurement of the skin temperature, or by the use of reflectance measurements (Breit & Kligman, 1969; Tronnier, 1969). The albino guinea-pig is the most commonly used experimental animal, and human skin can be readily studied by standardized methods. The light source usually used is a Krohmayer hot quartz lamp. After a short latency period following exposure to ultraviolet radiation, erythema appears, reaches a maximum at 4–5 h, and lasts for 24–36 hours.

The effects of aspirin and aspirin-like drugs can be studied when applied locally on the irradiated skin and when given orally. Both in guinea-pig and man, aspirin and related drugs will suppress the erythema and delay its onset, although they cannot eliminate it completely (Snyder & Eaglstein, 1974*a*; Gruber, Ridolfo, Nickander & Mikulaschek, 1972). The mechanism of erythema after exposure to ultraviolet irradiation has been related to prostaglandin formation in the skin as a result of ultraviolet-induced injury (Mathru & Gandhi, 1972; Snyder, 1976). Intradermal injection of prostaglandins produces long-lasting erythema. The vasodilatation is resistant to vasoconstrictors and is probably related to a direct action of prostaglandins on vascular smooth muscle and an inhibition of noradrenaline release from sympathetic fibres (Hedqvist, 1971; Chapnick, Paustian, Kluiner, Joiner, Hyman & Kadowitz, 1976). Other mediators of inflammation cause skin vasodilatation and it is likely that these play a role in the erythema observed following exposure to ultraviolet irradiation. This explains why aspirin and related drugs will delay, but cannot completely abolish the erythema (Wilhemi & Domenjoz, 1951). Aspirin, indomethacin and other cyclo-oxygenase inhibitors, when applied locally or when given orally, have been found useful in reducing the erythema due to sunburn in man (Snyder & Eaglstein, 1974*b*; Gruber *et al.*, 1972; Plummer, Hensby, Black *et al.*, 1977). These

Table 5 Effect of indomethacin on the closure of the ductus arteriosus in infants (modified from Friedman, 1979)

	Indomethacin	*Ligation*
Closure of duct	32/35	24/24
Time on ventilator	41.3±33 h	88.6±48 h
Mortality	14.3%	12.5%
Complications (Apnoea)	46.4%	42.8%
Pulmonary air leak	25%	33%
Renal dysfunction	0%	14.3%
Pneumonia	10.7%	28.6%
Enterocolitis	10.7%	9.5%

substances, when applied soon after exposure to ultraviolet light, will delay and reduce the erythema and temperature increase of the skin. The prostaglandin-related vasodilation occurs during the early phases of inflammation, whereas the later observed vascular changes are probably caused by other vasoactive agents. Thus, aspirin and other cyclo-oxygenase inhibitors have to be applied before or soon after exposure to sunlight or ultraviolet radiation to be effective in reducing the erythema and pain.

The above brief discussion indicates the ubiquitous actions of cyclo-oxygenase inhibitors. Some of these actions are of therapeutic importance, whereas others are still being studied or show varying degrees of effectiveness related to their inhibitor potency. Thus, the high sensitivity of platelets to aspirin, with irreversible inhibition of their function in conjunction with a lesser sensitivity of the prostacyclin system have given aspirin some important new medical indications.

It is probable that further research will increase our understanding of the involvement of the prostaglandin system in a variety of bodily functions, thus suggesting new uses for aspirin and other cyclo-oxygenase inhibitors.

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