INTRODUCTION
1.0 INTRODUCTION

1.1 REVIEW OF LITERATURE

Rheumatoid arthritis is the commonest form of inflammatory arthritis and affects about 1% of the population. The clinical presentation is heterogeneous with a wide variation in age at onset, degree of joint involvement, and severity. In addition, it is difficult to predict at diagnosis which patients will develop severe disease. Nearly 90% of patients with aggressive disease will become clinically disabled within 20 years. Furthermore, in patients with severe disease or extra-articular symptoms mortality is equal to that for patients with triple artery coronary artery disease or stage IV Hodgkin’s lymphoma. Thus the view that rheumatoid arthritis is a benign disease has been discredited.

For the past 20 years the treatment of rheumatoid arthritis has been developed on the premise that the prognosis of the disease is generally good. Treatment has been based on the sequential use of drugs starting with non-steroidal anti-inflammatory drugs progressing to disease modifying agents such as gold, sulphasalazine, and methotrexate. This pyramid approach has had limited success at preventing joint destruction or improving long term outcome. In fact, upto 90% of patients with aggressive synovitis have evidence of bone erosions within two years of the diagnosis despite treatment (Sharp et al., 1991). This has led to a move towards using disease modifying drugs early in the disease (Welske, 1996). The future of rheumatoid arthritis is currently at
an exciting cross roads with management focusing on early diagnosis, intensive induction therapy, and intensification of treatment for resistant disease. This review highlights how recent developments in our understanding of the pathogenesis of rheumatoid arthritis have led to the discovery of new targets for treatment.

1.1.1 Causes of rheumatoid arthritis

1.1.1.1 Cellular interactions in the synovium

Although there is abundant evidence that rheumatoid arthritis is immune mediated, it is still not clear whether it is primarily an autoimmune disease; whether the initiating agent is infectious, self antigen, or both; to what extent the course of the disease depends on systemic or joint specific events; or how the cells within the rheumatoid joint interact to produce the invasive and destructive environment observed in the disease (Harris, 1990). Rheumatoid arthritis is characterised by infiltration of the synovium with lymphoid cells, formations of new blood vessels, synovial proliferation, and joint destruction. The current view is that chronic inflammation is initiated by antigen induced activation of T cells which accumulate within the joint. Whether the perpetuation of the inflammatory process depends on T cells remains highly contentious, but vascular and synovial cell proliferation as well as cytokine production seem to sustain chronic synovitis and play an important part in joint destruction (Muller-Lander, 1995; Firestein, 1996; Panayi, et al., 1992; Feldman et al., 1996).
1.1.2 Genetic and environmental factors

Epidemiological data support the case for both environmental and genetic factors causing rheumatoid arthritis. Research in twins and other genetic studies suggest that the genetic component is at best 30%. An important genetic determinant for rheumatoid arthritis resides within the groove of the major histocompatibility complex molecule that binds antigen and presents it to T cells (typically the alleles DR4 and DR1) (Salmon, 1992). This region, however seems to be associated with only the severe forms of the disease. Current studies are exploring whether early screening programmes will be able to identify accurately which patients with early rheumatoid arthritis are likely to develop severe destructive joint disease and should therefore be treated aggressively at an early stage.

The idea that rheumatoid arthritis has an infectious trigger continues to fascinate rheumatologists, but no joint specific antigens have yet been discovered. Recent studies with genetically engineered mice suggest that a breakdown in the mechanism of self tolerance can lead to joint specific disease without the need for joint specific antigens (Kouskoff et al., 1996). Techniques for rapidly sequencing genes and identifying regions of interest are being used to look for new genetic loci associated with rheumatoid arthritis. Such searches for candidate genes have been effective for other polygenic diseases such as diabetes, and the discovery of predisposing genes outside the major histocompatibility complex may provide future avenues for directed treatment (Ziff, 1990).
1.1.3 Role of cytokines

The pattern of cytokine production within the rheumatoid synovium is very different from that observed in other immune mediated diseases. Most of the cytokines produced in rheumatoid arthritis seem to originate from macrophages or fibroblasts rather than activated leucocytes. Tumour necrosis factor α is thought to be important for controlling the proinflammatory cytokine network in rheumatoid arthritis (Mani, 1996). Neutralising antibody to tumour necrosis factor has a dramatic effect in humans, and recent studies have shown that recombinant soluble forms of the factor’s receptor can be used to mop up excess factor. The use of these soluble synthetic proteins (known as immunoadhesins) in conjunction with monoclonal antibodies offers the real possibility of inducing a partial remission in the normally relentless progression of the disease (Moreland et al., 1997).

Further studies examining the mechanisms by which the production of cytokines is regulated are yielding clues about their role in chronic inflammatory diseases. For example, some people with one polymorphism in an important regulatory region of the tumour necrosis factor α gene seem to produce too much of the cytokine during an inflammatory response and may therefore be predisposed to developing overexuberant chronic inflammatory responses.
1.1.4 New therapeutic targets

Because rheumatoid arthritis is so heterogeneous with a multi-step pathogenesis, it has proved difficult to treat systematically. Although current treatments have been relatively successful at controlling the symptoms of chronic synovitis, true long term remission in aggressive rheumatoid arthritis has not been achieved. This failure has sparked an interest in the use of early combination drug therapy in patients with aggressive disease. Furthermore, current strategies have been based on the assumption that T cells, antigenic peptide, and the major histocompatibility complex are the most appropriate targets for specific and sustained treatment. The success, albeit experimental, of anticytokine therapy has now focused attention on treatments aimed at alternative targets such as synovial macrophages, fibroblasts, and endothelial cells.

1.1.5 Adhesion molecules, cell matrix, and matrix degrading enzymes

Leucocytes do not have cilia and therefore cannot swim. They move throughout the body from the blood stream through an orderly sequence of molecular interactions involving several cell surface adhesion molecules. The direction of the migration is determined by specialised chemicals or chemoattractants called chemokines. Specialised cell surface proteins called integrins provide the mechanical support that allows the cells to migrate over and through the endothelium. During an inflammatory process, agents such as tumour necrosis factor α induce the expression and activation of families of adhesion molecules on both the leucocytes and the endothelium.
The availability of monoclonal antibodies and techniques for rapid cloning of these cell surface molecules has greatly increased our understanding of the molecular signposts or "area codes" that guide leucocytes to discrete compartments within the body. A specific synovial area code has not yet been identified, but it would provide an attractive target for anti-adhesion therapy. The first wave of anti-sticky drugs (a neutralising antibody to the platelet integrin αIIbβ3) has recently been licensed for use in preventing coronary artery restenosis after balloon angioplasty.

It is now clear that the extracellular matrix and the presence of other inflammatory cells can influence the behaviour of lymphocytes. For example, culturing monocytes with synovial fibroblasts leads to the production of proinflammatory cytokines such as interleukin 6. In addition, such cellular interactions can lead to the production and secretion of enzymes called matrix metalloproteases, which can remodel the extracellular matrix. Although the controlled expression and regulation of these enzymes is crucial for wound healing and migration of cells through tissues, unchecked expression can lead to joint destruction and scarring. Naturally occurring and synthetic inhibitors of these enzymes are currently in clinical trials for rheumatoid arthritis and show early promise.

1.1.6 Inhibition of angiogenesis

Angiogenesis (the formation of new blood vessels) is a cardinal feature observed in the proliferating synovial membrane in rheumatoid arthritis. Proliferation of endothelial cells requires interactions between adhesion molecules on the endothelial cell (such as integrins) and the
extracellular basement membrane or matrix (such as fibronectin). Both antibodies and small chemicals based on the sequence in the ligands recognised by integrins have proved effective in inducing the death (by apoptosis) of endothelial cells (Stromblad and Cheresh, 1996). Clinical trials of anti-angiogenic drugs are now under way in patients with rheumatoid arthritis.

1.1.7 Cell proliferation and apoptosis

Molecular and cellular interactions within the inflammatory site directly modify cell behaviour and cause chronic inflammation. Recent studies have shown that as well as regulating cytokine secretion and cell proliferation, the synovial microenvironment inhibits T cell apoptosis. Other studies have shown that the interaction of immunoglobulin secreting plasma cells with synovial fibroblasts prevents them from undergoing apoptosis in a contact dependent manner, which may account for the continued production of rheumatoid factor during the disease. These studies strongly suggest that the tissue microenvironment is crucial in determining the balance between cell proliferation, survival, and death (Akbar and Salmon, 1997). They also raise the possibility that one of the main defects in the rheumatoid joint is abnormal environmental cues leading to inappropriate cell survival, cytokine secretion, and cell retention. Treatments aimed at enhancing apoptosis or preventing leucocyte accumulation within the rheumatoid synovium may thus turn out to be particularly effective.
1.1.8 Role of traditional medical system in the treatment of rheumatoid arthritis

Review of literature on traditional system of Indian medicine viz. ayurveda and siddha will reveal the therapeutic potentialities of single herbal medicine and formulations in the treatment of chronic refractory inflammatory diseases like rheumatoid arthritis and osteo arthritis.

The ingredients used in these preparations are obtained from natural resources like plants, minerals, animals and planktonic algae. According to ancient literature the edible algae are described as sea grass and they play an important role in therapeutics. The other grass varieties used in traditional system of medicine are Dhruva grass, Wheat and barley grasses. Uniformly they are rich in nutrients like β-carotene, α-tocopherol, GLA, B₁₂ and vegetable proteins. They are used as nutritional supplement in folk medicine in the form of chutney, marmalade and avaleha (a modified form of jam) for the treatment of chronic illness of varied etiology. These grasses are also used as rejuvenative tonic (rasayana) for its antiageing and antioxidant properties.

1.1.9 Newer strategies for research in rheumatoid arthritis

Rheumatoid arthritis is not a benign disease. Future treatments will require a radical change in our understanding of the mechanisms involved in initiating and perpetuating the disease. A false sense of security, engendered by better control of symptoms, has been cruelly exposed by studies of long term
outcome showing that the prognosis of rheumatoid arthritis remains poor. Newer strategies based on early aggressive treatment, although still in their infancy, show some improvement over conventional treatments. Therapeutic targets such as the synovial fibroblasts are now providing exciting possibilities for future treatment.

Hence, in the present work, the sea grass, *Spirulina fusiformis* (SF) (MCRC isolate) was evaluated for its anti-inflammatory, antiarthritic and antioxidant activities in rats.

1.1.10 Objectives of the present work

1) To evaluate the antiinflammatory, analgesic and antipyretic activities of *Spirulina fusiformis* (SF) MCRC isolate) in appropriate experimental models in rats.

2) To evaluate the antiarthritic activity of SF in developing and developed experimental arthritis in rats.

3) To evaluate the antioxidant activity of SF in arthritic rats.

4) To study the biochemical changes in experimental arthritis and the therapeutic effect of SF, if any, to resolve the altered biochemical parameters to normal.

5) To study the acute and sub-acute toxicity of SF in normal rats.
6) To study the ulcerogenic potential of SF, if any, on administration for short period.

7) To compare the above parameters with a standard nonsteroidal antiinflammatory drug Nimesulide usually used for the treatment of rheumatoid arthritis and adjudge the therapeutic advantage, if any of SF treatment over standard NSAID.

1.2 **SPIRULINA**

*Spirulina* is a planktonic blue-green algae found in warm water alkaline volcanic lakes. Wild *Spirulina* sustains huge flocks of flamingos in the alkaline East African Rift Valley Lakes. It possesses an amazing ability to thrive in conditions much too harsh for other algae. As might be expected, it has a highly unusual nutritional profile. *Spirulina* has a 65% protein content, is the world's richest natural source of Vitamin B$_{12}$ and contains a wide spectrum of natural mixed carotene and xanthophyll phytopigments. *Spirulina* has a soft cell wall made up of complex sugars and protein and is different from most other algae which makes it easily digestible.

Millions of people worldwide eat *Spirulina* cultivated in scientifically designed algal farms. Current world production of *Spirulina* for human consumption is more than one thousand metric tons annually. The United States leads world production followed by China, India and Thailand. More countries are planning production as they realize that it is a valuable strategic resource.
1.2.1 **Spirulina the ‘wholesome food’**

It contains the most remarkable concentration of nutrients known in any food. It's the highest protein food, which contains over 60% of all digestible vegetable protein. It has one of the highest concentration of beta-carotene, vitamin $B_{12}$, iron and trace elements and rare essential fatty acids GLA, making *Spirulina* a great whole food alternative to isolated vitamins and minerals.

Scientists, on the path of old scriptures and tradition have found that four grasses are known for human consumption. Dhruva grass, Wheat grass, Barley grass and Sea grass are the four - in this category. Sea grass or *Spirulina* becomes the optional and the most conventional choice of natural supplement of nutrition due to its environment-friendly production and hassle-free utilization. The other three grasses include the practice of home production and require further processing before the actual intake, which is tedious and involves greater time at disposal. *Spirulina*, whereas is a ‘Superfood’ of convenience too. Available in tablet, capsule and powder form, it is provided directly for consumption by human.

This green superfood fills the void in any diet today. *Spirulina* provides, easy to metabolize protein, which body builders and physiotherapists use for soft tissue healing. The high protein and vitamin $B_{12}$ (which is lacking in many common vegetables) support vegetarians. These greens also have powerful antioxidants and minerals which are easy to absorb. The artificial minerals supplied otherwise to the body are not easily digestible by the human
system thus, making Spirulina the only alternative which is the best among its contemporaries.

It is desired that there should be no confusion over synthetic vitamins and the natural vitamins derived from food. The American Cancer Society says that food is the best source of vitamins and minerals as it provides other potential cancer-protection benefits, not offered by synthetic and isolated supplements. Spirulina, Dhruva, Wheat and Barley grasses are protein packed nutritious foods. Spirulina, ranges upwards to 65% protein whereas the other grasses run around 25%. They are all high in antioxidants, minerals, vitamins and phytonutrients. The betacarotene content of 1 g of Spirulina is equivalent to about \( \frac{1}{2} \) kg of raw carrots. Six ounces of milk gives 6 g of protein whereas 10 g of Spirulina provides 6.5 g of protein along with 8 g of fat and 150 calories. Spirulina not only caters to people on high protein, low calorie diet, but for those who are lactose intolerant or strict vegetarians.

Spirulina is not only synonymous with good health but also achieves the goal of sustaining a green and less polluted environment. Cultivating algae in harmony with the environment ensures further safety. Grown in controlled, pure cultures along with strict testing standards, guarantees quality and safety. Spirulina does not cause any kind of soil erosion since it can be grown in brackish water, fresh water ponds and warm water lake. It is higher in chlorophyll content than many of the green foods.

Besides the unique phytonutrient character of Spirulina that boost the immune system, prevent disease and improve health - the very fact that
nature cures whatever it afflicts becomes very true. The usage of Spirulina by man in the conventional complexities of stress prone life, can allow him to take liberty since Magna Mater or The Great Mother Nature herself is on his guard.

1.2.2 Spirulina fusiformis (MCRC isolate)

Spirulina fusiformis (MCRC isolate) was isolated, identified and characterised taxonomically in an earlier study (Jeeji Bai and Seshadri, 1980). This has been successfully adopted in mass culture (Seshadri et al., 1980). In this study the MCRC isolate of Spirulina has been used.

The naturally occurring Spirulina is quite often a mixture of species and strains. The original toxicological experiments was carried out using naturally occurring population (Chamorro Unido, 1980).

The biochemical and toxicological studies were done in Spirulina administrated rats and it showed no harmful effects (Venkataraman, 1980).

In Spirulina treated rats no serious abnormalities in functional organs of kidney, liver, heart and spleen had been observed. The absence of negative effects was also seen in other studies where Spirulina was used as a sole source of protein (Backer, 1988).

The detailed hematological and histopathological tests for toxicity of Spirulina fed rats (Yashino et al., 1980) showed no abnormalities.
Spirulina fed rats showed a 3 fold increase in lactobacillus content and 43% increase in Vit.B, level in the caecum (Takai, 1987) and also seen reduced kidney toxicity from mercuric poisoning (Yamana, 1988).

The nutritional study showed a body weight gain by Spirulina fusiformis supplemented in the poor rice diet intake (Krishnakumari, 1982).

Spirulina contains iron. An increase in the blood haemoglobin level was observed in the Spirulina treated rats, compared to control (Kapoor and Mehta, 1991).

Spirulina fusiformis diet was found to be slightly inferior to pure diet in rats in terms of protein and lactose levels in milk. Considerable increase in Vit-A and riboflavin storage capability of liver in Spirulina treated rats have been reported (Kapoor and Mehta, 1991).

1.3 THERAPEUTICALLY ACTIVE CONSTITUENTS OF BLUE-GREEN ALGAE SPIRULINA

Oral cancer is one of the commonest cancers in India. Tobacco is the leading cause, but other causes like alcohol are also present. Use of antioxidants and vitamins have been known to prevent cancers to a large extent. Experimental studies in animal models have demonstrated an inhibitory effect of Spirulina and Dunaliella algae on oral carcinogenesis.
An extract of *Spirulina* and *Dunaliella* algae was shown to prevent tumour development in hamster buccal pouch when a 0.1% solution of 7,12-dimethylbenz[a]anthracene (DMBA) in mineral oil was applied topically three times weekly for 28 weeks (Schwartz, 1988). The algae extract was delivered by mouth in continued dosages of 140 µg in 0.4 ml mineral oil three times per week. After 28 weeks, the animals given vehicle and untreated controls all presented gross tumours of the right buccal pouch. Animals fed canthaxanthin presented a notably and statistically significant reduction in tumour number and size compared with controls. Animals fed betacarotene demonstrated a smaller but statistically significant reduction in tumour number and size. The algae treated animals presented a complete absence of gross tumors. However, microscopic sections of the buccal pouch in the algae treated group showed localized areas of dysplasia and early carcinoma-in-situ undergoing destruction.

Mathew, (1995) evaluated the chemopreventive activity of *Spirulina fusiformis* (SF) (1 g/day) in reversing oral leukoplakia in pan tobacco chewers in Kerala, India. Complete regression of lesions was observed in 20 of 44 (45%) evaluable subjects supplemented with SF, as opposed to 3 of 43 (7%) in the placebo group. When stratified by type of leukoplakia, the response was more pronounced in homogeneous lesions: complete regression was seen in 16 of 28 (57%) subjects with homogeneous leukoplakia, 2 of 8 with erythroplakia, 2 of 4 with verrucous leukoplakia, and 0 of 4 with ulcerated and nodular lesions. Within one year of discontinuing supplements, 9 of 20 (45%) complete responders with SF developed recurrent lesions. Supplementation with SF did
not result in increased serum concentration of retinol or beta-carotene, nor was it associated with toxicity. This is the first human study evaluating the chemopreventive potential of SF.

*Spirulina* holds much promise in the prevention of cancers. Natural prevention is proving to be a potent therapy.

The hepatoprotective action of *Spirulina maxima* (Torres-Durran, 1998) and *Spirulina fusiformis* (MCRC isolate) (Murugan, 1996) against *CCl₄* induced hepatotoxicity has been reported. The hepatoprotective action of *Spirulina* (MCRC isolate) against Paracetamol and Aflatoxin B₁ has also been reported (Subramanian, 1996).

The effect of *Spirulina platensis* treatment to increase in the numbers of splenic antibody-producing cells in the primary immune response to sheep red blood cells (SRBC) has been reported (Hayashi, 1994).

An aqueous extract of the blue-green filamentous algae *Spirulina platensis* inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC) and Langerhans cells (LC). The extract inactivated HIV-1 infectivity directly when preincubated with virus before addition to human T-cell lines. Fractionation of the extract revealed antiviral activity in the polysaccharide fraction and also in a fraction which is depleted of polysaccharides and tannins. Aqueous *S.platensis* extracts contain antivetroviral activity that may be of potential clinical interest (Ayehunie, 1998).
Modulatory potential of *Spirulina fusiformis* was observed on the hepatic and extrahepatic carcinogen metabolizing enzymes in Swiss albino mice at a dose of 800 mg/kg b.w. given orally. A significant reduction in the hepatic cytochrome P-450 content was observed in the group treated with *Spirulina* in comparison with the control group. The hepatic glutathione-S-transferase activity was induced significantly by *Spirulina* treatment. There was no change in the extrahepatic glutathione-S-transferase activity after the animals were fed with *Spirulina* (Mittal, 1999).

### 1.3.1 Beta Carotene

Beta carotene, which is found in *Spirulina*, is a precursor of vitamin-A. The body converts beta carotene to vitamin-A. It occurs mainly in fruits and vegetables that are deep yellow, orange or dark green in colour, such as carrots, squash, yams, peaches, apricots, spinach, collard or mustard greens and broccoli. It is an anti-oxidant and prevents cancer-causing substances from damaging DNA. Epidemiological studies have linked high intake of foods rich in beta carotene and high serum levels of the micronutrient to a reduced risk of cancer, particularly lung cancer (Mayne, 1994).

### 1.3.2 Natural and synthetic β-carotene

Natural β-carotene holds several advantages over the synthetic form. The natural form is a more effective antioxidant than the synthetic form.
The physicochemical properties of 9-cis-β-carotene are substantially different from those of all-trans β-carotene, especially in relation to fat solubility and crystallization. Recently, the anti-oxidant activity of 9-cis-β-carotene was compared in vitro with that of all-trans β-carotene and the 9-cis isomer was shown to have higher antioxidant potency (Ziegler, 1989) and (Ziegler, 1991). An in vivo animal study showed that the stereoisomer mixture of D. bardawil, a unicellular algae protects against oxygen toxicity in the central nervous system, whereas no protection was observed with all-trans β-carotene or with oxidized natural β-carotene.

1.3.3 Phycocyanin

_Spirulina_ has a dark blue green colour, because it is rich in a brilliant blue polypeptide called phycocyanin. C-Phycocyanin (from _Spirulina platensis_) effectively inhibited CCl$_4$-induced lipid peroxidation in rat liver in vivo. Both native and reduced phycocyanin significantly inhibited peroxyl radical - induced lipid peroxidation in rat liver microsomes and the inhibition was concentration dependent with an increasing concentration (0-50 μM)-IC$_{50}$ of 11.35 and 12.7 μM, respectively. The radical scavenging property of phycocyanin was established by studying its reactivity with peroxyl and hydroxyl radicals and also by competition kinetics of crocin bleaching. These studies have demonstrated that phycocyanin is a potent peroxyl radical scavenger with an IC$_{50}$ of 5.0 μM and the rate constant ratios obtained for phycocyanin and uric acid (a known peroxyl radical scavenger) were 1.54 and 3.5, respectively. These studies clearly suggest that the co-valently linked
chromophore, phycocyanobilin, is involved in the antioxidant and radical scavenging activity of phycocyanin (Vadiraja, 2000).

1.3.4 Other Nutrients

Apart from nutritional values, Spirulina is loaded with unique life enhancing compounds called phytonutrients like, polysaccharides and sulfolipids that enhance the immune system, possibly reducing risk of infections, cancer and autoimmune disease. Spirulina is rich in natural carotenoid antioxidants that promotes cellular health and reduces the risk of cancer. Spirulina is also rich in chlorophyll, which helps to detoxify the environmental pollutants.

In addition to the above, Ca++, Fe++, Mg, K, Zn, Se and other trace minerals both in natural and colloidal form are present which are well utilized as cofactors in many enzyme reactions.

1.4 NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

NSAIDs account for a big market share in the pharmaceutical industry. The wide range of therapeutic applications for NSAIDs from the simple sprain to the management of chronic inflammatory conditions like rheumatoid arthritis and joint inflammatory conditions extend the use of NSAIDs from one day to few months or even years. The anti-inflammatory drug therapy starting from Acetylsalicylic acid to the newer molecules like Meloxicam exhibit the gastric intolerance as the side effects to drug therapy
from the day one itself or after the administration for few days. This is an inherent side effect of NSAIDs irrespective of their chemical nature. The anti-inflammatory drugs which produce the anti-inflammatory action through inhibition of cyclooxygenase pathway and subsequent inhibition of Prostaglandin (PG) synthesis produce gastric intolerance side effect due to the non-selective blocking of COX-I and COX-II cyclooxygenase pathways.

The Nonsteroidal Anti-inflammatory Drugs (NSAIDs) are a group of chemically dissimilar agents that differ in their antipyretic, analgesic and anti-inflammatory activities. They act primarily by inhibiting the cyclooxygenase enzymes but not the lipoxygenase enzymes. Aspirin is the prototype drug of this group; it is the most commonly used and the drug to which all other anti-inflammatory agents are compared. However, about 15% of patients show an intolerance to aspirin. Therefore, these individuals may benefit from other NSAIDs. In addition, some of the newer NSAIDs are marginally superior to aspirin in certain patients, because they have greater anti-inflammatory activity and/or cause less gastric irritation, or can be taken less frequently. However, the newer NSAIDs are considerably more expensive than Aspirin, and some have proved to be more toxic in other ways.

1.4.1 Mechanism of Action

The antipyretic and anti-inflammatory effects of the salicylates are primarily due to the blockade of prostaglandin synthesis at the thermoregulatory centers in the hypothalamus and at peripheral target sites.
Furthermore, by decreasing prostaglandin synthesis, the salicylates also prevent the sensitization of pain receptors to both stimuli at subcortical sites.

1.4.2 Therapeutic Actions

The NSAIDs including Aspirin, have the three major therapeutic actions, namely reduction in inflammation, pain and fever. However, not all of the NSAIDs are equally potent in each of these actions.

1.4.3 Anti-inflammatory Actions

Because Aspirin inhibits cyclooxygenase activity, it diminishes the formation of Prostaglandins and thus modulates those aspects of inflammation in which Prostaglandins act as mediators. Aspirin inhibits inflammation in arthritis, but it neither arrests the progress of the disease nor does it induce remission.

1.4.4 Analgesic Action

Prostaglandin $E_2$ is thought to sensitize the nerve endings to the action of bradykinin, histamine, and other chemical mediators released locally by the inflammatory process. Thus, by decreasing $PGE_2$ synthesis, Aspirin and other NSAIDs depress the sensation of pain. The salicylates are used mainly for the management of pain of low to moderate intensity arising from integumental structures rather than that arising from the viscera. NSAIDs are superior to opiates in the management of pain in which inflammation is involved; combinations of opiates and NSAIDs are effective in treating pain in malignancy.
1.4.5 **Antipyretic Action**

Fever occurs when the set-point of the anterior hypothalamic thermoregulatory center is elevated. This can be caused by PGE$_2$ synthesis, which is stimulated when an endogenous fever-producing agent (pyrogen) such as cytokine is released from White Blood Cells (WBC) which in turn are activated by infection, hypersensitivity, malignancy, or inflammation. The salicylates lower body temperature in patients with fever by impeding PGE$_2$ synthesis and release. Aspirin resets the "thermostat" towards normal and rapidly lowers the body temperature of febrile patients by increasing heat dissipation as a result of peripheral vasodilation and sweating. Aspirin has no effect on normal body temperature.

1.4.6 **Gastrointestinal Effects**

Normally, Prostacyclin inhibits gastric acid secretion, whereas PGE$_2$ and PGF$_{2\alpha}$ stimulate synthesis of protective mucus in both the stomach and small intestine. In the presence of Aspirin, these prostanoids are not formed, resulting in increased gastric acid secretion and diminished mucus protection. This may cause epigastric distress, ulceration, and/or hemorrhage. At ordinary Aspirin doses, as much as 3 to 8 ml of blood may be lost in the feces per day.

1.4.7 **Effect on Platelets**

Throboxane (TXA$_2$) enhances platelet aggregation, whereas PGI$_2$ decreases it. Low doses of Aspirin can irreversibly inhibit thromboxane production in platelets without markedly affecting TXA$_2$ production in the
endothelial cells of the blood vessel. As a result of the decrease in TXA₂, platelet aggregation is reduced, producing an anticoagulant effect with a prolonged bleeding time.

1.4.8 Actions on the Kidney

Cyclooxygenase inhibitors prevent the synthesis of PGE₂ and PGl₂ - prostaglandins that are responsible for maintaining renal blood flow, particularly in the presence of circulating vasoconstrictors. Decreased synthesis of prostaglandins can result in retention of sodium and water and may cause edema and hyperkalemia in some patients. Interstitial nephritis can also occur with all of the NSAIDs except Aspirin.

1.5 CHEMICAL CLASSIFICATION OF NSAIDs

1.5.1 Propionic acid derivatives

1.5.1.1 Ibuprofen

Ibuprofen was the first in this class of agents to become available in the United States. It has been joined by Naproxen, Fenoprofen, Flurbiprofen, Ketoprofen and Oxaprozin. All of these drugs possess anti-inflammatory, analgesic and antipyretic activity and have gained wide acceptance in the chronic treatment of rheumatoid and osteoarthritis because their gastrointestinal effects are generally less intense than that of Aspirin. These drugs are reversible inhibitors of the cyclooxygenases and thus, like Aspirin, inhibit the synthesis of prostaglandins but not that of leukotrienes. All are well absorbed on oral administration and are almost totally bound to serum albumin. They undergo hepatic metabolism and are excreted by the kidney.
The most common adverse effect is gastrointestinal, ranging from dyspepsia to bleeding. Side effects involving the CNS, such as headache, tinnitus and dizziness, have also been reported (Adams et al., 1969).

1.5.2 Indole Derivatives

This group of drugs includes Indomethacin, Sulindac and Etodolac. All have anti-inflammatory, analgesic and antipyretic activity. They act by reversibly inhibiting cyclooxygenase. They are generally not used to lower fever.

1.5.2.1 Indomethacin

This NSAID is more potent than Aspirin as an anti-inflammatory agent, but it is inferior to the salicylates at doses tolerated by rheumatoid arthritis patients. In certain instances, however, Indomethacin is more effective in relieving inflammation than Aspirin or any of the other NSAIDs (Rhymer and Gengos, 1979).

1.5.2.2 Sulindac

The inactive pro-drug is closely related to Indomethacin. Metabolism by hepatic microsomal enzymes produces the active form of the drug, which has a long duration of action. Although the drug is less potent than Indomethacin, it is useful in the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and acute gout. The adverse reactions are similar to but less severe than those of the other NSAIDs, including Indomethacin (Brogden et al., 1978).
1.5.2.3 **Etodolac**

This drug has effects similar to those of the other NSAIDs. Gastrointestinal problems are less common. However, other adverse effects such as fluid retention and abnormal kidney and liver functions have been reported. Etodolac may increase the serum levels of other drugs and thus raise the risk of adverse reactions caused by Digoxin, Lithium, Methotrexate, and enhance the nephrotoxicity of Cyclosporin (Glaser, 1995).

1.6 **OXICAM DERIVATIVES**

1.6.1 **Piroxicam**

Piroxicam is an oxicam derivative and its mechanism of action has not been established. But Piroxicam is used to treat rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. Its mean half-life of 50 hours permits administration once a day. GI disturbances are encountered in approximately 20% of patients. The drug and its metabolites are excreted in the urine. Piroxicam can interfere with the renal excretion of Lithiums (Brogden *et al.*, 1984).

1.7 **ANTHRANILIC ACID DERIVATIVES**

1.7.1 **Mefenamic acid**

Mefenamic acid and Meclofenamate have no advantages over the other NSAIDs as anti-inflammatory agents. Their side effects, such as diarrhoea, can be severe and associated with inflammation of the bowel (Winder, 1966).
1.8 PYRAZOLONE DERIVATIVES

1.8.1 Phenylbutazone

Phenylbutazone has powerful anti-inflammatory effects but weak analgesic and antipyretic activities. At present it is not a first line drug for the treatment of arthritis. The usefulness of Phenylbutazone is limited by its toxicity (Von Recheuberg, 1962).

1.9 SULFONANILIDE DERIVATIVE

1.9.1 Nimesulide

Nimesulide: The newer NSAID, a relatively weak inhibitor of PG synthesis (may be somewhat selective for COX-2), appears to exert its effect by other mechanisms like reduced generation of superoxide by neutrophils, inhibition of PAF synthesis and TNFα release, free radical scavenging and inhibition of metalloproteinase activity in cartilage. The analgesic, antipyretic and anti-inflammatory activity of Nimesulide has been rated comparable to other NSAIDs. It is used primarily for short lasting painful inflammatory conditions like sport injuries, sinusitis and other ENT disorders, dental surgery, bursitis, low backache, dysmenorrhoea, post-operative pain, osteoarthritis and rheumatoid arthritis.

Adverse effects of Nimesulide are gastrointestinal (nausea, loose motions), dermatological (rash, pruritus) and central (Somnolence, dizziness). Most asthmatics and those who develop bronchospasm due to intolerance to Aspirin and other NSAIDs, do not cross react with Nimesulide.
Nimesulide is completely orally absorbed; 99% plasma protein bound, extensively metabolised and excreted mainly in urine with \(T\frac{1}{2}\) of 2 to 5 hours (Ward and Brogden, 1988; Davis and Brogden, 1994).

Nimesulide is used as a reference drug in this study since it exhibits analgesic, antipyretic antiinflammatory and antioxidant properties.

1.10 **ARYL ACETIC ACID DERIVATIVES**

1.10.1 **Diclofenac**

A cyclooxygenase inhibitor, Diclofenac is approved for long-term use in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is more potent than Indomethacin and Naproxen. An ophthalmic preparation is also available. Diclofenac accumulates in synovial fluid. The urine is the primary route of excretion for the drug and its metabolites. Its toxicities are similar to those of the other NSAIDs, for example, gastrointestinal problems are common, and the drug can also give rise to elevated hepatic enzyme levels (Fowler, 1979).

1.11 **TOLMETIN AND NABUMETONE**

Tolmetin (Wong *et al.*, 1973) and Nabumetone (Boyle *et al.*, 1982) are as potent as Aspirin in treating adult or juvenile rheumatoid arthritis or osteoarthritis, but may have fewer adverse effects.
1.12 PYRROLO-PYRROLE DERIVATIVES

1.12.1 Ketorolac

This drug acts like the other NSAIDs. In addition to the oral route, Ketorolac can be administered intramuscularly in the treatment of postoperative pain, and topically for allergic conjunctivitis. Ketorolac undergoes hepatic metabolism; the drug and its metabolites are eliminated via the urine. It causes the same side effects as the other NSAIDs (Rooks et al., 1982).

1.13 EICOSANOIDS AND THEIR ROLE IN INFLAMMATION

Eicosanoids, unlike histamine, are not found pre-formed in the tissues; they are generated de novo from phospholipids. They are implicated in the control of many physiological processes and are among the most important mediators and modulators of the inflammatory reaction (Diagram 1.1) (Moncada et al., 1978; Samuelsson, 1983 and Davies et al., 1984).

Interest in eicosanoids arose in the 1930s after reports that semen contained a substance which contracted uterine smooth muscle. The substance was believed to originate in the prostate and was saddled with the misnomer, Prostaglandin. More than two decades later it became clear that prostaglandin was not just one substance but a whole family of compounds. In the 1960s, two prostaglandins (PGE and PGF$_{2\alpha}$) were isolated in crystalline form. Subsequently, several more prostaglandins were found to be generated in tissue, and it was shown that these compounds were derived from Arachidonate. In the early 1970s, Vane advanced the hypothesis that inhibition
Diagram 1.1 Summary diagram of mediators derived from phospholipids and their actions, and the sites of action of anti-inflammatory drugs. The arachidonate metabolites are 'eicosanoids'. Established drugs are shown with full thickness arrows, drugs still under test with dashed arrows. The glucocorticoids inhibit transcription of the gene for cyclooxygenase-2, which is induced in inflammatory cells by inflammatory mediators. The effects of PGE₂ depend on which of the three receptors for this prostanoid are activated; (PG = prostaglandin; PGI₂ = prostacyclin; TX = thromboxane; LT = leukotriene; HETE = hydroxyeicosatetraenoic acid; HPETE = hydroperoxyeicosatetraenoic acid; PAF = platelet-activating factor; NSAIDs = non-steroidal anti-inflammatory drugs)
of prostaglandin synthesis was the mechanism of action of Aspirin-like drugs. Later, intermediate substances in the synthetic pathway; two unstable cyclic medoperoxides were isolated and identified, and two rather different compounds derived from these intermediates were discovered-Thromboxane A\textsubscript{2} (TXA\textsubscript{2}) and Prostacyclin by Vane and their colleagues. Still later, the elucidation of a different pathway of arachidonate metabolism, resulting in the production of the leukotrienes, led to a further understanding of the role of arachidonate metabolites in physiological and pathological processes (Bergstrom, 1968; Samuelsson, 1972 and 1975; Vane, 1971). In 1982, Bergstrom, Samuelsson and Vane received the Nobel Prize for Medicine for their work in this area.

1.14 STRUCTURE AND BIOSYNTHESIS

The main source of the eicosanoids is arachidonic acid (5,8,11,14-eicosatetraenoic acid), a 20-carbon unsaturated fatty acid containing four double bonds (hence ‘eicosa’ referring to the 20 carbon atoms, and ‘tetraenoic’ referring to the 4 double bonds). Arachidonic acid is found esterified in the phospholipids, usually in the 2\textsuperscript{nd} position and a lesser extent in the glycerides of cell membranes (Diagram 1.2). The principal eicosanoids are the prostaglandins, the thromboxanes and the leukotrienes, though other derivatives of arachidonate, for example the lipoxins, are also produced. (The term prostanoids is used for both prostaglandins and thromboxanes). The initial and rate-limiting step in eicosanoid synthesis is the liberation of arachidonate, either in a one-step process or a two-step process. The one-step
process involves phospholipase A₂, the two-step process involves either phospholipase C then diacylglycerol lipase, or phospholipase D and then phospholipase A₂ (Diagram 1.3). Phospholipase D has been shown to be important in signal transduction in phagocytic cells. There are two forms of phospholipase A₂(PLA₂); one found intracellularly in the cytosol and another one present in the extracellular fluids. It is mainly the intracellular form which is implicated in the generation of inflammatory mediators, and its action can give rise not only to arachidonic acid and thus the eicosanoids, but also to lyso-glyceryl-phosphoryl-choline (lyso-PAF), which is the precursor of another powerful mediator of inflammation i.e., Platelet-Activating Factor (PAF).

Many stimuli can liberate arachidonic acid, and they vary with the cell type, for example thrombin in platelets, C₅ₐ in neutrophils, bradykinin in fibroblasts and antigen-antibody reactions on mast cells. General cell damage also starts this process.

The free arachidonic acid is metabolised by several pathways by one of two fatty acid cyclooxygenases which initiate the biosynthesis of the prostaglandins and thromboxanes, and by various lipoxygenases which initiate the synthesis of the leukotrienes, the lipoxins and other compounds.

The anti-inflammatory action of the glucocorticoids is largely due to inhibition of cyclooxygenase induction. These drugs also stimulate production of the inhibitor lipocortin.
Diagram 1.2  Outline of structure of phospholipids and site of action of phospholipases - indicating how arachidonate can be released by a one-step processes. The numbering of the carbon atoms in the glycerol 'backbone' is given on the left. Unsaturated fatty acids, such as arachidonic acid, are usually located at R on the 2nd carbon. This figure shows O-acyl residues on carbon atoms 1 and 2, but O-alkyl residues can occur (X = choline, ethanolamine, serine, inositol or hydrogen)

Diagram 1.3  Pathways of release of arachidonate from phospholipids by two-step processes. (InsP = inositol phosphate; DAG = diacylglycerol)
The anti-inflammatory action of the non-steroidal anti-inflammatory drugs is mainly due to the fact that they inhibit the action of one of the fatty acid cyclooxygenases. Other compounds which act selectively on the cyclooxygenase induced inflammatory cells or at specific sites of eicosanoid synthesis (e.g. inhibitors of 5-lipoxygenase, and thromboxane synthetase) are under test, as are specific antagonists of the prostaglandins and leukotrienes.

1.15 THE PRODUCTS OF THE CYCLO-OXYGENASE PATHWAY

1.16 THE PROSTANOIDS

Cyclo-oxygenase (COX) exists in two forms - COX-1 and COX-2. COX-1 is found in most cells as a constitute enzyme (i.e it is always present) and it is thought that the prostanoids it produces, are involved in normal homeostasis (e.g. regulating vascular responses and coordinating the actions of circulating hormones). COX-2 is induced in inflammatory cells by an inflammatory stimulus. This has relevance for the mechanism of action of present and future NSAIDs (Diagram 1.4).

Cyclo-oxygenase is found bound to the endoplasmic reticulum. It has two actions:

1. An endoperoxide synthase action, that first oxygenates arachidonate, followed by cyclisation to give the cyclic endoperoxide PGG$_2$. 

Diagram 1.4 The biosynthesis of prostaglandins, prostacyclin and thromboxane from arachidonate. Solid lines indicate known enzymic reactions, and dotted lines the transformations not known to be enzymic. Compounds with biolgical action are shown in boxes. There are two forms of cyclo-oxygenases (COX): One (COX-1) is constitutive and occurs in most cell types, and the other (COX-2) is induced in inflammatory cells by inflammatory stimuli. The current NSAIDs act mainly on COX-1. (PG = prostaglandin; TX= thromboxane; NSAIDs = non-steroidal antinflammatory drugs).
2. A peroxidase action that converts PGG_2 to another cyclic endoperoxide, PGH_2.

Subsequent steps in arachidonate metabolism differ in different cells. In platelets, the pathway leads to Thromboxane A_2 (TXA_2) synthesis, in vascular endothelium it leads mainly to prostacyclin synthesis and in macrophages it mainly leads to synthesis of Prostaglandin E_2 (PGE_2).

1.17 NOMENCLATURE

The confusing nomenclature of the eicosanoids derives from the fact that the names of the first two prostaglandins were based on the separation procedure. PGE partitioned into Ether and PGF into the phosphate buffer. PGA and PGB (which are artefacts) were so called because of their stability or otherwise in acids and bases. Thereafter other letters of the alphabet were filled in. The subscripts refer to the number of double bonds; thus PGE_2 has two double bonds. The Greek letter subscript, the α in PGF_{2α} refers to the orientation of hydroxyl above or below the part of the ring. PGE_2, PGI_2, PGD_2, TXA_2 and PGF_{2α} are the most important products of the cyclooxygenase pathway. If the cyclooxygenase acts on eicosatrienoic acid instead of arachidonic acid, the resulting prostanoids have only a single double bond, for example, PGE_1 (Nakano, 1973).
1.18 CATABOLISM OF THE PROSTANOIDS

Several intracellular enzymes are involved in inactivation of the prostaglandins. After carrier mediated uptake, there is rapid inactivation by 'prostaglandin-specific' enzymes, then slow inactivation by general fatty-acid-oxidizing enzymes. The metabolites of the prostaglandins are excreted in the urine. The prostaglandin-specific enzymes are present in high concentration in the lung, and 95% of infused PGE_2, PGE_1 or PFG_2α, is inactivated on first passage. The T1/2 of most prostaglandins in the circulation is less than 1 minute. PGI_2 is not taken up into cells in the transport system in the lung, and thus survives passage through the lung. However, it is very short-lived (T1/2 < 5 min), being hydrolyzed to 6-keto PGF_1α. Thromboxane A_2 hydrolyses rapidly to the biologically inactive TDB_2 (T1/2 = 30 sec) (Robert et al., 1981).

1.19 PROSTANOID RECEPTORS

A classification of prostanoid receptors has been proposed by Coleman (1995). Using data of the rank order of potency of five natural prostanoids on a range of different preparations, five main prostanoids receptors have been defined, one each for the natural prostanoids, PGD_2, PGF_2α, PGI_2, TXA_2 and PGE_2 termed DP-, FP-, IP-, TP- and EP- receptors respectively. Synthetic analogues of the natural prostanoids support and extend this classification, which has been further confirmed as some receptor antagonists have become available. Data obtained with the synthetic compounds have led to the proposal that there are three subgroups of receptors
of PGE$_2$- termed EP$_1$, EP$_2$ and EP$_3$. Binding studies have also provided supportive evidence for this classification (Hall et al., 1987; Halushuka et al., 1989).

1.20 ACTIONS OF THE PROSTANOIDS

The prostanoids affect most tissues, having a bewildering variety of effects. The fact that there is marked species variability in the response of different tissues has made information from experiments more confusing and has compounded the difficulties of unravelling the pharmacological actions of these agents. However the general actions of the prostanoids can now be expressed in terms of the actions on their respectively receptors as follows.

The action of PGD$_2$ on DP-receptors causes vasodilatation, inhibition of platelet aggregation, relaxation of gastrointestinal muscle, uterine relaxation, modification of release of hypothalamic/pituitary hormones. (Its bronchoconstrictor effect is due to an actin on TP-receptors). Signal transduction involves adenylate cyclase and an increase in cAMP (Hamberg et al., 1974).

The action of PGF$_{2\alpha}$ on FP-receptors causes myometrial contraction in humans, luteolysis in some species (e.g. cattle) and bronchoconstriction in other species (cats and dogs). The receptors involved in PGF$_{2\alpha}$- mediated release of gonadotrophins and prolactin are not yet known. Signal transduction involves InsP$_3$ generation and increase of cytosolic (Ca$^{2+}$) (Goldberg and Ramwell, 1975; Horton and Poyser, 1976).
The action of PGI$_2$ (Prostacyclin) on IP-receptors causes vasodilatation, inhibition of platelet aggregation, renin release and natriuresis via effects on tubular reabsorption of Na$^+$. Signal transduction involves adenylate cyclase and an increase in cAMP.

The action of TXA$_2$ on TP-receptors causes vaso-constriction, platelet aggregation and bronchoconstriction (the last more marked in guinea pig than in man). Signal transduction involves InsP$_3$ generation and increase of cytosolic (Ca$^{2+}$) (Aiken and Vane, 1973).

The actions of PGE$_2$ are as follows:

On EP$_1$-receptors it causes contraction of bronchial and gastrointestinal smooth muscle - the transduction mechanisms being InsP$_3$ generation and increase of cytosolic (Ca$^{2+}$).

On EP$_2$-receptors it causes bronchodilatation, vasodilatation, stimulation of intestinal, fluid secretion and relaxation of gastrointestinal smooth muscle - the transduction mechanisms being activation of adenylate cyclase and an increase in cAMP (Piper, 1984).

On EP$_3$-receptors it causes contraction of intestinal smooth muscle, inhibition of gastric acid secretion increased gastric mucus secretion, inhibition of lipophysis, inhibition of autonomic neurotransmitter release and stimulation of contraction of the pregnant human uterus - the transduction mechanisms being inhibition of adenylate cyclase and a decrease in cAMP (Sontang, 1986).
Actions of PGE₂ for which the receptors type is not yet known include the production of fever, inhibition of T cell proliferation, inhibition of macrophage activation, stimulation of release of adrenal steroids and of erythropoietin release from the kidney (Aiken and Vane, 1973).

1.21 THE ROLE OF THE PROSTANOIDS IN INFLAMMATION

The inflammatory response is always accompanied by the release of prostanoids, so they are certainly present at inflammatory sites, the predominant product being PGE₂, though PGI₂ can also be found. In areas of acute inflammation PGE₂ and PGI₂ are generated by the local tissues and blood vessels, and mast cells release PGD₂. In chronic inflammation, cells of the monocyte - macrophage series also release PGE₂. The prostanoids have a sort of Yin-Yang action in inflammation - stimulating some responses and decreasing other as follows:

PGE₂, PGI₂ or PGD₂ are powerful vasodilators in their own right and synergistic with other inflammatory vasodilators such as histamine and bradykinin. It is this combined dilator action in precapillary arterioles which contributes to the redness and increased blood flow in areas of acute inflammation. These prostanoids do not directly increase the permeability of the postcapillary venules, but they potentiate this effect of histamine and bradykinin. Similarly, they do not themselves produce pain, but potentiate the effect of bradykinin by sensitizing afferent C fibres. The anti-inflammatory effects of the NSAIDs are largely due to inhibition of these actions of the
prostaglandins. Prostaglandins of the E series are also implicated in the production of fever. High concentrations are found in the CSF in infections, and there is evidence that the increase in temperature generated by endogenous fever-inducing agents such as IL-1 is mediated by PGE$_2$. The antipyretic action of NSAIDs is partly due to inhibition of the synthesis of PGE$_2$ in the hypothalamus. Thus PGE$_2$ decreases lysosomal enzyme release and the generation of toxic oxygen metabolites from neutrophils and histamine release from mast cells. It also inhibits macrophage activation, lymphocyte activation and the generation and secretion of some cytokines (Moncada et al., 1978; Davies et al., 1984).

1.22 THE PRODUCTS OF LIPOXYGENASE PATHWAYS

1.23 THE LEUKOTRIENES

The lipoxygenases, soluble enzymes located in the cytosol, are found in lung, platelets, mast cells and white blood cells. The main enzyme in this group is 5-lipoxygenase - the first enzyme in the biosynthesis of the leukotrienes ('leuko' because they are found in white cells and 'triene' because they contain a conjugated triene system of double bonds). On cell activation this enzyme translocates to the cell membrane where it becomes associated with a protein termed the 'five-lipoxygenase activating protein' (FLAP) which is necessary for leukotriene synthesis in intact cells. The 5-lipoxygenase adds a hydroperoxy group to C5 in arachidonic acid. The next step in the pathway is the synthesis of leukotriene A$_4$ (LTA$_4$). This compound may be converted enzymically to LTB$_4$ and is also the precursor for an important class of
cysteinyl containing leukotrienes - LTC₄, LTD₄, LTE₄ and LTF₄ (also referred to as the sulphidopeptide leukotrienes). The first three of this latter group together constitute ‘slow reacting substance of anaphylaxis (SRS-A)’, a substance shown many year ago to be generated in guinea-pig lung during anaphylaxis. LTB₄ is produced mainly by neutrophils, and the cysteinyl-leukotrienes mainly by eosinophils, mast cells, basophils and macrophages. Lipoxins and other active products are also produced from arachidonate (Diagram 1.5) (Feuerstein, 1984; Piper, 1984).

1.24 ACTIONS OF LEUKOTRIENES

They are potent spasmogens causing dose-related contraction of human bronchiolar muscle in vitro. LTE₄ is less potent than LTC₄ and LTD₄, but its effect is much long lasting. All cause an increase in mucus secretion. Given by aerosol in vivo to human volunteers they cause marked reduction in specific airway conductance and in maximum expiratory flow rate, the effect being more protracted than that produced by histamine.

Small amounts of LTC₄ or LTD₄ given intravenously cause a rapid, short lived fall in blood pressure, and significant constriction of small coronary resistance vessels. Given subcutaneously they are equipotent with histamine in causing wheal and flare. Given topically in the nose LTD₄ increases nasal blood flow and increases local vascular permeability (Hamberg et al., 1975).
Diagram 1.5  The biosynthesis of leukotrienes from arachidonic acid. It is not clear whether LTF₄ occurs in vivo. Compounds with biological action are shown in grey boxes. (HETE = hydroxy-eicosatetraenoic acid; HPETE = hydroperoxyeicosatetraenoic acid)
1.25 THE ROLE OF LEUKOTRIENES IN INFLAMMATION

LTB$_4$ can be found in inflammatory exudates and is present in the tissues in many inflammatory conditions, including rheumatoid arthritis, psoriasis (a chronic skin disease) and ulcerative colitis. The cysteinyll-leukotrienes are present in the sputum of chronic bronchitis in amounts which are biologically active. On antigen challenge they are released from samples of human asthmatic lung in vitro and into nasal lavage fluid in vivo in subjects with allergic rhinitis. There is evidence that they contribute to the underlying bronchial hyper-reactivity in asthmatics and it is thought that they are among the main mediators of both the early and late phases of asthma. An LTD$_4$ antagonist, Accolate, has shown promise in the treatment of asthma. It is also possible that cysteinyll-leukotrienes has a role in the cardiovascular changes of acute anaphylaxis. Agents which inhibit the enzymes generating the leukotrienes-5-lipoxygenase inhibitors-are under development as anti-asthmatic agents (e.g. Zileutin) and anti-inflammatory agents (Vane, 1971 and Vane, 1995).