REVIEW OF EXISTING LITERATURE
DRUG RESEARCH - AN OVERVIEW
The last few decades have witnessed the rapid strides of modern system of medicine. Modern system specifies remedies as far as possible, for particular diseases based on scientific data. Modern system of medicine is costly as well as most of them have toxic effect even at therapeutic dose levels which is reported from clinical documents for which many medicines are declared banned subsequently. Naturally the obvious temptation would be to find out some alternative drugs which would be, cheaper, safer and easily obtainable. This might be provided by the ancient medicinal preparations which has been developed through various stages and require scientific recognition in the modern system.

The history of medicine, its beginning and its progress from the dawn of human evolution in India is little known. The stages of its evolution, as evident from the available sources of information can be conveniently be divided into:

1. Palaeomedicine - existent before 10000 B.C. amongst the early men in the palaeolithic stage.
2. Prehistoric Medicine - existent between 10000 B.C.-2500 B.C., when men passed successively through palaeolithic, mesolithic and neolithic stages.
3. Primitive Medicine - existent between 2500 B.C.-1500 B.C. during the Bronze age civilisation in India.
4. Archaic Medicine - Practised by Aryans after 1500 B.C., during Iron Age with the progress of Vedic Society. Its proper compilation in Ayurveda was
done in halting steps in the post-Vedic period. The knowledge of medicine then passed through the intermediate stages to reach finally the period of, 5. Modern Medicine — which started about three centuries earlier in India (1700 A.D. onwards).

Development of Medical knowledge

The development of medical knowledge in the Indian subcontinent that may be assigned to a particular period, supported by evidences, may be divided into —

1. Prevedic period
2. Vedic period
3. Post-vedic period and classical age
4. The middle age
5. The modern period

Thatte and Dahanukar (1986) explained that Ayurveda, Siddha and Unani systems of medicine provide health and care for a large part of the population of India. Roychowdhury (1988) explained that "the vedic scriptures and Ayurveda mentioned some of the diseases and their archaic treatment in ancient time". He also mentioned about the attempts to find out their chronological development and its links with the past, as well as, with the post-vedic periods when Siddha, Unani, Homoeopathy and ultimately allopathic systems successively stepped into the field of medical
science in India.

Ayurveda is one of the most ancient systems of medicine known today. The word Ayurveda is composed of two parts Ayu (life) and Veda (knowledge) i.e. the knowledge of the science of life. The origin of Ayurveda though difficult to pinpoint, have been placed by scholars of Ayurveda and ancient Indian literature to be at somewhere around 6000 B.C. (Thatte and Dahanukar, 1986).

Revival of Ayurveda and present state of research

During the early part of this century there has been a revival of interest in the Ayurvedic remedies. Many research workers in modern India, Drs. Kartick Bose, Col. Sir R. N. Chopra, Ganapath Sen, K. M. Radhakarni and others were the pioneers in this field. Their works were mainly directed towards the study of pharmacological and therapeutic effects of Ayurvedic remedies, which gave a new fillip to the rational understanding of Ayurveda.

Government of India has now realised the vast potential of this long unexplored remedial measures, mentioned in Ayurveda. There the medicinal plants play the major role and remedies prepared from these, now cater to the need of nearly 70% of the Indian population. To organize the research on medicinal plants many research centres have been set up where detection and isolation of active principles, chemical and botanical standardisation and determination of structural configuration are being pursued with the active ingredients obtained from different plants having
medicinal value.

Research in Ayurvedic medicines had moved from the realm of proving the efficiency of a number of plant products (Satyavati et al., 1976) to elucidating their mechanisms and sites of action. Reserpine, an antihypertensive drug, was isolated from the plant Rauwolfia serpentina (called 'sarpagandha' and prescribed for hypertension in Ayurveda) many decades ago. Another agent, curcumin, which is the active principle isolated from the rhizome of Curcuma longa, has been found to exert an anti-inflammatory effect by inhibiting prostaglandin synthesis. Further it has been shown to inhibit selective platelet prostaglandin production while sparing vascular endothelial prostaglandin synthesis.

Many plant preparations are prescribed in Ayurvedic literature to strengthen the general host resistance. These drugs are called Rasayana, Jeevaniya or Balya drugs, all of which essentially mean drugs which increase tissue resistance to disease. This concept appears similar to that of 'prohost therapy' as put forward by Hadden (1982). Prohost therapy is claimed to augment cellular response and consequently improve disease states.

Five plants Asparagus racemosus, Withania somnifera, Tinospora cordifolia, Piper longum and Terminalia chebula which are prescribed in Ayurveda as Rasayana, their effects on immune functions and nonspecific resistance to infections were evaluated.

Modern India has realized the vast potential value of research
concerning scientific evaluation of Ayurvedic remedies. By such efforts once more the remedies may find suitable place in the armamentarium of clinical treatment in near future.

Evaluation of Ayurvedic drugs

A number of difficulties are experienced by investigators while evaluating Ayurvedic drugs in modern scientific terms there is a wide variation in the quantity of pharmacologically active substance in each plant and changes in activity after extraction. Differences in methods of purification, standardization and compounding of formulations influence results. Selection of different animal and disease models have their own limitations. Since pathogenesis of various diseases, their treatment with different drugs, the choice and dose of the drug as well as the mechanism of action of a drug are variable it becomes difficult to conduct controlled clinical trials. Pharmacokinetic studies too are difficult, particularly when assessing a compound formulation.

It has been observed that ancient system of medicine which has developed over centuries includes several exercises in logic and gives detailed instructions for the preservation of health and treatment of diseases. Ayurveda faced on set back when modern medicine introduced a new way of subjecting all assumptions to experimental and statistical verification. It may be a general evaluations that this is the high time of accumulating knowledge
of various disciplines like cellular physiology and availability of modern biomedical research tools for verification of some concepts of Ayurveda can be attempted.

Inflammation is a disease which now-a-days has been an acute problem and no specific drugs are available for prevention and cure of this disease.

Extensive research works are going on Indian medicinal plants out of which many plants are found to be effective against inflammation and multi disciplinary approach are emphasized to recognize them scientifically.
It has been known since long, when men or animals are injured, some characteristic series of reactions occur in the damaged area and such reaction may last for hours or even days which appears more or less independent of the nature of the damaging agent and a wide variety of injury being followed by a very similar response. This tissue response to injury is known as inflammation, the term being derived from the Latin word 'inflammare' which means 'to burn'.

This response to injury is itself a process- not a state and that dynamic and complex changes occur over long periods within the injured area. It is better to regard inflammation not as a single process but as a collection of distinct mechanisms, each of which is sensibly designed to aid, and may well have evolved for, the defence of the organism against external injury, but each of which has other uses as well (Thomas, 1970).

History of inflammation

The history of inflammation and its therapy is as old and extensive as the history of medicine. In ancient Greek and Roman era inflammation was regarded as a single disease entity and the result of a disturbed state of body fluids. The necessary basis for a more rational explanation of inflammation was provided by William Harvey (1628), who discovered the circulation of the blood, but the essential nature of inflammatory process was not appreciated.
at that time. The first clear statement of the modern concept of inflammation was given by John Hunter (1794), who concluded that inflammation itself should not to be considered as a disease, but as a salutary operation consequent either to some violence or some disease'.

Most recent writers agree that it is unwise to attach too narrow a meaning of the term, and favour a definition such as that of Burdon Sanderson (1871): "The process of inflammation is the succession of changes which occurs in a living tissue when it is injured, providing that the injury is not of such a degree of severity as to at once destroy its structure and vitality". More recently Ebert (1965) expressed a similar idea when he proposed that: "Inflammation is a process which begins with a sublethal injury to tissue, ending with complete healing."

The Cardinal signs of inflammation

In ancient times inflammation was known by the appearances produced in the skin and other surfaces of the body. Its manifestations were summarised by Roman encyclopaedist Celsus (30BC-38 AD) as "rubor et tumour cum calore et dolore" i.e. 'redness and swelling with heat and pain', and these changes are commonly termed as the cardinal signs of the inflammation and often a fifth sign, loss of function is added. For many years this concept of fifth sign was attributed to the Greek writer Galen (130-200 AD), until Rather (1971) pointed out that there is no mention of such sign
Fig. 1. Cardinal signs of Inflammation.
in any of Galen's work. It appears that the concept of the fifth sign originated, as did so much of modern pathology, in Virchow's cellular pathology, published in 1858.

The basis of the cardinal signs

The changes responsible for the cardinal signs of acute inflammation as per current concepts may be summarised as follows.

Rubor. Due to gross and persistent dilatation of all small blood vessels, arterioles, capillaries and venules within the injured area.

Calor. An area of inflammation is hotter than the surrounding tissues only when it is situated in a part of the body, which is normally at a lower temperature than the interior of the body and circulating blood. Skin, nasal mucous membrane or conjunctiva are the common examples. The rise in local temperature of such inflamed areas is a direct consequence of the great increase in local blood flow. Inflamed skin is also not due to the stimulation of heat sensitive nerve endings which occur only in the skin.

Tumour. Local oedema of an acutely inflamed area is the main factor responsible for swelling. This is due to increased permeability of small blood vessels which allows protein-rich fluid, known as exudate, to escape into the tissues of the damaged area. There is not only high protein content in the inflammatory exudate but the exudate also contains commonly enough fibrinogen to clot
spontaneously after its escape into the tissues, and as a result of which large amounts of fibrin may be deposited within the injured area.

Dolor. This is the least understood among the cardinal signs of inflammation. Some endogenous chemical substances are the possible chemical mediators of inflammation which have very marked pain-producing properties. It may be mentioned that bradykinin, 5HT, and some prostaglandins cause pain when applied in high dilution to the base of a blister in human skin.

Tissue tension is another important factor that undoubtedly cause pain and tenderness. Areas of inflammation which become tense after the formation of only small volumes of inflammatory exudate, as for example, the skin covering the anterior surface of the tibia, or the ala nasae, is much more painful than a similar lesion in a less tightly bound-down area of skin. Pus under tension in a boil or an abscess may be extremely painful. Evacuation of the pus, either surgically or by spontaneous rupture, produces an immediate and marked decrease in both pain and tenderness.

Loss of function. This appears to be due in part of reflex inhibition of muscular movement as a result of pain, aided by a variable degree of mechanical limitation of movement due to swelling of the inflamed area.

Inflammation in a glandular organ such as liver or the pancreas, may cause loss of function due to death of some parenchymal cells
and damage to others by the agent causing the inflammation. It is not known whether there is in addition with the function of apparently undamaged glandular cells (Hurley, 1983).

Pathophysiology of inflammation
The injury which causes inflammation may be brought about by physical agents, chemical agents, infections and immunological reactions.

Essentially there are two categories of inflammation: Acute and Chronic.

Acute inflammation
The basic pattern of acute inflammation is uniform. The intensity and the localization of the reaction is determined by both severity of the injurious agent and the reactive capacity of the host.

The classical signs of acute inflammatory reaction are warmth, redness, pain, swelling and loss of function as already discussed.

The morphologic similarities among all types of inflammation suggest common control mechanisms and mediators that initiate, sustain and terminate the inflammatory responses in tissues. The changes that occur in the first few hours after sublethal injury involve, in varying degree, the following three processes:

1. Changes in vascular calibre and blood flow.
2. Increased vascular permeability which results in the
Fig. 2.

Inflammatotry Process

**Flow Chart**

- Etiologic agents
- Cell death
- Release of mediators
  - Systemic reaction
  - Local inflammatory reaction
    - Vascular defenses
    - Cellular defenses
  - Activation of immune defenses
    - Emigration of Leucocyte
    - Inflammatory Exudate

- [Injurious agent unrestrained]
  - Further destruction of tissue
  - Attempted repair
  - Progression of agent through lymphatics
    - by direct contiguity
    - by natural passage
  - Blood stream entry and spread
  - Death of host
  - Chronic inflammation

- [Injurious agent restrained]
  - Toxic effect neutralized
  - Repair and Regeneration
    - Resorption
    - Granulation tissue
    - Fibrosis
    - SCAR formation

Fig. 2.
formation of inflammatory exudate and local oedema.

3. Escape of leucocytes from the blood into extra vascular tissues.

Changes in vascular calibre and blood flow

Until recently very little was known regarding this aspect of the inflammatory response. In living tissues the changes in vascular calibre and flow can be observed but it is extremely difficult to make quantitative observations in such preparations. Just before a few years some methods have been developed which help in accurate measurement of the blood flow through local areas of tissues and considerable additional information has been accumulated about the vascular changes which follow tissue injury and the present knowledge achieved in this field may be summarized as follows:

Arteriolar constriction, when present, appears to be caused by a direct response of the smooth muscle of the arteriolar wall to the damaging agent.

Both nervous and humoral factors are involved in the vasodilatation observed after injury to human skin. Three phenomena occur following mild mechanical or thermal injury to the skin as observed by Lewis (1927) are (a) an immediate reddening confined at the area of injury; (b) a more extensive bright red halo or flare spreading outwards from the injured area and (c) formation of a wheal within the area of injury. It is not clear to what extent these findings can be applied to other areas of the body.
Similar axon reflexes have been identified in the cornea (Bruce, 1910) and in the tongue (Krogh, 1920), but not in the brain (Florey, 1925) or exposed abdominal viscera (Lewis, 1927). It is by now clear that all features of acute inflammation may occur in completely denervated tissues. However, in some occasions the intensity of the reaction may be modified by nervous influences. Inflammatory vasodilation is usually resistant to vasoconstrictor agents, e.g., norepinephrine, and as well to the stimulation of vasoconstrictor nerves.

Vasodilation does not itself cause increased leakage of protein from the dilated vessels. On the other hand vasodilation and increased vascular permeability are separate phenomena whose time course differs markedly in different types of inflammation. For example after crush injury to muscle, prominent vasodilation is present for many hours after the permeability of vessels within the injured area has returned to normal (Hurley and Edward, 1969). After thermal injury similar temporal separation of vasodilation and increased permeability occurs (Hurley et al., 1967) which also occurs after chemical injury (Steels and Michaels, 1966). This separation has been confirmed by results of recent quantitative studies. For example, massive and immediate increase in vascular permeability with very little hyperemia caused by bradykinin, whereas prostaglandins E\textsubscript{1} and E\textsubscript{2} cause marked hyperemia with no significant increase in vascular permeability. Although increased vascular permeability and hyperemia are separate phenomena, for any given level of increased permeability,
the amount of protein lost into the injured area varies directly with the rate of blood flow through the leaking vessels. This effect can be seen very clearly in the interaction which occurs between prostaglandin E₂ and bradykinin (Williams and Moreley, 1973). Prostaglandin E₂ causes gross hyperaemia but no significant change in vascular permeability; bradykinin produces little vasodilatation but induces an immediate and massive increase in vascular permeability. Simultaneous injection of the two substances into the same area, the hyperaemia induced by prostaglandin E₂ increases the rate of leakage from vessels rendered permeable by bradykinin, and total protein leakage is almost one hundred times greater than on bradykinin injection alone. The opposite situation may be seen in areas of severe injury such as the central part of a moderate thermal burn (Movat et al., 1978). Here the severity of injury might even be leading to complete vascular stasis, and in these circumstances, no protein at all escapes from widely dilated vessels despite the presence of large defects in their endothelial lining. There is wide variation in the relative potency of individual mediators as vasodilators and in increasing vascular permeability. In addition, many substances known to be present in injured tissues, notably certain prostaglandins and lactic acid are powerful vasodilators but cause little or no change in vascular permeability. Clearly many substances known to be present in injured tissues are capable of causing sustained vasodilatations. However, very
little is known of their relative importance in different types of injury. It is available from published reports that there are wide variations in the vasodilator powers of individual mediators in different species as well different organs and tissues. It is not clear how much variation reflects a genuine heterogeneity of vascular response, and how far can be explained by variations in the experimental models used (Alutra, 1978).

All the vascular changes observed in injured tissues are superimposed on the normal regulations which control pressure and flow within the microcirculation. These normal regulatory processes are very complex and are not understood in detail till date. Hence it is not surprising that, despite the availability and employment of the modern quantitative methods, little details is available regarding the factors responsible for the production of the sustained vasodilatation which forms such a prominent feature of the acute inflammatory response.

Increased vascular permeability:

It is necessary to know the nature and composition of the fluid that accumulates in the extravascular spaces to understand the mode of development of oedema in the injured tissues. The main characteristics of the inflammatory exudate may be summarized as follows:

Inflammatory exudate has a relatively high protein content, the proteins present being all those normally found in blood
plasma. The protein content varies after different types of injury and at different stages of single type of injury, but the total protein concentration is less than that of blood plasma in the same animal. All proteins appearing in inflammatory exudate are present in plasma (Yoffey and Courtice, 1970). In particular fibrinogen is commonly present in amounts sufficient to lead to coagulation of exudate either within a body cavity or shortly after removal from the body.

Some degree of sieving of proteins according to their molecular size persists during the formation of inflammatory exudate. In all normal tissues small protein molecules like albumin escape more readily from the microcirculation than do larger protein molecules like plasma globulins and this result in a higher relative proportion of small proteins in lymph than in plasma. This phenomenon is known as molecular sieving. The degree of relative retardation of larger protein molecules decrease during tissue injury, but some degree of molecular sieving of proteins persist even after severe types of injury (Yoffey and Courtice, 1970). Inflammatory exudate is with a high turnover pool but not a static puddle. The quantity of protein passing through the extravascular spaces of any tissue is the total quantity of protein in the lymph draining from it. The volume of lymph draining from the injured area commonly increases five to tenfold or even more and lymph protein concentration also rises considerably during tissue injury and formation of inflammatory exudate. These findings indicate
a massive increase in the amount of protein passing from blood to lymph and show that there must be a rapid turnover of the large pool of protein present in the extra-vascular spaces of the injured area i.e., of the inflammatory exudate.

Tissue texture and tissue compliance play a dominant role in governing the volume of the exudate which accumulates within an area of inflammation. A rapid increase in the quantity of fluid in the extravascular spaces of the injured area caused due to formation of exudate. Formation of this fluid occurs too rapidly to allow sufficient time for adaptive remodelling of tissue. Hence when acute inflammation in dense tissues, as for example the tightly bound skin over the bridge of the nose, or within organs enclosed in a fibrous capsule such as kidney, escape of small amounts of exudate produces a rapid and progressive rise in tissue pressure. This limits the escape of further fluid from small blood vessels and formation of substantial volume of exudate. By contrast, when subcutaneous tissues of loose texture such as those in the perirenal region become inflamed, or when inflammation involves the lining of a cavity such as the pleura or the peritoneum, large volumes of exudate can accumulate rapidly without provoking any substantial change in tissue pressure. Hence in these situations inflammatory stimuli usually evoke the formation of large volumes of exudate. An extreme example of this second type of response is seen in injury to the lung, where it is well known that oedema, irrespective of its cause, can spread with almost explosive speed through
large areas of the lung. Pulmonary oedema is able to develop with such extreme rapidity because fluid which escaping into lung alveoli can spread to adjacent alveoli via the interalveolar pores of kohn where without producing any significant rise in intra-alveolar pressure, i.e., tissue pressure forms no significant barrier to the spread of oedema fluid.

Basic patterns of increased permeability

The immediate transient response is observed after application of histamine-type mediators as described earlier. It may occur in an isolated event, as after injection of a permeability factor, in very mild injuries of many kinds or in the allergic wheal of type I hypersensitivity.

The immediate sustained reaction is seen after severe injuries such as thermal burns, crushing injury or application in high concentration of chemical irritants or bacterial toxins. Leakage may be sustained at a high level for many hours which begins immediately after injury.

Delayed prolonged leakage is a very common pattern of response which occurs after mild degrees of physical injuries such as thermal or ultraviolet burns or x-ray-induced damage.

The composition of inflammatory Exudate:

Three salient features of the composition of inflammatory exudate are its high protein content, the variability of protein levels in exudates and persistence of sieving according to molecular
size between plasma and inflammatory exudate.

The variation occurs because of two independent processes; one is the formation of gaps in vascular endothelium allowing free escape of plasma into extravascular tissues and the other is increased ultrafiltration as a consequence of the sustained vasodilatation characteristic of acute inflammation causing the passage of protein-free fluid into the inflamed tissues.

The basis of sieving between plasma and exudate or lymph is more obscure. Lymph draining from normal peripheral tissues contains a higher proportion of small protein molecules like albumin than does plasma, and this difference persists to a lesser degree in lymph draining from an area of acute inflammation. Such findings indicate the presence of a sieving mechanism in the normal vascular wall or extravascular tissue.

Leucocytic migration

In very mild injuries, such as accidental mild trauma to a rabbit ear chamber, pavementing once established may reverse and conditions return to normal. However with injuries of the severity necessary to provoke an acute inflammatory response, many of the adherent leucocytes subsequently pass out through the vascular wall into extravascular tissues—a process known as leucocytic migration. Having reached extravascular tissues the escaping cells may wander about at random, or more in an apparently
directed manner towards a focus of damaged tissue or a clump of bacteria. This directed movement is believed to occur under the influence of chemical concentration gradients and is known as chemotaxis. There are three stages of leukocytic behavior: pavementing, leukocytic emigration, and chemotaxis.

The migration of leukocyte, a hallmark of the inflammation is an active process; the enhanced vascular permeability and the migration of leukocytes are different phenomena which may or may not occur simultaneously. During normal blood flow erythrocytes and leukocytes in the microvasculature flow mainly in the central column of blood which is separated from the vessel wall by a clear layer of plasma (axial stream). During inflammation when slowing of flow occurs the axial streaming is no more present, the W.B.C. in the vessels passed into the peripheral stream and then on contact with vascular endothelium are hereby arrested on it and finally from continuous layer (margination of leukocytes). This addition might be caused by either neutralization of negative charges or reduction of charge density of the leukocytes. Divalent ions are also involved whether the chemotactic factor increased the adhesion of neutrophils to the endothelial cells. The marginated polymorphs extend between the junction by the endothelial cells and thereby disrupt it and cells squeezing through it and the intercellular junction reforms quickly. The mechanism of both the process of such disruption and consequent repair
are poorly understood.

The migration of leucocytes through the venule walls and the subsequent movement in the tissues occurs due to chemotaxis, the phenomenon in which the movement of the leucocytes are directed towards an attractant known as "chemotactic agent" there are no of chemotactic substances for neutrophil polymorphs. Fibrinopeptides, fibrin degradation products and kallikrein, formed due to the activation of the clotting, fibrinolytic and kinin systems, all can act as chemotactic agents. Activation of the complement system also produces chemotaxins. The chemotactic factors are also released from the injured tissue cells including prostaglandin E₂ and enzymes which activate the complement system. Bacterial endotoxins, lipids or proteins, secreted by some bacteria and the secretions from migrated neutrophils can act as chemotactic agents. The entire mechanism of chemotaxis for polymorphs is yet to be fully established but some interesting observations in this aspect has been reported Becker (1977), Wilkinson et al. (1977), Morocho et al. (1977), Gallin and Dule (1978), Sigmond (1978).

Neutrophils emigrate more rapidly than monocytes, so that in short lived acute inflammation the peak of polymorph emigration has passed off before monocytes emigrate significantly. But in prolonged inflammation due to pyogenic bacterial infection, polymorphs emigrate until most of the bacteria have been destroyed and only then monocytes emigrate in large number.
In certain inflammatory conditions due to infection mostly monocytes and lymphocytes emigrate. Chemotactic substances for monocytes are C₃ and C₅ fragment of complement system, a factor formed in serum by interaction with antigen-antibody complexes; the factor is liberated from sensitized lymphocyte when exposed to specific antigen.

Eosinophils accumulate mainly in immunologically inflammatory reactions. Thus has been ascribed to the release of eosinophil chemotactic factor of anaphylaxis for IgE sensitized basophil or mast cells, when they meet a specific antigen.

The major role of neutrophil at the site of inflammation is towards disposing invaders, foreign bodies, cellular debris and its dissolution. The neutrophil polymorph are actively phagocytic while the emigrated monocytes are not so, although they become more active on their change into macrophage. The process is similar for both. The particle to be ingested becomes attached to the surface of the leucocytes and the process known as "opsonisation" occurs. Opsonin (protein) covers the foreign matter in a manner that leucocyte recognise them as foreign. IgG (subtypes I and III) and opsonic fragments of C (from activated complement system) are the two opsonins that are well characterized. The receptors of the neutrophils for opsonins have also been recognized. After the recognition of the particles being foreign, the pseudopod completely enclose the particle. The limiting membrane of phagocytic vacuole fuses and then there
is a discharge of the granule content into this phagolysosome. The leucocytes become progressively degranulated and during degranulation there is some leakage of hydrolytic enzymes and some metabolic product into the external medium. Phagocytosis is an energy dependent process stimulating numerous intercellular events including increased oxygen consumption, glycogenolysis increased glucose oxidation and hydrogen peroxide ($$H_2O_2$$) production. The $$H_2O_2$$ produced within the phagolysosome is important in bacterial killing being the ultimate step in phagocytosis.

Consequences of acute inflammation

Acute inflammatory reaction may lead to the following results.

(a) After the termination of the injury, the blood flow and vascular permeability become normal; paving smear of leucocyte ceases; cells and tissue debris are digested by the enzymes emigrated polymorphs die and macrophages are drained; in short, normal state of the tissue is restored.

(b) In certain inflammatory conditions (due to pyogenic bacteria), severe local toxic injury forms abscesses in the tissue which is filled with inflammatory exudate rich in polymorph, bacteria and fragments of necrotic tissue. Here, return to the normal state is no longer possible. Since the tissue has been destroyed and the abscess becomes enclosed in a wall of granulation tissue, which eventually matures to scar tissue.

(c) Some acute inflammatory lesions do not subside and return
to normalcy is hampered. Formation of granulation tissue with consequent fibrosis or scarring is very common. It complicates acute inflammation when there is necrosis of tissue or excessive fibrin deposition and when acute inflammation persists and develops into a chronic one.

Chronic inflammation

Chronic inflammation is also characterized by pain, redness and swelling but it does not subside in a period of a few days, but may instead have a relentless damaging course of several weeks, months or years, and may have far reaching on hosta well being. It is not known whether an inflammatory process, by its nature, is destined in certain cases to become chronic or whether any acute inflammation has the potential to become chronic. A chronic inflammation is caused by the persistence of an irritant which may be of biologic, physical, or chemical in nature. The irritant remains at the site due to an inability of macrophages to digest it. The cycle of cellular infiltration, necrosis and fibrosis will continue as long as the irritant remains. Chronic inflammation is more proliferative than an exudative one and necrosis commonly occurs and recurs. Granulation tissue will form in response to this and then connective tissue will follow. Increasing amount of fibrous tissue will characterize a positive long term chronic process.
MEDIATORS OF INFLAMMATION
Mediators of inflammation

The three processes - vasodilatation, increased vascular permeability and leucocytic migration have been discussed. Here the role of chemical substances liberated within injured tissues, i.e. endogenous chemical mediators in the genesis of these processes is being discussed.

Several features of the inflammatory response have been considered to indicate the participation of endogenous chemical mediators:

1. The close similarity of responses to a great variety of stimuli.

2. The finding that an injury lasting only a brief period, as for example a thermal burn, may evoke an apparently ordered succession of reactive changes extending over many hours.

3. The existence of a latent interval between injury and reaction. With many types of injury an immediate and transient increase in permeability is followed by an interval of 2-24 hours before the onset of leucocyte emigration and the main phase of increased vascular permeability. Such a latent period has been observed after thermal burns, x-ray injury, exposure to ultra-violet light and the application of certain bacterial toxins.

4. The presence of endogenous factors in injured tissues with actions on blood vessels similar to those produced by injury.

These features of the inflammatory response are suggestive but not conclusive evidence for the role of possible endogenous mediators.
Possible Mediators of the Inflammatory Process

Many of the drugs used to modify the inflammatory process have been directed at altering the metabolism, release, or peripheral effects of endogenous chemical substances potentially capable of mediating aspects of an inflammatory process.

A bewildering multiplicity of factors which can reproduce one or more of the features of the acute inflammatory response has been shown to be present in injured tissues and hence to be potential endogenous mediators of acute inflammation. These factors may be subdivided into those arising from tissue cells and those found in plasma. The factors released from tissue cells are mainly the amines e.g. histamine, serotonin and catecholamine; prostaglandins and lysosomal components. The factors found in plasma are the kinin system, the coagulation system and the complement system.

Mediators (factors) released from tissue cells

Histamine. It is present in inactive form in the granules of mast cells, the eosinophil and basophil leucocytes and in platelets. Active histamine can be released from these deposits by inflammatory stimuli, and can also be synthesised by other cell types, its formation being regulated by the enzyme, histidine decarboxylase.

The notable actions of histamine are found on vascular system, smooth muscle and exocrine glands. Arterioles and venules in most species are dilated by histamine. Vasodilatation induced by
Histamine is independent of innervation and is only partly suppressed by antihistamines, but may be completely overcome by sympathomimetic amines.

Free histamine is found only in trace amounts in most tissues but may be quite active in terms of the role it can play during an inflammatory process (Miller et al., 1978; Erjavec et al., 1967).

It has been reported widely that histamine releases from mast cells immediately after an injury. Release occurs at a time when the released histamine could contribute to the ensuing inflammatory response. The overwhelming body of evidence relating tissue injury to histamine release is based on acute injury caused by each of a wide variety of noxious stimuli ranging from anaphylaxis through both chemical and physical insults.

Local administration of histamine elicits the classical vascular response of acute inflammation i.e. increase in blood flow and content, an increase in microvascular permeability and formation of oedema. In addition, in the skin, histamine causes a flare response based on a local axon reflex leading to vasodilatation. These major parts of phenomena were first described in human skin by Sir Thomas Lewis in 1927. An important feature of the inflammatory response to histamine is that the increased microvascular permeability component (which is the major cause of tissue oedema due to histamine) is self-limiting and rapidly reverses even when the microcirculation remains exposed to histamine. This is
exceedingly important when histamine is considered as potential mediator of chronic inflammation. The role of histamine during an inflammatory process may be defined in part by the effects of pharmacologic antagonists on the peripheral effects of the amine. An early and delayed phase of increased small vessel permeability was demonstrated after heating the skin of guineapig (Sevitt, 1958). Two phases of inflammation may be observed in many other types of injury. The initial phase can be suppressed by pretreatment of the experimental animals with antihistamines (Spector and Willoughby, 1965). In some types of inflammation, such as severe tissue destruction produced by high temperature, or prolonged heating, the early response is less noticeable, and the overall response may appear to be an accelerated late phase that is not altered by antihistamines. The role that histamine plays in the development of an inflammatory process is early, transient, incomplete and not essential for the development of the most characteristic changes that produce lasting tissue alteration. So the antihistamines or inhibitors of histidine decarboxylase have limited but specific usefulness as anti-inflammatory agents. T lymphocytes with histamine receptors may act as suppressor cells inhibiting immune responsiveness which suggests that histamine's role in inflammatory disorders is a more complex one.

Histamine along with prostaglandins and adrenergic catecholamines may play a major role in modulating the inflammatory response in both delayed and immediate type hypersensitivity.
Serotonin. It is found in large amounts in mast cells and in platelets and in lesser amounts in many other cells. It has a vasoconstrictor action in man but does not increase vascular permeability. Administered of exogenous serotonin produces cardiovascular effects. These effects are complex and variable because the direct and reflex actions of the amine may occur sequentially or sometimes almost simultaneously. When serotonin is administered, the resultant vasodilation and increased blood flow resemble the changes observed during inflammation. In normal subjects when serotonin is infused into the brachial artery, it causes the fingers to redden and then assuming a dusky blue colour. In rodents, the combination of arteriolar dilatation, venular constriction and separation of endothelial cells occurring after subcutaneous administration of serotonin produces leakage of plasma from vessels. This amine has only a modest and most likely unimportant effect on the emigration of leucocytes from blood vessels (Spector and Willoughby, 1964). Administration of 5HT to the planter surface of the rat paw causes oedema with striking extravasation of albumin, although unlike most inflammatory mediators, 5HT does not increase local blood flow.

The results of studies on inflammation in different species show variability between species and often little relation to the changes seen in man. Most serotonin in man (90 to 95%) is synthesised and localized in the enterochromaffin cells of the gastrointestinal mucosa and serotoninergic cells of the brain; some are present in blood platelets and spleen. Serotonin present
in inflammatory exudates for as long as one hour after injury. Whenever serotonin is present in inflammatory exudates histamine is also present. Inhibitors of serotonin fail to influence the vascular changes in inflammatory process in man (Spector and Willoughby, 1963). The permeability-enhancing properties of serotonin during inflammatory process in man seems to be negligible because this property is species dependent. Serotonin is a natural constituent of rodent mast cells but not those of most other animal species or man. It follows naturally that the presence of 5-hydroxytryptamine in rodent mast cells, coupled with the proinflammatory action of 5-hydroxytryptamine in these species, leads to an important role of 5-hydroxytryptamine in acute inflammation associated with mast cell degranulation in rodents. Though limited information is available regarding possible interactions of serotonin with other vasoactive substances the precise role of the amine in inflammation must be kept open for review. It may not be dramatic but may be critical for direct effects of other mediators in respect to the active contribution of a mediator.

The mediators are interrelated in respect to physical, chemical and pharmacological points of view (Miller and Melmon, 1970; Kaplan et al., 1971). Under such circumstances it would not be surprising to find that antiserootonin activities may be manifested inconsistently as antihistaminic or antidromergic effects.

Catecholamines. These are not considered in general as mediators of inflammation. In certain situation, however, they, like
serotonin may alter manifestations of inflammation. Epinephrine may contribute to the development of hemorrhagic lesion observed in some types of inflammatory processes, such as the Shwartzman phenomenon (Mckay et al., 1969). The tissue injury results in augmented synthesis and destruction of catecholamines after experimental studies (Spector and Willoughby, 1965). The presence of receptors for catecholamines on leucocytes that are coupled to adenyl cyclase suggests that catecholamines are capable of modulating the inflammatory response in vivo (Bourne et al., 1974). Rocha e Silva (1964) suggested that epinephrine might cause an indirect increase in capillary permeability by release of kinins due to the activation of proteases (Biggs et al., 1947). Histamine and bradykinin were shown to release epinephrine (Frendelberg, 1955; Rocha e Silva, 1962) and thereby cause an increase in local concentration of catecholamines at the site of injury. Epinephrine might be natural anti-inflammatory substance as suggested by Spector and Willoughby (1964). It decreases permeability since iproniazid inhibited thermal oedema and this was prevented by pretreatment with dibenamine.

After several observations it seems that anti-inflammatory property demonstrated by epinephrine was due to its action on the pituitary-adrenal axis and unrelated to its local hormonal activity as suggested by Spector and Willoughby (1964). On the other hand, Rocha e Silva (1964) suggested a pro-inflammatory role of adrenaline since it activated proteases and thereby
released Kinines. From this suggestion one would expect a potenti- tion of inflammatory reaction by exogenous administration of epinephrine and by MAO and COMT inhibitors which increase tissue catecholamine levels. But it has been reported that epinephrine, pyrogallol, pheniprazine, nialamide and NO 592 significantly inhibited carrageenin-induced oedema. Thus, the possible profi inflammatory role of epinephrine as suggested by Rocha e Silva (1964) seems unlikely.

Prostaglandins (PGs) and Leukotrienes (LTs):

The prostaglandins are a group of long chain hydroxy fatty acids and the term 'prostaglandins' was first introduced by Von Euler 1935. It was believed that prostaglandins are smooth muscle-stimulating lipids and prostate gland to be a major source of prostaglandins. But this suggestion i.e. regarding prostate gland to be the major source is now known to be incorrect although the name has been retained.

The leukotrienes (LTs) were so named because they were originally demonstrated in leukocytes and they contain a conjugated triene system in their structure; unlike the prostaglandins (and thromboxanes) they do not contain a ring system.

Biogenesis of Prostaglandins (PGs) and Leukotrienes (LTs):

Oxidative metabolites of arachidonic acid is increased in inflamed tissues. Two principal enzymatic pathways of arachidonic acid oxygenation are involved in inflammatory processes. One is the
Cyclo-oxygenase pathway which produces prostaglandins and the second pathway is 5-lipoxygenase which produces leukotrienes (LTs).

Presence of free arachidonic acid within cells is very low but they are available comparatively in larger amount esterified in cell membrane phospholipid and glyceride. Arachidonic acid is located at 2-position of phospholipids and its release is controlled either by phospholipase A₂ (PLA₂) or the combined action of phospholipase C (PLC) and a diglyceride lipase.

Cyclo-oxygenase pathway. Fatty acid cyclo-oxygenase enzyme complex is available in the microsomal fraction of most animal cells. This is also termed as prostaglandin synthetase. Free arachidonic acid can be metabolized by this enzyme complex to prostaglandin endoperoxides (PGH₂ and PGF₂α). The endoperoxides, which are unstable at physiological pH and temperature, are pivotal in the formation of several other products. This endoperoxides can be converted enzymically to prostacyclin and thromboxane A₂ or primary prostaglandins (PGD₂, PGE₂, and PGF₂α). A C₂ double bond fatty acid (HET), malondialdehyde (MDA) are also formed either by enzymic or non enzymic reactions.

The formation of metabolites varies from cell to cell e.g., blood platelet convert arachidonic acid to TXA₂, whereas vascular endothelium produces prostacyclin.

Both prostacyclin and TXA₂ are unstable at physiological pH and temperature (half lives approximately 5 min and 30 secs respec-
Fig. 3. Schematic representation of Prostaglandins and Leukotrienes biosynthesis.
tively) and are immediately hydrolysed to the 6-keto PGF\textsubscript{2\alpha} and TXB\textsubscript{2} respectively.

Lipoxygenase pathway. An alternative pathway of arachidonate metabolism is an oxidation controlled by lipoxygenase enzymes (LO). There are several lipoxygenases occurring in both the animal and plant kingdoms which catalyse the oxidation by molecular oxygen of cis, cis - 1, 4 - pentadiene systems. In this way arachidonic acid can be metabolised to several HPETE positional isomers. The first lipoxygenation of arachidonic acid in mammalian tissues to be described was that occurring in blood platelets which resulted in formation of 12 HPETE (Fig.3). However, another hydroperoxy derivative namely 5HPETE, is of more interest. Since it can be converted to a novel series of biologically active compounds known as 'Leukotrienes'. The initial enzymic reaction in the conversion of 5-HPETE to leukotrienes is the loss of water to form the 5, 6 epoxide, LTA\textsubscript{4}. As with endoperoxides in the synthesis of prostaglandins, LTA\textsubscript{4} is pivotal in the formation of other leukotrienes (Fig 3). It is hydrolysed to 5(E), 12(R)-di-hydroxy-6, 14-cis, 8-10 - trans - eicosatetraenoic acid (LTC\textsubscript{4}) under influence of the enzyme LTA\textsubscript{4} hydrolase. LTC\textsubscript{4} can also be non-enzymatically hydrolysed to other 5, 12 and 5, 6 dihydroxy acids. Glutathione can react with LTC\textsubscript{4} to form the 5 hydroxy-6 glutathionyl derivative (LTC\textsubscript{5}) under the influence of specific glutathione S-transferase. LTC\textsubscript{4} can be metabolised successively by γ-glutamyl-transpeptidase
and cysteinyl-glycine dipeptidase to LTD\textsubscript{4} and LTE\textsubscript{4} respectively. Another leukotriene, LTD\textsubscript{4}, has been identified as having cysteine-glutamate at C-6.

These peptido-lipid leukotrienes are the components of the bronchoconstricting activity generated during anaphylaxis which is referred by pharmacologists as slow reacting substance of anaphylaxis (SRS-A) (Brocklehurst, 1962).

Different leukotrienes are formed in specific cell-types. Human eosinophils and neutrophils synthesise LTD\textsubscript{4} and LTE\textsubscript{4} respectively. Monocytes and macrophages are able to synthesise both LTD\textsubscript{4} and the peptido-lipid leukotrienes (Samuelsson, 1983).

Role of cyclo-oxygenase products in inflammation

Cyclo-oxygenase activity is stimulated by mechanical, chemical or immunological challenge and as inflammation is the response of living tissue to irritation and injury, prostaglandin synthesis always occurs in inflammed tissues. Various cyclo-oxygenase products are elevated in human inflammatory diseases which include allergic eczema, rheumatoid arthritis, osteoarthritis, psoriasis, ulcerative colitis and gout (Higgs et al, 1984). These products are PGE\textsubscript{2} which
is predominant although PGF$_{2\alpha}$, PGD$_2$, 6 Keto-PGF$_{1\alpha}$ and TXB$_2$ has also been reported.

The most important prostanoids in inflammation are PGE$_2$ and prostacyclin. They are potent vasodilator and hyperalgesic agents and are present in inflamed tissues in sufficient concentrations to account for erythema and increased sensitivity which is characteristic of acute inflammation (Solomon, et al., 1968). The prostaglandins can influence the course of inflammation through their interaction with other mediator substances, e.g., additive effects with histamine or bradykinin and alteration in vascular and metabolic responses to norepinephrine, bradykinin and angiotensin (Steinberg et al., 1964; Moncada et al., 1973; Williams and Morley, 1973; Mezenes et al., 1975). The prostaglandins do not cause overt pain but they sensitize afferent pain nerve endings to the actions of histamine, and bradykinin (Ferreira, 1972). Prostaglandins are released during fever and can produce fever themselves when administered into the cerebral ventricles in experimental animals or when given systemically to human subjects (Feldberg and Saxena, 1971; Hendricks et al., 1971; Feldberg and Gupta, 1973). Prostaglandins are produced by leucocytes. The production is enhanced during phagocytosis. Leucocytes can significantly...
Prostaglandins are considered pathogenically important in a number of settings, but they have not been definitely proven to be essential to any of them. Individually, each prostaglandin has its own spectrum of activities which is both tissue and species specific. Even in the same organ system prostaglandins can oppose the action of others. For example different prostaglandins \( \text{PGF}_2 \) and \( \text{PGE}_2 \) contract all segments of human fallopian tubes; \( \text{PGF}_2 \) relaxes the same segments; \( \text{PGH}_2 \) and \( \text{PGE}_2 \) contract the uterine end but relax the ovarian end (Sandberg et al., 1965). \( \text{PGF}_2 \) stimulates the uterus of the human, cat, guineapig and rat but relaxes rabbit uterus (Berti and Naimzada, 1965; Horton et al., 1965).

The detection of prostaglandins in inflammed tissues and the demonstration of their inflammatory properties led to the proposal that they are important inflammatory mediators. This theory was further strengthened by the discovery that the aspirin-like anti-inflammatory drugs are selective inhibitors of cyclooxygenase. It has been confirmed that the plasma levels attained after administration of anti-inflammatory doses of NSAIDS are sufficient to block prostaglandin synthesis. Therapeutic doses of aspirin...
and indomethacin are also reported to decrease the urinary excretion of prostaglandin (Hamberg, 1972). There is a good correlation between prostaglandin synthesis in vivo and the reduction of oedema (Higgs et al., 1964) and this specific action explains the analgesic, antipyretic and anti-inflammatory effects of the aspirin-like drug.

Role of Lipoxygenase products in inflammation:

The first indication that lipoxygenase activation occurs in inflammation came from the observation of presence of 12HETE in the involved epidermis of patients with psoriasis (Hammarstrom et al., 1975). LTB₄ has been measured in experimental inflammation and low concentration at LTB₄ have been detected in conditions of rheumatoid arthritis, Gout (Rae et al., 1982) and psoriasis (Krain et al., 1982). LTC₄ has been reported being present in synovial fluids from patients with arthritis (Heaney et al., 1986). The mono-substituted LO products, 12HETE and 5HETE, are weak chemokinetic and chemotactic agents in vitro and in vivo for human and rabbit PMNs (Palmer et al., 1980). The 5, 12-dihydroxy product LTB₄ has more powerful effects on PMN function. It is a potent chemokinetic, chemotactic and degranulating agent for PMNs of several species in vitro and causes PMN accumulation in vivo (Bray, 1983; Higgs et al., 1981). This compound also increases plasma exudation in presence of a vasodilator prostaglandin (Wedmore and Williams, 1981; Higgs et al., 1981; Bray et al., 1981).
In experimental inflammation, LTB$_4$ was reported to be found in exudates and reaching a maximum after 6 hours after induction of the inflammatory damage and declining to undetectable levels between 16 and 24 hours (Simmons et al., 1983).

The peak LTB$_4$ concentration correlated with the maximum rate of PMN influx in the exudate. It has been observed that the concentration of LTB$_4$ in the exudate as well as the PMN count was decreased when the experimental animals were pre-treated with colchicine (Salmon and Higgs, 1987). These observations suggest that PMNs are the source of LTB$_4$ and probably, therefore, LTB$_4$ is not the initial signal for PMN infiltration, but may contribute to an amplification of the response. The role LTB$_4$ in recruitment of inflammatory leukocytes is supported by the observation that a lipoxygenase inhibitor reduces cell migration in experimental inflammation (Higgs et al., 1979). The effects of peptido-leukotrienes on the vascular changes of inflammation appear to be species-specific. LTC$_4$ and LTD$_4$ are vasoconstrictor and cause plasma leakage in guinea-pig and rat skin (Peck et al., 1981; Ueno et al., 1981) but have little activity in rabbit skin (Ueno et al., 1981). In human skin, LTD$_4$ and LTE$_4$ cause transient wheal and flare responses (Lewis et al., 1982) either by a direct action or through the release of other endogenous mediators.

There are controversial and conflicting reports on the role of leukotrienes in inflammatory pain. Injection of LTD$_4$ (10-60 ng) into rat paws induced a prolonged reduction in the
nociceptive pressure threshold and LTB$_4$ was approximately equi-
potent with bradykinin in this activity (Levine et al., 1984) while in another model, LTB$_4$, LTC$_4$ and LTD$_4$ antagonised bradykinin-
induced algiesia in the rabbit ear (Schweizer et al., 1984).

To summarise, it can be mentioned that metabolites of arachidonic
acid formed via the cyclo-oxygenase pathway and lipoxygenase
pathway represent an important class of inflammatory mediators. Prostaglandins (Cyclo-oxygenase products formed) specially PGE$_2$ and
prostacyclin, in particular, cause or enhance the cardinal signs of
inflammation. Synergistic interaction with other mediators
suggests that prostaglandins have a central role in the development
of the inflammatory response. Selective inhibition of cyclo-
oxygenase by NSAIDS result in the relief of symptoms in diseases
ranging from sunburn to arthritis. Leukotrienes are formed via
lipoxygenase pathway and the possibility that the leukotriene
(LTB$_4$) is a mediator of leucocyte activation in inflammation
indicates that inhibitors of lipoxygenase may have potential
therapeutic value. Dual inhibitors of both Prostaglandin and
leukotriene could benefit over existing drugs (Higgs et al., 1979).

Lysosomal components. There are potential mediators influencing
inflammatory processes which are released from lysosomes, parti-
cularly from polymorphs but also from other cells including
macrophages and platelets. Lysosomal constituents can degrade
collagen, elastin, cartilage, hyaluronate, chondroitin sulfate,
nucleic acid, complement components, fibrin, plasminogen, coagulation factors and kininogen. The most important lysosomal products in inflammation appear to be cationic proteins and neutral proteases. Cationic proteins can increase vascular permeability either directly or via degranulation of mast cells, and appear also to be chemotactic to monocytes. Neutral proteases are probably important in the pathogenesis of tissue damage after many kinds of injury, including abscess formation, serum sickness, Arthus reaction and perhaps emphysema.

The lysosomes of leucocytes contain protease that can cleave C5, the fifth component of complement, to yield a product that, in turn selectively releases other lysosomal enzymes from intact leucocytes.

Lysosomal enzymes are released by two mechanisms. One is called "regurgitation during feeding"; the lysosomal contents are released into the surrounding medium by cells engaging in endocytosis (Weissmann et al, 1971). Another mechanism of discharge of lysosomal enzymes from intact leucocytes involves selective release (exocytosis) of lysosomal constituents when leucocytes encounter immune complexes on solid surfaces, this process has been termed reverse endocytosis (Weissmann et al, 1972).

Mediators derived in plasma.

Three important mediator systems each of considerable complexity, are present in blood plasma they are, the kinin system, the complement system and the coagulation system.
Each system is usually suppressed by inhibitors and activation requires a number of intermediary steps, the systems and process of activation are interrelated and share key components, so that activation of one leads to activation of components of the other systems.

The Kinin System. On a molar basis the most potent known permeability factor is bradykinin, a nonapeptide formed by digestion of plasma glycoprotein kininogen, by a proteolytic enzyme. Kallikrein found in normal plasma as its inactive precursor prekallikrein. Contact of plasma with damaged tissues or with a foreign surface activates Hageman factor (Factor XII of the blood coagulation system) which converts prekallikrein to Kallikrein. Enzymes with kallikrein activity are also present in many tissues, in urine and in glandular secretions.

Several peptides are similar to bradykinin and can be released by similar enzyme and also powerful permeability factors. The three kinins refer to several polypeptides similar to bradykinin in structure and pharmacologic effect. The three that occur naturally in man include bradykinin, lysyl-bradykinin, (Kallidin) and methionyl lysyl-bradykinin. Bradykinin may be considered a prototype for the kinins. It is a linear nonapeptide with a molecular weight of 1060 that has been isolated from plasma and as well synthesized (Webster and Pierce, 1963).

Bradykinin is a very powerful pain-producing agent when applied to a blister base or injected intra-arterially or intradermally.
in humans (Kellermeyer and Graham, 1968). This has been postulated to play a role in the development of ischemic oedema (Rocha e Silva, 1964) and may be concerned in pathogenesis of endotoxic shock (Nies et al, 1968a, 1968b; Nies and Melmon, 1971; Nies et al, 1972). A bradykinin-like substance that produces pain has been isolated from human blister fluid and from inflammatory exudates and synovial fluid during acute arthritic diseases of varying etiologies (Nies and Melmon, 1968).

Urinary kallikrein seems to have an important homeostatic role related to salt metabolism and blood pressure (Geller et al, 1972; Margolius et al, 1972a, 1972b, 1974; Webster et al, 1975). Plasma kallikrein might, independent of kinin formation, contribute to altered vascular permeability in inflammatory conditions.

The synthesis and release of kininogen from the liver represents a reliable indication of kininogen supply and it is to be preferred to the measurement of plasma kininogen concentration, which is the difference between production and consumption. Therefore plasma kininogen concentration usually does not reflect the increased precursor production rather the increased peripheral consumption as suggested by numerous studies as reviewed Gracia-Leme (1978).

Release and accumulation of kinins in peripheral tissues has been found to be increased in various types of inflammatory reactions. (Lewis, 1970 and Gracia-Leme, 1978). Local production of kinins is definitely increased in inflammatory lesions
produced by carrageenin, urate crystals, heat and other noxious manipulations (Regoli and Barabe, 1980). Activation of kallikreins occurs in inflamed tissues and degradation of kinins may be reduced by the decreased pH of the oedematous fluid. Because kallikreins are inactivated much more slowly than kinins, it has been suggested by Zachariae et al (1968) that the production of kinins may be prolonged in such a way that these peptides could participate not only in the initial but also in the intermediate and late phases of the inflammatory process.

Once released in peripheral tissues, kinins act on blood vessels (particularly on endothelia), through which they produce both peripheral vasodilatation by promoting the release of a smooth muscle relaxing factor and increase of capillary permeability by contracting the capillary endothelium (Regoli, 1984). Kinins find specific receptors on leucocytes, they inhibit polymorphonuclear chemotactic responses, modulate sarcolemma spreading and exert mitogenic actions on rat thymic lymphocytes. Once entered into the inflamed area, white blood and other cells may perpetuate the production of kinins, since they have been shown to contain kallikreins and possibly kinins (Zachariae, et al, 1968).

Kinins are among the most potent activators of prostaglandin release (Regoli and Barabe, 1980; Marceau et al, 1983) and indeed some of their major actions - for instance the endothelium-mediated vasodilatation, the production of pain, the smooth muscle contraction or relaxation in various organs - are associated with
release of prostaglandins. It has also been shown that kinins promote the release of prostacyclins from vessels (heart, kidney) and from cell cultures (rat adipocytes, human endothelial cells) possibly interacting with membrane phospholipases. Recent findings also indicate that kinins and other peptides activate PGE_2 production by deriving their arachidonic acid from phospholipids while noradrenaline derives it from triglycerides lipolysis (Axelrod et al, 1985).

The action mechanisms by which kinins exert all functions as mentioned have not been sufficiently investigated, it is however conceivable that the activation of phospholipases, which has been determined in some tissues, could be involved in some if not all the proinflammatory effects of these peptides.

The complement system. The complement system of blood plasma plays an important role in many immune defence reactions and absence of a functional complement system reduces many inflammatory reactions. Complement activation promotes acute inflammation, recruitment of leucocytes and killing of pathogens by phagocytosis, lysis or release of toxic products.

The human complement system consists of at least 11 distinct serum proteins and three inhibitors (those of Cl, C3 and C6). The 11 complement proteins in the classic pathway have been divided into three functional units that react in fixed sequences and are directed against cell membranes: the recognition unit (Cl, C1, C4, C2), the activation unit (C5, C6, C8), and the
Fig. 3(a): Sequence of complement activation by classic pathway and complement dependent biologic functions. (Reproduced after Miller et al., 1978.)
membrane attack unit (C5, C6, C7, C8, C9) (Alper and Rosen, 1975; Müller and Bernhard, 1975; Colten, 1976) (Fig. 3a). An alternate (or properdin-dependent) pathway involves for complement activation also exists. The alternate pathway involves at least five proteins with activation of C3 that bypasses C1, C4 and C2. The alternate pathway is initiated by complete polysaccharides, aggregates of IgA, and some bacterial endotoxins; the activation may be independent of immunoglobulin (Ruddy et al., 1972; Vogt, 1974; Pearson and Austen, 1975). The mechanism of activation of alternate pathway is not well understood. C3, once activated by either classic or alternate pathway, interacts with other serum components to activate the late-acting complement factors that cause extensive cellular damage. Leucocytes, lung macrophages and the cells those in the glomerulus appear to possess receptors for components of complement (Arnaiz-Vellema and Hey, 1975; Gelfand et al., 1975; Belillon and Metcalfe, 1975; Reynolds et al., 1975). When cells e.g., platelets or mast cells that contain biologically active mediators are the target of a complement reaction, their mediators may be released and activated. Activation of complement is not restricted to cell membranes of individual circulating cells. Activation of the complement system need not inevitably progress to its full expression of cell membrane lesions and cytotoxicity, but intermediate reaction products and complexes are formed that have properties important to the inflammatory process (Fig. 3a).
Anaphylatoxin, a compound generated from either the C3 or C5 components of complement, is a substance of low molecular weight that can release histamine from mast cells, cause smooth muscle contraction and change in vascular permeability. Activated components of complement can also participate in the inflammatory process by virtue of their chemotactic properties and their ability to enhance phagocytosis. In addition to forming anaphylatoxin, C3 causes immune adherence, conglutination, chemotaxis and enhanced phagocytosis by granulocytes, C5, C6 and C7 can form complexes that are chemotactic and can release histamine independently of anaphylatoxin formation (Muller-Eberhard, 1968).

Loss of a functional complement system reduces the ability to mount an acute inflammatory response to injection, with the result that the pathogen is less readily killed and eliminated from the body. In contrast, other genetic deficiencies can lead to inflammatory diseases. Deficiencies of the classical pathway tend to be associated with diseases, such as systemic lupus erythematosus and glomerulonephritis. Deficiency of a control protein which limits the proteolytic activity of Cl leads to recurrent bouts of angioedema (Jose, 1987).

Components of coagulation system. The coagulation system is extremely complex. Activation of the intrinsic clotting system is initiated by activation of Hageman factor (Factor XII); this agent then interacts with the complement and kinin-kallikrein systems. In addition to promoting the generation of vasoactive substances, activated Hageman factor by itself is capable
of producing increased vascular permeability. Other components of the clotting system may have important functions in the development of an inflammatory process, e.g., fibrin which can be leukotactic and is an essential component for the development of the classic Shwartzman reaction (Vassalli and McClusky, 1964, McKay et al., 1969). Fibrin interacts with other plasma proteins such as components of complement, fibrin, and the kallikrein system. Such interactions might be significant in terms of an inflammatory process, but proof of the relevance of these interactions to the inflammatory process has not yet been obtained (Kissin, 1969, Hambarg, 1969).

Interactions between mediators. There is no single chemical mediator that can mediate and sustain most of the events occurring during inflammation. Mediators may interact pharmacologically, their appearance may be sequential or all may simultaneously appear (Millar and Melmon, 1970). For example prostaglandins can intensify the effects of other inflammatory mediators such as histamine and bradykinin; there is also evidence that inhibitors of prostaglandin synthesis inhibit the vasodilatory effects of bradykinin and potentiate the vascular effects of serotonin and norepinephrine (Moncada et al., 1973; Williams and Morley, 1973, Messina et al., 1975). Histamine potentiates the inflammatory response produced by serotonin and bradykinin; serotonin and bradykinin may as well have synergistic effects (Millar and Melmon, 1970).
Immune inflammation

Miller et al (1978) described various inflammatory conditions, in which the immune systems play a substantial role. In these cases the immune response causes cellular injury by several different mechanisms. They are as follows:

Immediate hypersensitivity: These reactions are mediated by IgE antibody adherent to mast cells. When the cell bound IgE combines with circulating antigens, the mast cell discharges its granules containing histamine, SRS-A and other mediators. These agents cause contraction of smooth muscle that is clinically expressed as bronchoconstriction, urticaria, abdominal pain and vasodilatation.

Cytolysis: In causes injury or destroys the cells, usually mediated by IgG. This antibody binds to cell (especially blood cell). This combination may activate complement cascade or predispose to phagocytosis by macrophages with receptors that combine with the cell-bound antibody.

The formation of soluble antigen antibody complexes

In the presence of excess antigen causes cellular injury. The soluble complexes are deposited in well vascularized tissue and can cause remarkably intense inflammatory reaction. In the passive Arthus reaction the combination of antigen and antibody will activate complement; activated complement will release
Fig. 4. Cellular events in immune inflammation.
histamine, cause leukotaxis, contract smooth muscle and increase vascular permeability. The phagocytic cells ingest immune complexes and release lysosomal enzymes, resulting in extensive tissue damage. The size distribution and tissue localization of the complexes determine the resultant tissue damage and inflammatory process. Vasculitis, certain renal diseases and some arthritides have features suggesting components of a disease caused by immune complexes.

In delayed hypersensitivity: This is a fourth mechanism of immunopathology. There is no circulating antibody but committed small lymphocytes convey the immunologic information. These cells actually may control the influx of large number of inflammatory cells especially the mononuclear cells. The tuberculin reaction, contact dermatitis, organ graft rejection are examples of these diseases.

The principal cellular events evolved in immune inflammations are shown in (Fig.4) (reproduced from Shen, 1981). The three-way interaction of macrophages, subsets of T-lymphocytes (e.g., T suppressors, helpers, killers) and B-lymphocytes constitute a dynamic system, which is mainly responsible for immunologic inflammatory disease.

Inflammatory diseases
A persistent and self-generating stimulus may lead to number of inflammatory conditions like rheumatic diseases. Rheumatic diseases are comprised of a large family of clinical syndromes
with a common involvement of the joints and/or paraarticular structure. A brief account on the pathogenesis of some important diseases are given below.

Rheumatoid arthritis. Rheumatoid arthritis is a systemic disease usually manifested clinically by inflammation (Goetzl et al., 1971). It is a chronic inflammatory disease which is believed to be the result of autoimmune reactivity, presumably to some component of the joint. Spontaneous remissions and exacerbations are a part of natural history of rheumatoid arthritis.

Because the articular cavity is an anatomically closed space, the ongoing inflammatory process has been extensively studied. Both B and T lymphocytes are present and gammaglobulins are locally synthesized and transported from the circulation. In rheumatoid arthritis, immune complexes containing components of complement are found in the synovial tissues, in fluid and in large number of leucocytes, byproducts of complement activation and a large number of lysosomal enzymes are also present in the inflamed joints (Zvaifler, 1973).

The etiology of the disease is not clear but immunologic abnormalities may play an important role in the progression of disease. Following an unknown initiating factor the production of abnormal and antigenic IgG stimulates the synthesis of rheumatoid factors (IgM & IgG) and forms immune complexes, IgG aggregates. Activated complement system generate chemotactic factors for PMN leucocytes which enter into articular cavity. PMN leucocytes ingest the
immune complexes to become R.A. cells and then discharge a variety of hydrolases from lysosomal granules which damage the tissue and provoke proliferative inflammatory response in a rheumatoid joint.


gout: Gout usually manifests itself by an inflammation of joints of the larger toe. This is a local symptom of a general metabolic disorder caused by the accumulation of uric acid in the tissues. It is characterised by acute and chronic inflammatory responses to the deposition of microcrystalline sodium urate in the joints and tissue. The crystals lead to activation and release of lysosomal enzymes, and also activate the complement system,
Hageman factor, resulting inflammatory condition. The deposition of urate is due to the defective urate metabolism which may be rectified by antigout agents.
Evaluation of Anti-inflammatory Agents

The complexity of the inflammatory process and the diversity of the drugs which have been found to be effective in modifying this process have resulted in the developments of numerous methods of assay for detecting anti-inflammatory substances. Extensive review works of the various screening methods to detect anti-inflammatory activity of the test substances have been done by Winter (1966a), Weiner and Piliero (1970), Donar (1971), Paulus and Whitehouse (1972), Swingle (1974), Arrigoni-Martelli (1977, 1979) and Gryglewski (1977). A few of these methods have achieved popularity because of their simplicity, reproducibility and economic feasibility. Number of in vivo and in vitro models have been proposed to be able to detect anti-inflammatory effect, a few of these methods have been systematically evaluated for their potential usefulness in the screening programmes and/or have achieved popularity for their ability to select drugs known to exert beneficial effects in rheumatoid diseases.

Swingle (1974) grouped the entire screening procedures in four categories.

Category - 1. Interference with the manifestation of one of the cardinal signs of inflammation.

Category - 2. Modification of one of the events occurring during inflammatory process.

Category - 3. A biological or chemical characteristic (which
may not be related to anti-inflammatory activity) of class known to be anti-inflammatory drugs.

Category - 4. The modification of the syndromes in laboratory animals which are believed to represent models for various rheumatoid disease states.

Interference with the cardinal signs of inflammation

Inhibition of swelling (Tumour, 1st Cardinal sign)

A widely used technique, is to measure their ability to inhibit oedema produced in the hind paw of rat by phlogistic agent. The general procedure is to inject a small amount of solution or suspension of an irritant into the plantar tissue of a hind paw of the rat and then measuring the degree of swelling at various intervals or at the peak of swelling. The popular method for the determination of swelling is to measure the paw volume. The volume is usually measured by the mercury (WINTER et al, 1962, VAN ARNON et al, 1965) or water displacement (Bhatt et al, 1977) methods and in some other methods a pressure transducer with a recording galvanometer has also been used. A number of phlogistic agents have been used to induce oedema. Formalin-induced oedema (Bourne, 1951; Logan and Wilhelm, 1966; Northover and Subramanian, 1961) has been widely used but according to Winter (1966a), this is much less sensitive to other phlogistic agents tested in inhibiting swelling by standard anti-inflammatory drugs. This is also true for kaolin-induced oedema (Garattini et al, 1965), although both these oedemas appear to be selectively...
inhibited by non-steroidal anti-inflammatory drugs. The oedema induced by yeast is less sensitive to standard anti-inflammatory drugs (Winter, 1965). Carrageenin, a mixture of polysaccharides, composed of sulfated galactose units, derived from Irish sea moss, Chondrus crispus (Smith et al., 1955) was found to be a phlogistic agent of choice over the above mentioned agents. This was introduced as oedemogen by Winter et al. (1962) and its biological role has been reviewed by Di Rosa (1972). Not all samples of carrageenin are equally effective in eliciting inflammatory response and effective preparations contain lambda type galactan (McCandles and Lenox-Horner, 1961). As originally described by Winter et al., 1962 the assay consists of drug administration followed 60 minutes later by the carrageenin administration (0.05 to 0.1 ml, 1%) into the plantar tissues of one hind paw. The oedema is measured 3 and/or 5 hours later. The development of the oedema in the rat paws after the injection of carrageenin has been described as a biphasic event (Vinegar et al., 1969). The initial phase of the oedema has been attributed to the release of serotonin and histamine; the oedema is maintained during the plateau phase by the kinin-like substances and the second phase is due to the increase of prostaglandin-like compounds (Di Rosa et al., 1971a; Di Rosa and Willoughby, 1972). The recognition of different mediators for different phases may have important implications for interpreting the effects of drugs.

The importance of leucocyte emigration for the full development
of carrageenin oedema has been acknowledged by (van Arman et al., 1971; Vinegar et al., 1971). In the early phase of oedema, the dominant cells are polymorphonuclears whereas in the advanced stage mononuclears predominate. The importance of the latter type of cells in the advanced phases of oedema, their interrelation with prostaglandin activation and the inhibition of their immigration by non-steroidal anti-inflammatory drugs have been stressed by Di Rosa et al. (1971a, 1971b). Strain, sex and body weight of the adult rats are not significant variables in this assay (Arrigoni-Martelli and Conti, 1964). Carrageenin oedema is also not influenced by ambient temperature or humidity (Garrattini et al., 1965). However, non-specific inhibition of carrageenin oedema has been noticed with some irritants such as hypertonic saline, acetic acid, kaolin and inflammatory exudates (Atkinson and Hicks, 1971; Garrattini et al., 1965; Jori and Bernardi, 1966; Nalz et al., 1971b). Shanahan (1968) pointed that anti-inflammatory substances which exert their effect by virtue of their irritant properties can be distinguished from "true" anti-inflammatory agents by administering them locally in a mixture with the carrageenin, into the paw of rats. Irritant compounds cause a further increase in the size of the paw, whereas non-irritant (true) anti-inflammatory agents produce a reduction in the size of the paw.

Carrageenin-induced oedema test in rats is a very useful model for detecting anti-inflammatory substances (Arrigoni-Martelli and Conti, 1964; Di Rosa and Willoughby, 1971; Vinegar et al., 1971).
(1969), but it suffers from certain aspects of non-selectivity, since it can be inhibited by a number of drugs not usually thought of as anti-inflammatory (Silvestrini, 1965; Niemegere et al., 1964). However, Winter (1966b) has pointed out that many of these drugs were given at doses that would be expected to produce behavioral or autonomic effects. The results obtainable with carrageenin oedema assay are consistently reproducible (Arrigoni-Martelli, 1979) and this method can be used for a comparative bio-assay of standard anti-inflammatory agents (Nowakowski, 1971; Winter et al., 1962). Levy (1969) proposed the use of mice instead of rat in carrageenin-induced oedema method. Although the method is less sensitive to known anti-inflammatory drug but the assay may be used to confirm the anti-inflammatory effects in a second species. Many authors proposed to use some polypene antibiotics (e.g., lyostatic-induced oedema) as oedemogens. Both steroidal and non-steroidal anti-inflammatory agents are claimed to be effective in this model (Arrigoni-Martelli et al., 1971; Schiatti et al., 1970).

Mediators of inflammation such as histamine, serotonin, bradykinin, prostaglandins, hyaluronidase also have been recommended (Burner and Gohn, 1976) as oedemogens for testing anti-inflammatory activity.

Inhibition of redness (Rubor, 2nd Cardinal Sign)

Inhibition of the erythema of depilated guinea pig skin after exposure to U. V. radiation is an useful method for assessing
Topical and systemic anti-inflammatory drug activities. This erythematous response is associated with direct injury to epidermis and adjacent structures. A close correlation seems to exist between the potency of drugs in this assay and their anti-inflammatory potency in man (Adams and Cobb, 1958; Winder et al., 1958, 1965).

The assay procedure has been slightly modified since original description (Wilhelmi, 1950; Wilhelmi and Domenjoz, 1951). Depilated guinea-pigs are exposed to UV light from a UV lamp with a suitable heat filter, at a distance of 3-20 cm, for 20-120 seconds. Following irradiation, the initial vascular permeability, inhibitable by antihistamines, is followed by leukocytic emigration into the site and development of erythema which reaches maximum intensity at 2-4 hours. At 12-24 hours the second phase of increased permeability and accumulation of neutrophils at injured site reaches maximum. The erythemas are scored subjectively. Non-steroidal anti-inflammatory agents do not prevent but only delay the development of erythema. The results expressed in terms of the time required for the response to develop after drug administration (Gupta and Levy, 1973), the method is quite selective for drugs of the acidic non-steroidal anti-inflammatory type as it is not affected by steroidal anti-inflammatory agents (Barin, et al., 1955; Winder et al., 1958; Winter, 1966a) antimalarials (e.g., chloroquine), basic non-steroidal drugs (Winder et al., 1958) and otherwise pharmacologically active compounds (Winder et al., 1958; Adams, 1960).
Thurfuryl nicotinate (5%)-induced erythema has been elicited in the depilated skin of guineapig. Erythematous response reaches maximum at 15 minutes and begin to subside at about 1 hour. This type of erythema is more sensitive than the UV induced erythema to drug effects but it is fainter and difficult to read (Haining, 1963; Arrigoni-Martelli, 1979).

Inhibition of heat (Calor, 3rd Cardinal sign)

There are no widely acceptable standard experimental techniques which utilize the increased temperature of an inflamed site for the assessment of anti-inflammatory activity. Although blood flow, erythema and elevated temperature are strictly correlated (Chinoskey and Flanagan, 1974) and the measurement of temperature would appear to be more objective than the grading of degrees to redness (Lambelin et al., 1971). There have been relatively few attempts to utilize local hyperthermia as the measured response. This may be due to non-availability of thermometers capable of reliably and reproducibly measuring the temperature of the skin or the sophisticated and expensive thermometers required for this purpose. The detailed descriptions of them are available in the works of Collins and Ring (1972), Vinegar et al., (1969) and Lambelin et al., (1970, 1971), who compared the effects of standard anti-inflammatory agents by these methods. Antipyretic and anti-inflammatory activity are not inseparable, although these two properties occur together in acidic non-steroidal drugs. The mechanism of anti-pyretic action of these...
drugs are not fully clear, but prostaglandins mediate the pyrogen fever and inhibition of prostaglandin synthesis, helps to explain the antipyretic activity.

The most widely used method for assessing anti-pyretic activity is yeast-induced fever. Rats are injected subcutaneously with a suspension of (7.5-15%) brewers yeast. A rise in rectal temperature of about 2°C occurs and persists for more than 24 hours. At 18 hours after injection, test compound is administered. Temperature is recorded at ½ or 1 hour interval for 4 to 5 hours (Adams et al, 1969; Bolis et al, 1974; Winder et al, 1962; Bled and Ernpp, 1975). Winter (1965) compared four standard anti-inflammatory agents and obtained parallel dose response curves.

Most of the other methods used to evaluate the anti-pyretic activity of drugs, utilize bacterial pyrogens as fever inducing agent. In addition to rats, cats, rabbits and mice have been used where the pyrogens are injected intramuscularly (Cashin and Heading, 1968; Cranston et al, 1970; Feldberg and Sassera, 1975; Winter and Maua, 1963). All of the above methods are quite sensitive to standard anti-inflammatory-anti-pyretic drugs (Arrignon-Martelli, 1979).

Inhibition of pain (Solor, 4th Cardinal Sign)

The most successful of the methods used to evaluate the analgesic activity of anti-inflammatory drugs involves the assessment of drugs ability to modify what might be defined as inflammatory
pain. Such an assessment appears to be most relevant test, because this type of pain is presumably present in most of the conditions for which such drugs are employed.

The original method described by Randall and Sellito (1957) has been modified (Gilfoil et al., 1963; Winter and Flataker, 1965; Swingle et al., 1971a) to overcome some limitations and to suit the needs of a more reliable assessment of the analgesic activity of anti-inflammatory agents. The principle of the method is the application of pressure, which is applied in increasing magnitude, to the acutely inflamed hind paw of a rat (as a result of the injection of yeast suspension). The force is applied through an air driven plunger (tetalon or glass). The force (in mm Hg) at which animals begin to struggle (or vocalize) is assumed to represent pain threshold and serves as the end point. Drugs are administered before, at the time of, or more conveniently 2 hour after the injection of yeast, when oedema and hyperalgesia are fully developed and the measurement is performed 1 hour later. This procedure minimizes any effect the drug may have on the hyperalgesia as a result of its anti-oedemic action. However, the hyperalgesia and oedema are not correlated in this model and the doses of aspirin as low as 25-50 mg/kg reduce the pain without affecting the magnitude of oedema (Gilfoil et al., 1963). As it is possible to determine the threshold to pressure of the uninflamed paw in addition to the inflamed paw, the method is suited for distinguishing centrally-acting from peripherally-acting antinociceptive drugs. Centrally acting drugs, e.g., narcotic analgesics elevate the threshold to pressure of both paws. Peripherally-acting drugs have no effect on threshold to pressure of the uninflamed paw (Randall and Sellito, 1957).

Recently, a new hyperalgesic assay has been designed in which
carrageenin (500 μg) are used instead of yeast. The assay is sensitive to the analgesic effect of indomethacin and aspirin (Vinegar et al., 1976).

Inhibition of stretching (Writhing, Squirming, abdominal constriction) in the mouse or in the rat used as an index of analgesia since mild as well as strong analgesic were found to reduce the number of stretches produced by phenylbenzoquinone (0.2 ml of 0.025% solution, i.p.) (Siegmund et al., 1937) or by acetic acid (0.2 ml of a 3% solution, i.p.) (Koemer, et al., 1959).

The typical response after the i.p. injection of a nociceptive agent is, "wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs" (Collier et al., 1964, 1968). The drugs are usually administered 60 min before the irritant injection and number of stretches is counted for a period of 10 or 20 minutes after irritant injection. Bradykinin (E-mele and Shanaman, 1963) and acetylcholine (Collier et al., 1964) are also used as writhing inducing agent.

Non steroidal anti-inflammatory agents, antihistamines, anti-cholinergic, sympathomimetics, suppress the response. Writhing assays are till widely used, because no other simple test is yet available for rapid evaluations (Swingle, 1974).

Inhibition of loss of function (functio laesae 5th sign)

The fifth cardinal sign of inflammation, loss of function, has probably not been exploited much to evaluate the anti-inflammatory
activity because of relative difficulty in quantifying the response. Swingle (1974) cited following methods, which may be used to evaluate the activity of the test compound. An assessment of the improvement of grip strength of adjuvant arthritic rats as a measure of the effectiveness of therapy has been made (Perrine and Tatenoe, 1968; Walz et al., 1971a). Particularly no new information regarding the effectiveness of drugs has been obtained by using this parameter (Swingle, 1974).

The grip function measurement in rats made 'arthritic' with Mycoplasma arthritides, has also been made (Kiewinger, 1980). Aspirin, phenylbutazone, indomethacin, gold preparations are effective but morphine and steroids are not effective.

The intra-articular injection of talc into one leg of pigeon has been utilized, for testing anti-inflammatory drugs (Floersheim et al., 1963; Julou et al., 1971). After the injection the time required for the one-legged stance was determined. The assay can be used to detect both steroidal and non-steroidal drugs. Langford et al. (1972) utilized the spontaneous locomotor activity in mice that had received yeast in the planter tissue of the hind paws as an assay to detect loss of function following inflammation. They demonstrated that aspirin and phenylbutazone were effective but hydrocortisone was ineffective in this system. Antihistamines and CNS stimulants gave false positive results.
Modification of one of the events occurring during inflammatory process

Inhibition of inflammatory exudation: Cellular and non-cellular components are the constituents of inflammatory exudate (i.e., fluid part). The application of methods that assess the degree of inflammatory exudation to screening programs for anti-inflammatory drugs has not been particularly successful. Exudation of fluids into the pleural cavity of rats can be achieved with turpentine (Hurley and Spector, 1965; Spector and Willoughby, 1959), Evans blue (Sancilio and Rodrigues, 1966), a mixture of carrageenin and Evans blue (Sancilio, 1969), homologous and heterologous serums (Hurley and Ryan, 1967). The simple determination of the volume of exudate, especially combined with a protein determination and cell count can serve a satisfactory measure of the degree of inflammatory exudation. Plasma protein leakage may also be quantitated by measuring the amount of Evans blue bound to plasma albumin or of other suitable plasma markers present in the extravascular space. Felderman and Kovacs (1969) utilized peritoneal capillary permeability test in mice, since it has been shown (Northover, 1963, 1964) that steroids are in effective in this situation by extravasation of dye (pontamine sky blue) leakage. Different irritants have been used to induce pleurisy for the evaluation of anti-inflammatory activity (Koh...
et al., 1978). By utilizing a mixture of carrageenin and Evans blue, Sancilio (1969) was able to use a pleural effusion method for a comparative bioassay. The quantitative assessment of leucocyte emigration into inflamed areas has not received due attention, taking into account the dominant role played by white cell infiltration in the inflammatory process. A smaller number of experimental models have been specifically designed to evaluate the leucocyte emigration and these models provide less reproducible results than those commonly employed to assess anti-inflammatory activities. Some of the in vivo experimental models are:

1) Cell collection from natural cavities - peritoneal (Van Furth et al., 1973), pleural (Di-rays et al., 1971), Vinegar et al., 1973), dog knee joint (Phelps and McCarthy, 1966), Chicken intertarsal joint (Brune and Glatt, 1974).

2) Cell collection from artificial cavities - Skin window (Rebuck and Crowley, 1955), Skin Chamber (Gowland, 1964; Southan and Levine, 1966).

3) Cell collection from early granulomata (Paul et al., 1972)

4) Cell labelling and transfer (Perper et al., 1974).

From the survey of available data, it appears that nonsteroidal anti-inflammatory agents mainly inhibit migration of mononuclear cells and partly alter the PMN cell
migration into inflammed area (Di Rosa et al., 1972; Perper et al., 1974). The development of a suitable assay system for anti-inflammatory drugs using the criterion for activity as the inhibition of mononuclear cell exudation is very much needed which will be simple, quantifiable and selective for antirheumatic drugs.

Inhibition of inflammatory granulation: After the vascular and exudative changes, the repair phase of the inflammatory process begins to predominate. It is characterized by the proliferation of fibroblasts, the new connective tissue synthesis and the multiplication of small blood vessels by mitosis of connective tissue and endothelial cells. The cellular proliferation penetrates the exudate, producing a highly vascularized reddish mass termed 'granulation tissue' (Swingle, 1974).

The classical granuloma assay (based on the inhibition of the synthesis of granulation tissue) was developed initially to measure the effect of corticosteroids; it was later found to respond to non-steroidal anti-inflammatory drugs (Winter, 1965). 'Cotton pellet granuloma' test is perhaps the most widely used assay and rather a simple test for assessing the activity of anti-inflammatory drugs on the proliferative component of the inflammatory response. It was introduced by Meier et al. (1950) and subsequently modified by Meyer et al.
al (1953), Winder et al (1962) and Winter et al (1963). Two sterilized cotton pellets (5-50 mg) are implanted with sterile technique into the subcutaneous tissue of the flanks or in the axillae of rat. During the first 3 hours after implantation, the pellet undergoes a rapid saturation by the fluid of low protein content, escaping from the vessels (Meyer et al., 1953; Swingle and Shideman, 1967). Then an exudative phase occurs, lasting 2-4 days (Swingle and Shideman, 1967). The third component of the response is the proliferative phase and corresponds to the appearance of the collagen in the granuloma. The duration of implantation influences the granuloma formation which is a time related reaction. The greatest rate of increase occurs during the first few weeks; thus, the majority of assessment have been confined to 7-14 days. Assessment of the response is usually based on the weight of granuloma. An increase in the size of the cotton pellet that is implanted, results in increased amount of granulation tissue. Surface area, rather than weight of the pellet, may be more important to the response obtained (Robinson and Robinson, 1964; Benzi and Frigo 1964). Non specific stress may influence the amount of granulation tissue; by determining the weights of adrenals at the termination of the assay the contribution of endogenous corticosteroids to the effect of a drug on the response can be ascer-
tained (Winter, 1965). Dipasquale et al. (1967) reported, however, that the granuloma formation is relatively independent from the endocrine status of the animal. The dryweight of the granuloma is reduced, if food intake is restricted, or if body growth is impaired by administering drugs (Dipasquale and Mell, 1965). Winter (1965) has obtained parallel dose response curves in this assay both for steroidal and non steroidal anti-inflammatory agent but Winder et al. (1963, 1965) have found less steep curves for non steroidal drugs. Dorfman and Dorfman (1965) have obtained significant reductions of granuloma with the non-steroidal drugs but they obtained poor dose response relationship.

Besides cotton pellet granuloma, variety of other methods have been reported in the literature. Some of them are, the granuloma 'Pouch technique (Robert and Bedamis, 1957) which consists of introducing pouch in the back of the rat by injection of sterile air. Subsequently, croton oil or turpentine or mycobacterial adjuvant is introduced into pouch to induce granulomatous tissue formation. One or two days later, the pouch is deflated to stimulate exudate formation. The response is evaluated 4-14 days later on the basis of the volume of fluid collected and weight or thickness of the pouch. Carrageenan injected subcutaneously in the rat (Morris et al., 1968; Spector, 1969) induces a development of granulo-
tamous tissue which is not easily dissectable. However, it has been employed for studies on biochemical aspects of connective tissue formation and studying the effect of anti-inflammatory drugs on it (Trnavsky and Trnavska, 1961).

Other methods for assessing the antiproliferative effects are, measurement of granulation tissue formation, following inflammation of polyvinyl chloride conical ring in skin wounds (Rudas, 1960); measurement of granulation tissue formation after four days of implantation of filter paper discs in 8 days old chick embryo (D' Arey and Howard, 1967). The tensile strength of experimentally induced wounds have also been used to assess the effect of anti-inflammatory drugs (D'apasquale et al, 1967).

The possession of a property that has been associated with a class of drugs known to be anti-inflammatory:

Properties of non-steroidal anti-inflammatory agents:
Many of the methods developed for the acidic non-steroidal anti-inflammatory drugs are conducted in vitro. Although these are less time consuming and economic but they give large number of false positive results. These methods are: uncoupling of oxidative phosphorylation (Adams and Cobb, 1958; Whitehouse, 1967), inhibition of denaturation of protein (Mizushima, 1964, 1968; Mizushima and Suzuki, 1965), erythrocyte stabili-
Nonsteroidal anti-inflammatory agents inhibit the prostaglandin biosynthesis at concentrations comparable to their therapeutic levels in man and in animal models. Target for the non-steroidal anti-inflammatory agents is the fatty acid cyclooxygenase, a component of the prostaglandin synthetase system, provided a rational approach for in vitro screening of compounds for their anti-inflammatory activity. The inhibitory effect shows a high degree of stereospecificity, the same was observed between structure-activity relationships for in vitro prostaglandin synthetase inhibition and in vivo anti-inflammatory activity (Ferreira et al, 1971; Smith and Willis, 1971; Vane 1971; Vane and Ferreira, 1978).

The prostaglandin synthetase system is a membrane bound multienzymic complex which is found in the microsomal fraction of tissue homogenates. Sheep and bovine seminal
vesicle microsomes are frequently used as the source of the enzyme (Smith and Lands, 1971; Takeguchi et al., 1971). Besides these, perfused dog spleen (Ferreira et al., 1971), human platelets (Smith and Wills, 1971), cell free homogenates of guinea pig lung (Vane, 1971) are also used.

The incubation mixture usually contains arachidonic acid, glutathione and enzyme in buffer. The rate of reaction depends on the concentration of arachidonic acid, ratio of prostaglandin formed and the inhibitory potency of the test drug (Flower et al., 1973; Gryglewski, 1974). An inhibitor can be preincubated or without preincubation with the enzyme. The prostaglandins (predominantly PGE$_2$) are extracted, separated by TLC and quantitated biologically (Flower et al., 1972), or radiochemically (Flower et al., 1973) or radioimmunologically (Bauminger et al., 1973) or by gas chromatography - mass spectrometry (Hamberg, 1972).

Besides the prostaglandin synthetase assay, recently improved assay procedure to measure the leucocyte functions are continually being developed. They include inhibition of chemotaxis (Flower and Blackwell, 1979), adherence (Stecher and Chiles, 1978) and action of macrophages (Sonney et al., 1978). The inhibition of proteases are also being investigated (Butler and Kruse, 1978).
Properties of steroidal anti-inflammatory agents: It is suggested by Swingle (1974) that there are some basic properties of anti-inflammatory glucocorticoid steroids which are undissociable from their anti-inflammatory activity. Following these observations he recommended some methods. They are, lymphoid tissue involution in rodents (Dougherty, 1952; Dorfman, 1970; Swingle et al., 1971b) cell culture techniques (Hawson, 1972), deposition of glycogen (Newman and Gyermek, 1966), waterload excretion by adrenalectomised rats (Mass et al., 1968) granuloma assays (Meier et al., 1950; Mayer et al., 1953) inflamed mouse ear (Tonnell et al., 1965) and histological changes in chick duodenal mucosa (Hayes, 1965).

Properties of antirheumatic drugs: There are no specific methods available to assess the activity of the antirheumatic drug, like chloroquine, gold preparations and d-penicillamine.

Properties of immunosuppressive drugs: Various in vitro and in vivo methods have been proposed and Arrigoni-Martelli (1977) and Rosenthal (1974) have reviewed them in detail. Most of the immunosuppressive drugs also have anti-inflammatory property and it should be noted that anti-inflammatory response is an important component of most immunologically induced response. Finding new antirheumatic drugs by the use of immunosuppressive screening methods does not appear much more
relevant because the methods in use, are designed to detect agents that can turn off an established and ongoing process (Swingle, 1974).

Modification of syndromes purported to be animal models for human rheumatoid disease

Modification of experimental arthritis: Animal models for rheumatoid arthritis and most of the other rheumatic diseases of man are lacking and this has hindered the search for truly effective drugs for the treatment of these diseases. Various types of experimental arthritis can be induced in animals but their similarity to human diseases is a matter of debate. However, not entirely unsuccessful attempts have been made to devise animal models of the various rheumatoid diseases. Out of these, adjuvant-induced arthritis in rat is by far the best and most popular one. Adjuvant arthritis is a systemic disease induced by the intradermal injection of killed mycobacteria in mineral or Paraffin oil (Newbould, 1963; Pearson, 1956) characterized by the appearance of the swelling of paws, and nodules on ears and tail. Immunological mechanisms are involved in the development of the disease. Evidences appear to be in favour of a delayed hypersensitive response to a disseminated antigen which is probably derived from mycobacteria (Gary and Walkman, 1967; Newbould, 1963; Walkman and Wennmacher, 1963). Much of the evidence, however, would apply equally well to an antibody-dependent mechanism (Quagliata and Phillips-
Quagliata, 1972). Nature and characterization of the initiating immunogen has also been done (Pearson and Chang, 1978). Killed mycobacteria (M. butyricum, M. tuberculosis, M. Phlei) are most commonly used species) are either suspended or emulsified in mineral or paraffin oil injected (0.2-1.0 mg of mycobacteria/0.1 ml) into hind paw or tail. Some authors preferred latter site (Perper et al., 1971; Winder et al., 1969). A localized primary inflammatory response at the injection site is followed by a disseminated immunologic disease which develops in between 7 to 26 days. The systemic disease is characterized by the swelling of contralateral non-injected limb, local hyperpyrexia, nodules on the ears and tail and multiple joint lesions. Various hematologic and biochemical changes in rat have been observed (Swingle, 1974).

The most objective assessment of the severity of the disease can be noted by determining the magnitude of swelling of the hind paws. If the adjuvant has been injected into one hind paw, then the swelling of that paw may be used to assess the acute inflammatory reaction and the swelling of the contralateral paw to assess the severity of the secondary lesion (Arrigoni-Martelli, 1977). Other types of assessment include, subjective assessment on the degree of joint involvement or severity of lesions occurring in paws, ears and tail (Brown et
Several nonspecific factors have been claimed to inhibit the adjuvant arthritis, e.g. hepatic injury, high dose of oestrogen, PGE\textsubscript{1} and PGE\textsubscript{2}, alloxan-induced diabetes, insulin, thyroidectomy and respiratory infection (Swingle, 1974). Various strain of rats differ in their response to the injection of the adjuvant (Rosenthale, 1970). The severity of the disorder may be modified by steroidal and nonsteroidal anti-inflammatory drugs. The amount of steroidal and non steroidal anti-inflammatory drugs needed to produce a significant effect on adjuvant-induced arthritis seems to correlate with clinically effective human doses. Efficacy of Gold preparation in this model is a matter of debate (Arrigonni-Martelli, 1977). Induction of acute and chronic arthritis by various bacterial cell walls and their water soluble peptido-glycan components (Koga et al, 1976) by preformed collagen-anticollagen complexes and variety of other antigens have also been described (Steffen et al, 1977; Brackertz et al, 1977). Several other methods have been devised to induce experimental arthritis.
in animals, e.g. repeated oral administration of 6-sul-
fanilamidoindazole produces inflammation in rat, which
can be inhibited by standard nonsteroidal anti-inflam-
matory agents (Miller et al., 1970; Sigg et al., 1967).
Intra-articular injection of a variety of antigens in
previously immunized rabbits produces arthritis, which
is truly chronic, lasting more than one year and the
histological features bore resemblances to human arthri-
tis (Dumonde and Glynn, 1962; Condon et al., 1971).
Non-steroidal anti-inflammatory agents are able to con-
trol some of the parameters of disease activity (Black-
ham, 1978). Arthritis has also been induced in chick
by feeding zinc deficient diet. Steroidal and non-steroi-
dal anti-inflammatory agents are effective in this model
but not the gold preparations (Nielsen, et al., 1968;
Hoekstra, 1969).
Modification of experimental gout: It is well known
that microcrystalline sodium urate is responsible for
initiating an attack of gout and this knowledge was
employed in the development of animal model of this
disorder. Models of gout include: the response of dogs
(McCarty et al., 1966) or rabbits (Spilberg and Osterland,
1970) to the injection of urate crystals into the joints
of rats (Trnavsky and Kopecky, 1966) or mice (Fitz-
gerald et al., 1971) to the injection of this crystal
into the plantar tissue or into their (rat’s) interscapular region to produce granuloma. Colchicine and several of its analogs and effective in these models. Indomethacin and Phenylbutazone are effective in the dog model (Shoenenthal et al., 1966). A bird model of pseudogout, based on intra-articular injection of microcrystalline sodium urate has also been developed. The length of time during which the bird stood on one leg was taken as a measure of the extent of inflammatory reaction (Floersheim et al., 1973a, 1973b).

Modification of spontaneous disease states
Three spontaneous disease states of animals that have received most attention as models for rheumatoid diseases (Jones, 1969). They are: Aleutian disease of mink (Horton, 1970), disease of New Zealand mice (Horton, 1970; Mellors, 1971) and canine systemic lupus erythematosus (Lewis et al., 1965).

These are the methods which are generally used to detect the anti-inflammatory activity of a new compound. But it should be admitted that still there is a considerable lack of suitable foolproof method. Spector and Willoughby (1968) rightly pointed out the limitation of the methods. According to them, “one of the difficulties of searching for new anti-inflammatory agent is that it is tempting to select the present, inadequate drugs,
design tests, that give positive results and then with the aid of these tests to search for further compounds giving positive results. Such methodology may lead only to 'old' anti-inflammatory agents instead of new.
Anti-inflammatory agents

Inflammation is a complex process initiated by a number of mechanisms and the entire process of inflammation with multiple manifestations are not yet fully understood. A large number of drugs are currently being used against various inflammatory diseases. These drugs only suppress the clinical manifestations, arising from the inflammatory reactions. The anti-inflammatory agents may be defined as those compounds which depress the generally accepted models of experimental inflammation and afford symptomatic relief in arthritic disorders at the doses not producing toxic, behavioral or autonomic effects (Arrigoni-Martelli, 1977). There are some other types of drugs which are probably not anti-inflammatory in the above sense but do more than simply suppressing the symptoms and signs of the disease. The latter type of drugs are termed as antirheumatic agents.

The drugs used in inflammatory disorders have been extensively studied. They are a heterogeneous group with varied chemical and physical properties. Their clinical usefulness as anti-inflammatory agents could not be predicted before they were tested for this purpose. The clinicians must be wary about his knowledge of the mechanism of action of anti-inflammatory agent because (1) they are multipotential substances that have many different properties and effects, and it is different to isolate a predominant anti-inflammatory effect; (2) knowledge of the inflammatory process and therefore of potential sites and modes of drug action...
is incomplete and has been partly based on experimental models that may bear no definite or consistent relationship to the mechanisms of inflammation in human disease; (3) anti-inflammatory agents rarely reverse the entire process of a specific inflammatory disease (e.g., during treatment of rheumatoid arthritis with corticosteroids, the swelling may diminish, the redness and pain may disappear, and the patient may feel better but the destruction of the joint continues. In contrast, cyclophosphamide (not ordinarily considered as an anti-inflammatory drug) may reverse the course of the pathology (Miller et al., 1978) and diseases that superficially share many pathogenetic and clinical features may not respond to the same anti-inflammatory agent. For example preparations of gold, when properly used, may ameliorate the manifestations of rheumatoid arthritis but are without any effect in other types of inflammatory disorders.

Paulus (1974) suggested that an ideal drug to treat anti-inflammatory condition should be aimed in the following four target areas.

(1) The etiological factors.
(2) Mediators of initial tissue injury
(3) The non-specific, normal protective inflammatory response produced by tissue injury, and
(4) The processes attempting to restore normal function.

Anti-inflammatory agents in use may be broadly divided in two classes which are steroidal anti-inflammatory agents and Non-
teroidal anti-inflammatory agents.

Anti-inflammatory steroids

Anti-inflammatory corticosteroids usually are glucocorticoids; the mineralocorticoids are ineffective as anti-inflammatory agents and corticosteroids having both actions, exert a wider range of side effects. Liddle (1961), Chen and Borroweag (1970), Popper and Watnick (1974), Jasani (1979), Miller et al. (1978, 1979) and Wolf (1979) have reviewed the anti-inflammatory activity of the steroids. Corticosteroids and their synthetic analogues are able to suppress the early phenomenon and also the latter manifestations of the inflammatory reactions without affecting the underlying cause of the disease. Anti-inflammatory corticosteroids reduce the inflammatory reactions in the following manners:

They inhibit alterations of vascular tone, capillary permeability and proliferation of capillaries and fibroblasts and deposition of collagen. They also prevent the release of mediators. Corticosteroids increased the stability of mast cell granules and thus reduce the histamine forming capacity of the tissues and stabilise the lysosomal membranes. Anti-inflammatory steroids block the extraneuronal uptake process for inactivation of catecholamines and also have vasoconstrictor action. Inhibition of chemotaxis of leucocytes also has been noticed with corticosteroids.

In addition to those mentioned above, anti-inflammatory steroids inhibit the release of phospholipids required for the biosynthesis
of prostaglandins (Gryglewski et al., 1975, Hong and Levine, 1976). It has been shown that stimulation of release of thromboxane A2 by agents such as histamine, serotonin and rabbit aorta-constricting substance releasing factor is inhibited by anti-inflammatory steroids. They also inhibit the phospholipase A2 activity and thereby inhibit the release of arachidonic acid. Anti-inflammatory steroids can prevent the biosynthesis of a whole cascade of lipid mediators of inflammation, including the prostaglandins and SRS-A or leukotriene C4, which are derived from arachidonic acid (Bach et al., 1977; Jakschick et al., 1977). Several discrete effects of steroids relevant to their anti-inflammatory properties are gradually becoming clear, e.g. inhibitory effect on fibroblasts (Gray et al., 1971); inhibition of formation and accumulation of macrophages (Vassali et al., 1976). Although anti-inflammatory steroids suppress the inflammation and are valuable life-saving drugs but the quantity of corticosteroids necessary to produce anti-inflammatory effects, result in a number of profound metabolic alterations and the chronicity of many inflammatory diseases may require the use of these agents for a long period. The inevitable side effects that are associated with the use of corticosteroids are: hyperglycaemia, peptic ulcer, osteoporosis, infections, fluid retention, Cushing's syndrome, growth retardation, suppression of normal adrenal-pituitary axis etc.

Hundreds of corticosteroid derivatives have been synthesized over the years with the hope of increasing the potency and of
decreasing the side effects. Though highly potent derivatives have been obtained but the incidence of side effects restricted their use.

Nonsteroidal anti-inflammatory drugs

A large number of compounds of diverse chemical characteristics are currently being used as anti-inflammatory drugs. Although they have a varying degree of anti-inflammatory activity, generally they do not possess the side effects, that are commonly associated with steroidal drugs. Biological properties of non-steroidal anti-inflammatory drugs (NSAID) have been reviewed by Domenjoz (1966); Murray and Pilliero (1970); Paulus and Whitehouse (1973); Mills (1974); Scherrer & Whitehouse, 1974; Ratona and Blengio (1975); Arrigoni-Martelli (1977); Bressloff (1977); Hurkmans (1977; 1978a); Vane and Perrein (1979); Nickander et al (1979); Flower et al, (1980) and Simmons and Mills (1980). The chemistry of NSAID have been discussed by Scherrer and Whitehouse (1974) and Shen (1981). NSAID are generally effective in various models of inflammation as described earlier and suppress the clinical manifestations and histological picture of the diseases of inflammatory origin (e.g. rheumatic diseases) but they do not cure the disease.

According to chemical structure NSAID can be divided into two broad classes, (1) Aryl acidic agents and (2) Nonacidic agents. 

Aryl acidic agents:

Acidic NSAID constitute a large number of compounds of following
chemical nature:

(a) N-aryl anthranilic acid-fenamates, e.g., mafenamic acid, meclofenamic acid, flufenamic acid, tolufenamic acid, clorixin etc.

(b) Salicylic acids - acetyl salicylic acid, diflunisal, fenbufen, benorylate.

(c) Phenyl propionic and phenyl acetic acid- ibuprofen fenoprofen, naproxen, flurbiprofen, ketoprofen, tolmetin, aldometac, diclofenac, fenbufen, suprofen, carprofen etc.

(d) Indole acetic acid - indomethacin, melindac

(e) Enolic and other acidic agent-phenylbutazone, oxyphenbutazone, suprofen, ixiancon, piroxicam.

Non-acidic agents

A variety of non-acidic agents that have been reported to possess anti-inflammatory activity, may be categorized as (a) Substituted triaryl-indoxole, bimetopayrol, flumizole, ditaenol, thienbenzamide (b) Quainazolinones : Proquazone, ciproquazone and (c) Others - diflazone, tribenoside, nictindole.

Among the numerous compounds in each group of drug so far synthesized and investigated, the above list includes some of the important drugs with their structural modifications. Detailed information about the individual clinically important drug can be obtained from the reviews cited in Table-A.

The pharmacological effects of nonsteroidal anti-inflammatory
Table A: List of important reviews concerning the pharmacological properties of some clinically important NSAID.

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>REVIEWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenamates</td>
<td>Kendal (1966); Scherrer (1974)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Dixon et al. (1963); Smith and Smith (1966); Adams and Cobb (1967); Tainter and Perris (1969); Krane (1972); Cohen (1976).</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Adams and Buckler (1979).</td>
</tr>
<tr>
<td>Fenoprofen</td>
<td>Brogden et al. (1977b).</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Brogden et al. (1975).</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Adams and Buckler (1979).</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Haubek (1976); Mason and Bolton (1977).</td>
</tr>
<tr>
<td>Tolmetin</td>
<td>Ward (1975); Brogden et al. (1978b).</td>
</tr>
<tr>
<td>Alclofenac</td>
<td>Brogden et al. (1977a).</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Shen and Winter (1978); Huskisson and Franchimont (1976); Brogden et al. (1978a).</td>
</tr>
<tr>
<td>Sulindac</td>
<td>Shen and Winter (1978); Huskisson and Franchimont (1976); Brogden et al. (1978a).</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Von Roehnemberg (1962); Kendall (1963); Whitehouse (1965); Yu (1974).</td>
</tr>
</tbody>
</table>
### Table B: Effects of some NSAID on various experimental models of inflammation

<table>
<thead>
<tr>
<th>NSAID</th>
<th>Carrageenin oedema in rat</th>
<th>UV erythema in guineapig (ED50 mg/kg, o.s.)</th>
<th>Therapeutic arthritis in rat (ED50 mg/kg)</th>
<th>P.G. Synthesis in vitro (sheep seminal vesicle ID50 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td><strong>0.9 - 10.3</strong></td>
<td><strong>0.45 - 10.0</strong></td>
<td><strong>0.1 - 0.18</strong></td>
<td><strong>12.6 - 58.0</strong></td>
</tr>
<tr>
<td>Indomethacin</td>
<td><strong>0.5 - 1.5</strong></td>
<td><strong>0.8 - 30.0</strong></td>
<td><strong>4.7 - 6.1</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Sulindac</td>
<td><strong>2.7 - 8.4</strong></td>
<td><strong>1.7 - 3.3</strong></td>
<td><strong>1.5 - 2.0</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td><strong>5.0 - 14.0</strong></td>
<td><strong>7.0 - 15.0</strong></td>
<td><strong>1.7 - 3.2</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td><strong>10.0 - 23.0</strong></td>
<td><strong>52 - 86</strong></td>
<td><strong>1.7 - 3.2</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td><strong>20.0 - 52</strong></td>
<td><strong>52 - 86</strong></td>
<td><strong>1.7 - 3.2</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td><strong>22.0 - 55.0</strong></td>
<td><strong>52 - 86</strong></td>
<td><strong>1.7 - 3.2</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Naproxen</td>
<td><strong>21.0 - 56.0</strong></td>
<td><strong>52 - 86</strong></td>
<td><strong>1.7 - 3.2</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td><strong>25.0 - 45.0</strong></td>
<td><strong>52 - 86</strong></td>
<td><strong>1.7 - 3.2</strong></td>
<td><strong>1.50 - 2.80</strong></td>
</tr>
</tbody>
</table>

* N.A. - Not available.
drugs (NSAID) are more or less similar although chemical nature is somewhat different to each other. Most of them possess analgesic and antipyretic effects, although there are variations in the degree of these activities.

NSAID are effective in various experimental models of inflammation (Table B). Salicylates and other NSAID are effective in rheumatoid arthritis and musculoskeletal disorders of inflammatory origin. Nickander et al. (1979) cited various inflammatory diseases in man to which each individual agent is prescribed. Although large number of drugs developed but no drug, so far has been recommended specifically for the treatment of rheumatoid arthritis that are basically different from salicylates and none of them seems to hold more promise for patients other than a temporary relief.

Various mechanisms of action of Salicylates and NSAID have been proposed (Whitehouse, 1964, 1967; Smith, 1966, 1978). But these are not acceptable as there is a poor correlation between the in vitro and in vivo concentrations of drugs that are required. Aspirin, indomethacin and many other NSAID inhibit the prostaglandin biosynthesis as suggested by most authors. Aspirin prevents the prostaglandin endoperoxide by cyclo-oxygenase enzyme inhibition (Flower, 1974). Aspirin irreversibly inactivates the cyclo-oxgenase by acetyllating lysyl amino group of the enzyme (Bu*ch et al., 1978; Roth and Dick, 1978). Salicylic acid has no acetylating capacity and is inactive against cyclo-
oxygenase in vitro system but is as active as aspirin in vivo, in reducing the synthesis of prostaglandin (Hamberg, 1972). Mode of inhibition of indomethacin (the most potent irreversible inhibitor of cyclo-oxygenase) is particularly complex and probably involves a site of on the enzyme different from that which is acetylated by the aspirin. Indomethacin inhibits several pathways associated with arachidonic acid cascade, such as phospholipase A₂ of PMN leucocytes (Kaplan et al., 1978) and also inhibits the release of arachidonic acid (Churchland Levine, 1978) and 15 hydroxy prostaglandin dehydrogenase (Hansen, 1974) at low concentrations under certain experimental conditions. Sulindac (an indomethacin analogue) is a prodrug which is less potent as a cyclo-oxygenase inhibitor but its sulfide metabolite is more potent in the above system. The fenamates (aryl anthranilic acids) inhibit cyclo-oxygenase and fenamates (especially meclofenamic acid) appear to antagonize certain effects of prostaglandins, e.g. PGF₂α-induced contraction in isolated bronchial smooth muscle (Coller and Sweatman, 1968; Shen, 1978). Other NSAID like phenylpropionic acids, enolic acids (Flower et al., 1980) and other non acidic agents (Shen, 1981) also inhibit prostaglandin synthesis. The concentrations required to inactivate this enzyme are within the plasma levels achieved by these drugs during normal therapy, even when plasma binding is taken into account. There is a good evidence that the therapeutic doses of aspirin reduce the prostaglandins biosynthesis in men and such doses inhibit the production of prostaglandins.
by human platelets and reduce the prostaglandin content of human semen. After the treatment with therapeutic doses of aspirin the concentration of the metabolites of PGE$_1$ and PGE$_2$ is reduced in the urine. There is a good rank of other correlation between antiemetic activity and carrageenin-induced oedema test in rat (Hamberg, 1972; Ren et al, 1972). NSAIDS by virtue of inhibition of the cyclo-oxygenase, reduce the levels of prostaglandin E$_2$, F$_2\alpha$, and thromboxane A$_2$. NSAIDS are not the inhibitors of prostacyclin and thromboxane synthetase. A few inhibitors of these enzymes have been identified. Selective inhibitors of these enzymes would well provide desired therapeutic effects with a reduced incidence of side effects. Presently the cyclooxygenase inhibitors reduce the level of various mediators causing alterations in the normal physiological functions of the intestinal tract, kidney and uterus (Nickander et al, 1979).

Besides inhibition of prostaglandin synthesis salicylates, phenylbutazone, indomethacin and other NSAIDS also inhibit synthesis of histamine release, 5 hydroxytryptophan decarboxylase and synthesis of mucopoly saccharides. Salicylates stabilize lysosomal membranes and Phenylbutazone stabilizes lysosomal membranes and decreases tissue response to lysosomal enzymes. Salicylates and other NSAIDS uncouple oxidative phosphorylation. Salicylates have action to suppress antibody production and antigen-antibody reaction and indomethacin reacts with serum and tissue proteins.

Doyer and Krane (1978) discussed the role of NSAIDS in inhibiting collagenase production and Panush (1978) suggested that some
of them may suppress the lymphocyte functions. But according to Flower et al. (1980), some of the proposed mechanisms of actions like fibrinolysis, uncoupling of oxidative phosphorylation etc., by the NSAID are not acceptable, since higher concentrations are needed to achieve a significant degree of inhibition. Perhaps these nonspecific actions may have a secondary effect on the efficacy and toxicity.

There is also evidence of a non steroidal anti-inflammatory agent e.g., lutifensol (F. 1686) having a wide range of anti-inflammatory activities in animals while differing from the activities of the classical non-steroidal drugs (Ikewiye et al. 1994). Lutifensol is not a major inhibitor of prostaglandins and lacking any action on the Freund's adjuvant arthritis. Some flavonoid compounds e.g. Gossypin, epicatechin, napirin have been reported as anti-inflammatory action like classical non-steroidal anti-inflammatory agents (Tarayre et al. 1984). Lotifazole is not a major inhibitor of prostaglandins and lacking any action on the Freund's adjuvant arthritis. Some flavonoid compounds e.g. Gossypin, epicatechin, napirin have been reported as anti-inflammatory action like classical non-steroidal anti-inflammatory agents (Tarayre et al. 1984).

The analgesic effect of salicylates, phenylbutazone and other NSAID involves a peripheral component of action, probably due to an interference with the function of pain producing substances (Collier, 1969). NSAID may avert the sensitization of pain receptors to mechanical stimulation or to other mediators by preventing the synthesis and release of inflammatory mediators. The role of prostaglandins in pain production has clearly been established (Katz, 1971; Collier and Schneider, 1972). Most data are consistent with the presumption that aspirin as an analgesic, is effective in pathological conditions or in experimental models
where prostaglandins are synthesized locally. Although it does not affect the hyperalgesia or pain caused by the direct action of prostaglandins, i.e., when injected locally (Montoya et al., 1975; Pereira, 1972; Pereira et al., 1973), although the pain evoked by bradykinin are inhibited by aspirin (Arrigo-Martelli, 1977).

Salicylates and some other NSAID have varying degree of antipyretic action, but they do not affect the body temperature of normal individuals. Antipyretic action may be due to the anti-prostaglandin activity since Feldberg and his associates (Feldberg and Saxena, 1971; Feldberg and Gupta, 1973; Milton 1973) showed the role of prostaglandin in fever, but the probability of CNS component of their activity cannot be ruled out (Flower et al. 1980).

Side effects of nonsteroidal anti-inflammatory agents

One of the most common side effects of NSAID is the high incidence of gastrointestinal disturbances ranging from the subjective feeling of discomfort to haemorrhage and development or reaction of mucosal lesions (Hin et al., 1971). There is a considerable variation in their tendency to cause such sequelae. Recently an increasing number of NSAID have been introduced, many of which have been claimed to exhibit a low incidence of gastrointestinal side effects. However, the fact is that after period of evaluation in the clinic and further detailed investigation in animals, many of these allegedly "well tolerated" NSAID
eventually prove more ulcerogenic than originally assumed (Rainsford and Whitehouse, 1980). Various structural modifications of the parent chemical structure have been made to decrease their gastrointestinal effects, but no satisfactory results have been obtained. In case of aspirin, its derivatives like trilisate, benorylate, diflunisal are less irritant but not absolutely free from side effects. Some of the esters of aspirin are claimed to have much lower gastric ulcerogenic activity (Rainsford and Whitehouse 1980). Likewise, many claims have been made that new formulations of aspirin (e.g. sustained or slow release, soluble or enteric coated preparations) which have been developed and promoted in recent years cause less gastrointestinal blood loss than observed with plain aspirin tablets. On further investigations the claims of improved gastric safety often proved unjustified (Rainsford and Whitehouse, 1980). In search for better indomethacin analog with better patient tolerance led to the discovery of sulindac (Shen and Winter, 1978). Sulindac is a prodrug - its sulfide metabolite is a potent anti-inflammatory agent. The advantage of using sulindac as a reversible prodrug being that it "prevents the initial exposure of gastric mucosa to the active drug, as a result the gastrointestinal side effects are minimal. Ibuprofen is an analgesic and anti-inflammatory agent, generally, ibuprofen causes less gastrointestinal side effects than aspirin (Miller et al., 1978).

The inhibition of biosynthesis of mucous glycoprotein (Rainsford, 1978) and the prostaglandins are the recognised biochemical actions
of aspirin and other NSAID, contributing to ulcer. The gastric mucosa synthesizes PGlz, which may well be involved in functional hyperemia and gastric secretion. The gastric erosions induced by indomethacin and many ulcerogenic agents in experimental animals are most effectively prevented by prostaglandins or their analog. Certainly, the fact that this effect is shared, to a greater or lesser degree, by the common inhibitors of prostaglandin synthetase, suggests that it is related to the withdrawal of a protective prostaglandin (Bennet and Curwen, 1977; Whittle et al., 1978; Guth, et al., 1979).

Many NSAID have been reported to cause renal damage after prolonged administration. The primary lesions appear to be papillary necrosis with secondary chronic interstitial nephritis. The injury is often insidious in onset, usually is manifested initially as reduced tubular function and concentrating ability and may progress to irreversible renal insufficiency (Gault, 1968; Abel, 1971; Argst, et al., 1979). As prostaglandin synthesis-inhibitors, NSAID decrease renal blood flow and excretion of water and sodium especially in patients with impaired renal function (McGiff and Wong, 1979).

Higher doses of long acting NSAID have undesirable effect on liver and low incidence of agranulocytosis has been found with pyrazolones (Huskisson, 1977).

Inhibition of prostaglandin biosynthesis may also cause some other undesirable side effects like disturbances of platelet...
function and prolongation of gestation period and may have undesirable side effect on male fertility. Hypersensitivity reaction has been reported to occur during salicylate therapy (Flower et al., 1980).

Antirheumatic drugs

There are some drugs which are not mentioned in the previous section have a slow onset of action and a beneficial effect on various rheumatic disorders in man, although they are not able to cure the inflammatory diseases and have potential side effects. These antirheumatic drugs currently in use are: gold preparations, antimalarials like chloroquine and d-penicillamine. Detail informations about these drugs may be obtained from the reviews cited below :

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Reviews</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td>Freyberg (1972) ; Walsegal (1974), Blum (1975) ; Bresaloff (1977) ;</td>
</tr>
<tr>
<td>D-Penicillamine</td>
<td>Arrigonni-Martelli (1977)</td>
</tr>
</tbody>
</table>

A good number of immunosuppressants are also used for the treatment of various diseases of inflammatory origin (Arrigonni-Martelli, 1977).
Antigout agents

Gout is a metabolic disorder caused by derangement of purine metabolism as a result of which crystals are deposited in the joints. Colchicine, allopurinol and a number of uricosuric agents are used in the treatment of gout. Fitzgerald (1974), Yu (1974) and Kelley and Weiner (1978) reviewed various aspects on the subject.

Indian medicinal plants as Anti-Inflammatory agents

Diverse descriptions of a variety of arthritic conditions are available in the Ayurvedic texts. A large number of drugs useful in such situations especially from different medicinal herbs, (single or combined) have been suggested, e.g. Rasna, Punarnava, Rasandi, Mulethi, South, Mahayogaraja, Gugglu, etc.

The eight selected medicinal plants are mentioned below with special reference to their anti-arthritis properties. These are not the only anti-arthritis herbs in the treasury of Ayurveda but they were chosen on the basis of their popularity and therapeutic excellence. Several components preparations of these herbs are described in ayurvedic classics and marketed by large scale manufacturers. To supplement the effects of these drugs, to make preparations palatable to make their effects multi-dimensional and to increase their self-life, several other drugs are added and preparations made according to different pharmaceutical processes (Dash, 1986). The medicinal plants are listed below:

1. Allium sativum (Lasuna)
2. Commiphora mukul (Guggulu)
3. Paederia foetida (Prasarani)
4. Pluchea lanceolata (Bhuna)
5. Ricinus communis (Kandha)
6. Strychnos nuxvomica (Kupilu)
7. Vitis rotundifolia (Nigundhi)
8. Zingiber officinale (Ardraka, Sunthi)

Dash (1986) mentioned these eight drugs to have a large safety of margin even when consumed in doses higher than the prescribed one for therapeutic purposes. Instead of side (toxic) effects, these drugs produce side benefits. They can as well be used by healthy persons and patients alike. In healthy persons, the herbs prevents the occurrence of the disease and promote positive health. In patients, these plants cure the disease and make body immune to future attacks of this ailment (Dash, 1986). This is not unlikely since the basic principle of Ayurveda is concerned with maintenance of a balanced state of health rather than curing of diseases.

Even now-a-days many Ayurvedic practitioners are using various indigenous plants for treatment of varied types of arthritic conditions. Although, the application of these medicaments has a sound tradition and a rationale background according to the Indian system of medicine, perhaps, it is essential to investigate the rationality of their use in modern scientific terms. The scientific studies to work out the actual efficacy and other
limitations to these drugs would definitely widen the scope of these drugs for further use if they come out to be really effective. This particular problem is specifically important, firstly due to the gravity of the problem of rheumatism and arthritis itself drug and lack of entirely suitable modern drug (of synthetic origin) for the treatment. As because the synthetic drugs provide only symptomatic relief and are not devoid of side effects, target should be to evaluate new drugs from plant kingdom which may provide a therapeutic cure and would be free from undesirable effects as well as cheaper in cost which would be accepted by the people of the developing countries like India. The facts emphasized above have been realized by the Indian investigators, since a review of recent literature shows evidences of efforts made in this direction. A systematic study of anti-inflammatory effect of Indian medicinal plant had been started by Gujral and his associates (1956) and they screened a number of plants for antiarthritic effect. Subsequently various workers from different laboratories in India have made significant contributions in this aspect. Gujral and Vasava (1956), Kanakadhar et al. (1960) and others in the sixties mainly used formaldehyde-induced arthritis and cotton oil-induced granuloma pouch as the experimental models. Later with the introduction of carrageenin oedema, cotton pellet granuloma and adjuvant arthritis models, workers, in different laboratories tested their drugs with the help of the latter models. A comprehensive list of the Indian medicinal plants with anti-
<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthus illicifolius</td>
<td>Agshikar et al. (1979)</td>
</tr>
<tr>
<td>Acacia senegal</td>
<td>Dhawan et al. (1977)</td>
</tr>
<tr>
<td>Allicium sativum</td>
<td>Bhakuni et al. (1969)</td>
</tr>
<tr>
<td>Aloe barbadensis</td>
<td>Bose (1978)</td>
</tr>
<tr>
<td>Alpinia calcarata</td>
<td>Sharma and Singh (1980)</td>
</tr>
<tr>
<td>Anisomeles ovata</td>
<td>Gafur et al. (1978)</td>
</tr>
<tr>
<td>Asparagus racemosus</td>
<td>Singh and Chaturvedi (1966)</td>
</tr>
<tr>
<td>Asparagus chloroanthus</td>
<td>Rastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Balsamodendron mukul</td>
<td>Singh (1978)</td>
</tr>
<tr>
<td>Barleria cristata</td>
<td>Singh (1978)</td>
</tr>
<tr>
<td>Bergenia lissopetale</td>
<td>Gehlot et al. (1976)</td>
</tr>
<tr>
<td>Blumea lacera</td>
<td>Nag et al. (1981)</td>
</tr>
<tr>
<td>Bonninghausenia albiflora</td>
<td>Rastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Boerhavia diffusa</td>
<td>Satyavati et al. (1976)</td>
</tr>
<tr>
<td>Boswellia serrata</td>
<td>Atal et al. (1980); Panchananda et al. (1981)</td>
</tr>
<tr>
<td>Calotropis procera</td>
<td>Singh (1978)</td>
</tr>
<tr>
<td>Cassia fistula</td>
<td>Satyavati et al. (1976)</td>
</tr>
<tr>
<td>Cassia alata</td>
<td>Rastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Chrysanthemum indicum</td>
<td>Lesher et al. (1982)</td>
</tr>
<tr>
<td>Citharexylum suberectum</td>
<td>Dhawan et al. (1977)</td>
</tr>
<tr>
<td>Clerodendron pholadige</td>
<td>Satyavati et al. (1976)</td>
</tr>
<tr>
<td>Name of the plants</td>
<td>References</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Cocculus cordifolius</td>
<td>Singh (1978)</td>
</tr>
<tr>
<td>Cocculus hirsutus</td>
<td>Singh (1978)</td>
</tr>
<tr>
<td>Commiphora molil</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Costus speciosus</td>
<td>Satyavati et al. (1976)</td>
</tr>
<tr>
<td>Cordia dichotoma</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Crataeva religiosa</td>
<td>Singh (1978)</td>
</tr>
<tr>
<td>Cirus deflux</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Cyperus compactus</td>
<td>Dhawan et al. (1980)</td>
</tr>
<tr>
<td>Cythes gigentia</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Citherslyun suberaratum</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Datiesa cannabina</td>
<td>Chandhoke (1979)</td>
</tr>
<tr>
<td>Desmodium laciflorum</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
<tr>
<td>Desmodium sanguine</td>
<td>Satyavati et al. (1976)</td>
</tr>
<tr>
<td>Dioscorea pentaphylica</td>
<td>Dhawan et al. (1980)</td>
</tr>
<tr>
<td>Diospyros cardifolia</td>
<td>Dhawan et al. (1977)</td>
</tr>
<tr>
<td>Euphobria pentacariferum</td>
<td>Raastogi and Dhawan (1982)</td>
</tr>
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<td>Euphobria macrophylla</td>
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<td>Glycyrrhiza glabra</td>
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<td>Garcinia mangostana</td>
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<td>Gopat Krishnan et al.</td>
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<td>Hedychium coronarium</td>
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<td>Hedychium spicatum</td>
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<td>Hibiscus rosasinensis</td>
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<tr>
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<td>Inula helenium</td>
<td>Gujral and Saxena (1956)</td>
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<td>Ipomoea turpethium</td>
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<td>Jambosa laeta</td>
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<td>Lawsonia innermis</td>
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<td>Nikasia cordata</td>
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<td>Mezoneuron cucullatum</td>
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<td>Medicago longifolia</td>
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<td>Moringa pterygosperma</td>
<td>Chatterjee, et al. (1983)</td>
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<td>Myrtus communis</td>
<td>Singh et al. (1978)</td>
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<td>Nyctanthes arboristria</td>
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<td>Olax nana</td>
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<td>Pandorea pinnata</td>
<td>Gujral and Saxena (1956)</td>
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<td>Pimenta deodara</td>
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<td>Pipter longum</td>
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<td>Plecospermum spinosum</td>
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<td>Raphanus sativus</td>
<td>Tiwari et al. (1992)</td>
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<td>Rhus chinensis</td>
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<td>Roylea elegans</td>
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<td>Semecarpus anacardium</td>
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<td>Sida rhombifolia</td>
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<td>Scrophulus brachyptus</td>
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<td>Syzygium cumini</td>
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<td>Thunbergia mysorensis</td>
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<td>Pendse et al. (1977, 1981)</td>
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<td>Trigonella foenum</td>
<td>Khare et al. (1982)</td>
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<td>Withania somnifera</td>
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<td>Vatica roxburghii</td>
<td>Prasad et al. (1968)</td>
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<td>Verbena officinalis</td>
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<td>Vitis negundo</td>
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<td>Xeromphis uliginosa</td>
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<td>Zingiber officinale</td>
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<td>Sharma and Singh (1980)</td>
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Table D: Active principles that have been isolated from the Indian medicinal plants having anti-inflammatory activity.

<table>
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<tr>
<th>Nature of compound</th>
<th>Name of the compound</th>
<th>Plant</th>
<th>Animal models used to detect anti-inflammatory activity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1. Alkaloids</td>
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<td>Berberine</td>
<td>Berberis aristata 2, 3</td>
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<td>Crotalaburnine</td>
<td>Crotalaria lobanifolia 3</td>
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<td>Tylophorine</td>
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<td>Total alkaloids</td>
<td>Allamghan lamarckii 3, 5</td>
<td>3, 5</td>
<td>Praad et al. (1966)</td>
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<td>2. Flavonoids</td>
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<td>Sevachinin</td>
<td>Puerals coryfolia 1, 8, 6</td>
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<td>Anand et al. (1978)</td>
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<td>Goosepin</td>
<td>Hibiscus vitifolius 1, 9, 5, 3, 4, 10</td>
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<td>Parsar and Ghosh (1974)</td>
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<td>Nepitrin</td>
<td>Nepeta hindostana 1, 3, 2, 4</td>
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<td>Agarwal (1980)</td>
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<td>Epicatechin</td>
<td>Anacardium occidentale 2, 4</td>
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<td>Swarnalakshmi et al. (1981)</td>
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<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
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<td></td>
<td>Lancoelarin</td>
<td>Calabaria lanceo-</td>
<td>1,3</td>
<td>Tripathi and</td>
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<td></td>
<td></td>
<td>larifla</td>
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<td>Kaemferol</td>
<td>Rhamnos procumbens</td>
<td>1,5</td>
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<td>Taxifolin</td>
<td>Medicago longifolia</td>
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<td>Gupta &amp; dh (1971)</td>
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<td>3. Xanthone</td>
<td>Mangiferin</td>
<td>Cascaera decussata</td>
<td>1,2</td>
<td>Shankarnarayan &amp;</td>
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<td>Dehydrocyclo-</td>
<td>Callophyllum innophyllum</td>
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<td></td>
<td>o-mandoline</td>
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<td>Jacareubin,</td>
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<td></td>
<td>6-deoxyjac-</td>
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<td></td>
<td>reubin</td>
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<td></td>
<td>Meanaxanthone-B</td>
<td>Messua ferris</td>
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<td>4. Others</td>
<td>Curcumia</td>
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<td>1,2,3,4</td>
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<td>Beta sitosterol</td>
<td>Cyperus rotundus</td>
<td>1,2,8</td>
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<td>Cellosphylline-B</td>
<td>Callophyllum innophyllum</td>
<td>1,3,4</td>
<td>Male &amp; Ak (1980)</td>
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<td>Table (Contd.)</td>
<td>A</td>
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<td>C</td>
<td>D</td>
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<td>Disoibinin</td>
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<td>Pyroxylin</td>
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<td>Oleonolic acid</td>
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<td>binecariferum</td>
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<td>3-3 glucoside</td>
<td></td>
<td>Randia dumetorum</td>
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</table>

* Annual models used for testing anti-inflammatory activity:
1 - Carrageenin-induced oedema; 2 - Cotton pellet granuloma; 3 - Formaldehyde-induced arthritis; 4 - Freund's complete adjuvant-induced arthritis; 5 - Granuloma pouch; 6 - Writhing response; 7 - Antihyaluronidase activity; 8 - Pyrexia; 9 - Mediator-induced oedema; 10 - Turpentine-induced pleurisy.
inflammatory activity have been presented in Table C. It is interesting to note that, although, a considerable number of plants have been studied and these investigations have suggested definite anti-inflammatory activity of several plants but a large number of studies have not been pursued only further. However, recently in some cases, the investigations were extended further and several active principles have been isolated from the crude plant extracts (Table D). Another shortcomings of these investigations is that most of these studies did not report the effect of the plant extract (or of the purified fractions) on the gastrointestinal side effects, which are the most common undesirable effects associated with almost all nonsteroidal anti-inflammatory drugs. Recently a few workers reported the studies on gastrointestinal side effects of the herbal anti-inflammatory agents out of which Rhamnus procurvata (active ingredient kaemferol, a flavone) (Goei, et al., 1988), Azadiricata indica (active ingredient nim bidin; (Pillai and Sathishkumar, 1984) Mikania cordata (Pal, Bhattacharyya and Nag Chaudhuri, 1988) and Pluchea indica (Pal and Nagchoudhuri, 1988) have been reported to possess anti-inflammatory effect simultaneously with anti ulcer activity. It is a matter of pity that except in a few studies, none have pursued further upon the stage of clinical trails. This may be due to the fact that most of the research works done in this field were purely academically oriented and there are no sound industrial support behind those projects. Some of the herbal drugs which have been subjected to clinical trails are Boswellia
serrata (Panchanand et al. 1981); Curcumin (Dhar et al. 1973; Dhawan et al. 1980) Combination of Balsamodendron mukul resin, Alpinia chinensis and Pharma bhasma (Towde, 1980), Alliin from Allium sativum, Lemonol from Dalbergia lanceolaria and active principles from Glycyrrhiza glabra (Satyabati et al. 1976). All of them were reported to improve the clinical symptoms in arthritic condition in man.
MIKANIA CORDATA
Mikania cordata

Mikania is a large genus of scandent herbs or shrubs distributed in topical America, Africa and Asia, belonging to the family Compositae. The only species occurring in India is *M. cordata* (Dum.) B.L. Robinson, Synonym, *M. scandens* Hook. (Fl.Br.Ind.).

The vernacular names are as follows:
- English-climbing hempweed
- Bengali - Taratala
- Sindhi - Gampalu

*M. cordata* is a herbaceous climber found as a weed in West Bengal, Eastern Assam, Aka Hills and also south India.

The herb is a noxious weed occurring in tea gardens, other timber forests and waste lands predominantly in West Bengal and Assam.

The leaves are deltoid ovate, cordate ovate, entire or more or less coarsely crenate or undulate; heads four flowered in compound corymbs; corolla dirty white or white, achenes linear - oblong with reddish pappus.

Stems and leaves are relished by cattle in Malaya when fodder is scarce.

On analysis of green materials of the herb for chemical composition it has been reported (Shastri, 1962) to...
Fig. 5: *Nikania cordata*
contain the following.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>90.9%</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Fat</td>
<td>0.3%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>4.1%</td>
</tr>
<tr>
<td>Fibre</td>
<td>1.1%</td>
</tr>
<tr>
<td>Ash</td>
<td>0.13%</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.08%</td>
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<tr>
<td>Phosphorus</td>
<td>(P₂O₅)</td>
</tr>
</tbody>
</table>

It is a rich source of Vit.A and Vit.C and contains also Vit.B. Mikanin was isolated together with epifriedinol from the root, and together with fumaric acid from the leaves and stems of *M. cordata* (Kiang, et al. 1965). It has also been reported that in addition to Mikanin, epifriedinol and fumaric acid, stigmasterol, friedelin, glucose and fructose were isolated from the roots and mikanolide and dihydromikanolide from leaves and stems of the plant (Kiang et al., 1968).

As far as the medicinal uses are concerned the leaves are used in some parts of Africa as a soup vegetable. They are used in Malaya for itches and in Java and S.Africa, for poulticing wounds (Shastri, 1962). Occurrence of growth inhibitory substance(s) has also been reported (Wong Phei Wong, 1964).
So far the Pharmacological actions are concerned, practically nothing seems to be known except the only available reference obtained which stated that the whole plant excluding the root possesses the gross C.N.S. depressant effect (Bhakuni et al. 1969).