CHAPTER II

Review of Pharmacological Activities
2.1. INFLAMMATION

Inflammation is a reaction of the living cell to irritation or injury. The inflammatory process involves a series of events that can be elicited by numerous stimuli like infectious agents, ischemia, antigen-antibody interactions and thermal or other physical injury. The events that follow the stimulation have been well characterized. The mast cells of the adjacent tissue liberate histamine,\textsuperscript{10,11} which causes, the initial vasodilatation and increased capillary permeability. This is maintained by bradykinin, released from the substrate kininogen by kinin-forming enzymes activated during the early phase of inflammation.\textsuperscript{12} Subsequently, small molecular weight substances such as prostaglandin, further maintain the increased vascular permeability and in combination with kinins are responsible for pain.\textsuperscript{20,21} These early vascular events are responsible for the four cardinal signs namely heat, redness, swelling and pain, described by Celsus.\textsuperscript{15}

Following these early events, there is a wave of migration of polymorph nuclear leucocytes (PMN) from the blood vessels to the inflammed tissue.\textsuperscript{16} It is assumed that their role is to phagocyte the inflammatory stimulus. During this process, which involves the release of hydrolytic enzymes,\textsuperscript{17} it is possible for host tissue to be damaged and perhaps to become antigenic.\textsuperscript{18} Following the early wave of PMN migration there is a later phase of monocyte migration.\textsuperscript{19} Monocytes, during the process of margination, adherence, diapedesis, and chemotactic movement to the site of inflammation, are activated to become macrophages, which are more actively phagocytic than are PMN.\textsuperscript{20} These two cell types, PMN and monocytes in combination with the vascular changes, are able to resolve the acute inflammatory response, which then regresses, and the tissue returns to normal.

The key question is the process by which an acute response is transformed into a chronic event. This presumably involves the interaction of monocytes or other accessory cells\textsuperscript{20} with lymphocytes, the stimulation of the immune system, and the eventual formation of antibody.\textsuperscript{21} The antigen may be persistent bacterial cell wall components, viral particles or damaged host tissue, or possibly a combination of these.\textsuperscript{22} Hence, the chronic inflammation of rheumatoid arthritis is driven by immunological processes, with both genetic and environmental factors playing a role.
Lymphocytes are the cells that are presumed to initiate and perpetuate the chronic inflammatory response in the rheumatoid synovium. A large proportion of leucocytes within the synovium and synovial fluid migrate to this location from the blood. This is dominantly mediated by the expression within local microvasculature of adhesion molecules. These molecules are recognized by passing leucocytes, resulting in adhesion, and migration through the endothelium into the synovium. A large number of different adhesion molecules have been identified which belong to several different classes. Their expression is induced and/or upregulated by cytokines such as tumour necrosis factor alpha (TNFα), interferon gamma (IFNγ), and interleukin-1 (IL-1). Chemotactic factors direct migration of leucocytes, via a chemical ingredient to the precise inflammatory site. These factors are quite diverse and include cytokine products of both immune and connective tissue cells.

Macrophages play a central role in chronic inflammatory process. Their importance derives from the enormous variety of cytokines, enzymes and other bioactive molecules that they produce, and consequently their capacity to greatly amplify the inflammatory response. The synovial fibroblasts greatly increase in number in rheumatoid arthritis. This is due to the local production of fibroblast growth factors, dominantly of macrophage origin. These include platelet-derived growth factor (PDGF), TNFα, and perhaps IL-1. The fibroblast is the major source of several important products in the chronic inflammatory response in rheumatoid arthritis.

To cope with the increased demands generated by an inflammatory response, bone marrow production of leucocytes is also increased usually. The end result of this process is that there is increased production of the leucocytes, which are directed through the homing mechanisms to migrate to the synovium where they participate in the local inflammatory response.
2.1.1 INFLAMMATORY MEDIATORS

2.1.1.1. Cytokines

This is a general name for a class of molecules, which are active in cell-to-cell communication, usually over a short range and in extremely low concentrations. Molecules in this class are categorized as lymphokines, monokines and growth factors. A small and highly selected number of vast array of these important peptides are discussed briefly.

Interleukin-1

IL-1 along with TNFα is one of the most important cytokines involved in directing the components of the inflammatory response and influencing a wide variety of cells. There are two forms of this protein, IL-1α and IL-1β. IL-1 has important effects on prostanoid production. It stimulates the production of prostacyclin (PGI₂) by endothelial cells. IL-1 stimulates phospholipase-A₂ (PLA₂) resulting in enhanced formation of prostaglandin and modulates the formation of other inflammatory mediators such as platelet-activating factor (PAF) and leukotrienes. IL-1 also stimulates the production of collagenase and prostaglandin E (PGE) by synovial cells and the production of C-reactive protein (CRP) by hepatocytes. Finally, it stimulates the proliferation of endothelial cells which are important for the growth of new blood vessels associated with inflammation.

Tumour necrosis factor alpha

Functionally there are remarkable similarities between TNFα and IL-1. Although it may be produced by a number of different sources, the most important is the macrophage. It is an important inducer of adhesion molecules and procoagulants on endothelial cells. It acts as a growth factor for fibroblasts and stimulates the production of colony stimulating factors, matrix proteins and enzymes for these cells.

Interferon gamma

This molecule is produced exclusively by activated lymphocytes and is important in communicating information between lymphocytes and other cells of both immune and
non-immune origin. It enhances lymphocyte-mediated cytotoxic responses and is the most important macrophage-activating factor.

Platelet-derived growth factor, Transforming growth factor beta, Interleukin-4, Interleukin-6 and Colony stimulating factor are examples for other important cytokines. Inhibition of cytokine synthesis is thus a potential point for anti-inflammatory drug intervention.

2.1.1.2. Eicosanoids

Essential fatty acids are incorporated into phospholipids in cell membranes. Under the action of phospholipases, notably phospholipase A₂, arachidonic acid is released. The subsequent action of cyclooxygenase or 5-lipoxygenase produces a cascade of products, the prostaglandins (PGs), leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETES), respectively which are collectively known as the eicosanoids (Fig. IIa). These primarily have a range of proinflammatory actions, although some, notably prostaglandin of the E series, have anti-inflammatory characteristics.

**Prostaglandins**

Cyclooxygenase (COX) converts arachidonic acid to Prostaglandin, which is further metabolised to prostanoids. Two isoforms of COX exist, a constitutive (COX-1) and an inducible (COX-2) enzyme. Constitutive cyclooxygenase is present in cells under physiological conditions, whereas COX-2 is induced by some cytokines, mitogen and endotoxins presumably in pathological conditions, such as inflammation.

The description by Vane (1971) of the inhibition of prostaglandin synthesis by most of the Nonsteroidal antiinflammatory drugs (NSAIDs) stimulated immense effort in this area of research. There was a general correlation between the potency of NSAIDs as inhibitors of prostaglandins and their anti-inflammatory actions. The NSAIDs like aspirin, indomethacin and ibuprofen were more potent inhibitors of COX-1 than COX-2. Recent studies suggest that inhibitors of COX-2 are potent anti-inflammatory agents which do not produce the typical side effects (e.g. gastric ulcers) associated with the nonselective COX-1 directed anti-inflammatory drugs. However, by only inhibiting prostaglandin synthesis with the NSAIDs and leaving the formation of leukotrienes
untouched, more arachidonic acid may be available for metabolism to these potent inflammatory mediators.

Some prostaglandins also have anti-inflammatory properties. PGE₁, PGE₂, and the stable 15-methyl-substituted PGE (misoprostil), have been shown to attenuate inflammation in various models. PGE can inhibit release of the pro-inflammatory leukotriene, LTB₄, by activated neutrophils. PGEs also inhibit leukocyte chemotaxis, adherence to various substrates (including endothelium), phagocytosis and generation of oxygen derived free radicals. So, PGs play an important role in regulating inflammatory reactions as well as both humoral and cellular immune reactions. PGs act as modulators of inflammation rather than as pure mediators.

The contrasting actions of different PGs suggest that the inhibition of the overall synthesis of PGs may not always be as beneficial as was thought initially. Clearly, a balance is required between the pro- and the anti-inflammatory products of the PG cascade and a more specific antagonism of key parts of the cascade might be optimal.

**Leukotrienes**

Leukotrienes are formed by the action of 5-lipoxygenase or 12-lipoxygenase on arachidonic acid released from membranous phospholipids by the action of PLA₂. LTB₄ was the first leukotriene to be characterized. Its actions have been best documented and suggest a key role in the inflammatory process. Evidence has been provided showing the enhanced production of 5-LO products such as LTB₄ and 5-HETE in psoriasis, asthma, arthritis and allergy. LTB₄ is a potent chemoattractant for both neutrophils and eosinophils. It activates neutrophils by inducing aggregation, calcium mobilization, superoxide release and up-regulation of certain adhesion molecules. LTB₄ also causes the release of toxic oxygen metabolites by eosinophils. Additionally, LTB₄, along with LTD₄ stimulates production of the potent inflammatory mediator, IL-1 by human monocytes after activation by a variety of cell membrane stimulants. Lipoxygenases, like other enzymes involved in the eicosanoid cascade, are selectively distributed in different cell types, thus increasing the diversity of eicosanoid-stimulated response.
Leukotrienes are believed to play a significant role in the amplification of the inflammatory response in inflammatory bowel disease. \(^\text{49}\) Lipoxygenase products may also play an important role as second messengers modulating neurotransmitter release by regulating K\(^+\) ion channels and protein kinases. \(^\text{50}\) This may be of relevance to neurogenic inflammation.

Regulation of 5-LO pathway has become an important target for therapeutic intervention. Inhibition of the lipoxygenase can be elicited by direct binding to the enzyme, by preventing the translocation of the enzyme from the cytosol to the membrane, or by interfering with the binding of the lipoxygenase to a binding protein in the membrane. This 5-lipoxygenase activating protein must be present for intact cellular leukotriene biosynthesis. \(^\text{51}\)

The 5-LO pathway in PMNs has been reported to be inhibited by NSAIDs like indomethacin, ibuprofen and aspirin. But the data also suggest that NSAIDs do not affect the lipoxygenase pathway \textit{in vivo} at usual doses. Pharmacological intervention at the conversion of arachidonic acid to LTA\(_4\) and LTD\(_4\) by the development of 5-LO inhibitors is clinically viable approach and has shown efficacy in clinical asthma. \(^\text{52}\) Recently, development of LTB\(_4\) receptor antagonists as general antiinflammatory agents has received significant attention. Compounds that are dual inhibitors of both cyclooxygenase and 5-lipoxygenase are being studied as potential antiinflammatory agents with an improved safety profile in comparison to classical NSAIDs. \(^\text{53}\)

\subsection*{2.1.1.3. Complement}

The activation of complement by immune complexes results in the production of a wide variety of biologically active breakdown products. Of particular importance in inflammation are C\(_{3a}\) and C\(_{5a}\), which act as chemoattractants and neutrophil activators. \(^\text{54}\) Additionally, the complement product C\(_{3b}\) is deposited on membranes, which promotes attachment of phagocytes and phagocytosis. \(^\text{55}\)
**Esterified Fatty Acids**
**cell Membrane**

**PLA**
**Arachidonic acid**

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**Cyclooxygenase**

- Prostacyclin-6-keto PGF1α (PGI₂)
- Thromboxanes
  - TXA₂− TXB₂-2, 3-dinor TXB₂
- Prostaglandins
  - PGE₂
  - PGD₂
  - PGF₂α

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**Lipoxygenase**

- Products (12-lipoxygenase)
  - 12-HPETE
  - 12-HETE
- Products (5-lipoxygenase)
  - 5-HPETE- LTA₄ –LTB₄
  - LTC₄, D₄, E₄, F₄

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**PLA**: Phospholipase

**HPETE**: Hydroperoxyeicostetraenoic acid

**HETE**: Hydroxyeicosatetraenoic acid

**FIG:- II** a Eicosanoid biosynthetic pathway
2.1.1.4. Degradative enzymes

A considerable number of different enzymes are produced within the rheumatoid synovium. Their role is to participate in the activation of different inflammatory pathways and degrade connective tissue matrix. The mechanical damage to joint and cartilage is to a large extent mediated by proteases, which include collagenase, elastase and plasminogen activator released under the influence of cytokines such as IL-1. Inhibition of proteases is thus another potential point for anti-inflammatory drug intervention.

PLA₂ that mediates the release of arachidonic acid from cellular phospholipids is elevated in synovial fluid. Consequently this enzyme is a target for anti-inflammatory drug action. However, the common NSAIDs does not inhibit it and although it is inhibited by the corticosteroids, their adverse systemic effects limit the chronic use of these drugs.

2.1.1.5. Reactive oxygen species.

There has been a great interest and focus on the role of free radicals, and other reactive species usually derived from oxygen products in the inflammatory process. Formation of these highly reactive oxygen species (ROS) is integral to the phagocytic action of PMNs. Stimulation of the cell by chemoattractants such as LTB₄, or products of the complement cascade such as complement C₅a, results in activation of the NADP/NADPH oxidase system and a cascade which generates ROS products. These include superoxide radical and hydrogen peroxide, and possibly the very reactive hydroxyl radical.

The oxygen free radical, superoxide, O₂⁻ has been identified as a proinflammatory reactive oxygen species. Cytokines such as IL-1 and INF enhance the production of O₂⁻ by endothelial cells. This process causes connective tissue damage, which precedes the proliferation of endothelial cells. An interesting concept has been that oxygen radicals may act on self-antigens, e.g. IgG, rendering them autoantigenic and thus driving an autoimmune response.
The significance of superoxide generation in the therapy of inflammation can be seen by the fact that administration of superoxide dismutase (SOD) or metal complexes having SOD-like activity act as powerful inhibitors of acute and chronic inflammatory conditions. It has been shown in numerous in vitro studies that NSAIDs decrease the release of superoxide (ibuprofen, piroxicam, tenoxicam, indomethacin, salicylic acid, acetylsalicylic acid) and inhibit the chlorinating activity of myeloperoxidase. It has been suggested that copper complexes of NSAIDs may form in vivo, and these may be the bio effective forms of these drugs. Despite the suggestion that NSAIDs inhibit the release of superoxide by stimulated PMNs, data also suggest that such action may be due to direct scavenging of free radicals by the NSAIDs.

Hydrogen peroxide has also been implicated in various steps in the inflammatory process and in the action of some antirheumatic drugs. Data also suggest that hydrogen peroxide can activate endothelium and has a modulatory role in the respiratory burst of neutrophils by altering the response of the neutrophils to surface stimulants. The anti-inflammatory effects of antioxidants and free radical scavengers could be due to their ability to mop up these high-energy compounds and radicals.

**Pain in Inflammation**

Pain is one of the cardinal symptoms of inflammation. It is produced by histamine, 5-hydroxytryptamine, bradykinin and various inflammatory exudates. Prostaglandins do not produce pain in small doses. However, they can potentiate pain impulse generation induced by bradykinin and histamine. This led to the hypothesis that PGs are able to sensitise pain receptors to the effects of other agonists. The analgesic effects of NSAIDs can be explained by their inhibiting action on prostaglandin synthesis.

**Fever in Inflammation**

Fever may be produced by many inflammatory reactions such as tissue damage, malignancy, antigen-antibody reaction and tissue graft rejection. The formation of cytokines such as IL-1 or tumour necrosis factor by neutrophils and other cells is enhanced which induces the synthesis of PGE2. Prostaglandins (PGE2) have been
implicated as responsible for fever. NSAIDs bring down the elevated body temperature, which appears to be mediated by cyclic AMP by inhibiting the synthesis of PGE₂.

2.1.2. CLASSIFICATION OF ANTIINFLAMMATORY DRUGS

The drugs, which are generally considered to be anti-inflammatory, belong to the general categories of steroidal and nonsteroidal agents.

Steroidal Antiinflammatory drugs

The glucocorticosteroids suppress the symptoms of inflammation and give relief from debilitating conditions especially in diseases like rheumatoid arthritis. Steroids include hydrocortisone, cortisone, corticosterone, prednisolone, fludrocortisone, betamethasone, dexamethasone etc. They prevent the formation of prostaglandins and leukotrienes. Unlike the NSAIDs, the compounds prevent the liberation of arachidonic acid by inhibiting the enzyme PLA₂. The synthesis of another putative factor of inflammation, namely, platelet activating factor (PAF), could also be reduced by inhibition of PLA₂. The steroids offer every effective treatment of several inflammatory diseases. Unfortunately, these drugs are not without harmful effects. Continued intake of these drugs leads to a number of unwanted effects. The most frequent undesirable consequences of prolonged glucocorticoid therapy are suppression of ACTH secretion, antagonism of most insulin actions, protein wasting and involution of lymphoid tissues. Muscle weakness and atrophy can also occur. Treatment with glucocorticoids carries an increased risk of infection. Abrupt withdrawal of glucocorticoid therapy may result in manifestation of adrenal insufficiency due to suppression of ACTH section. Many of these side effects are quite serious warranting their discontinuance.

Nonsteroidal Antiinflammatory drugs

The nonsteroidal anti-inflammatory drugs have attained medical importance because of their ability to mitigate the symptoms of inflammation, particularly pain and swelling. In terms of the management of rheumatoid arthritis, it is generally held view that NSAIDs help control the inflammation, provide analgesia and positively influence the quality of life. NSAIDs, however do little to inhibit the rheumatic disease, and
further that, used alone they will be inadequate. NSAIDs may thus be defined as the
drugs which ameliorate the symptoms of inflammation but in doing so have little effect
on the course of the disease. They are characterized by having a rapid and reversible
effect on inflammation. Most NSAIDs are analgesic, antipyretic and antithrombotic
besides being anti-inflammatory.

The mechanisms by which NSAIDs act may be multifactorial but they primarily
act via inhibition of prostanoid production. With the recognition of the role of other
mediators of inflammation such as leukotrienes, interleukins and reactive oxygen species,
there is still debate on the significant actions of NSAIDs, which are not mediated by
inhibition of prostanoid synthesis. An important part of the actions of these drugs is their
analgesic action. Inhibition of prostaglandin synthesis will have both anti-inflammatory
and analgesic consequences. Interestingly, some NSAIDs may block pain independently
of their action on prostaglandin synthesis.

Disease-modifying antirheumatic drugs (DMARDs) such as D-penicillamine,
gold compounds, antimalarials, chloroquine and hydroxychloroquine, methotrexate and
sulphasalazine have the potential to alter the course of the disease. They are slow acting.
They do not have analgesic actions although pain should be ameliorated as the process of
chronic inflammation is inhibited. The division between NSAIDs and DMARDs is not
absolutely clear in terms of the biochemical response markers of inflammation. Clearly a
DMARD must also be antiinflammatory, and antiinflammatory drugs have not been
absolutely demonstrated to have no effect on the course of the disease. DMARDs are
also associated with potentially serious adverse effects, and there is often a high rate of
withdrawal in patients taking these agents.

2.1.3. EVALUATION OF NSAIDs

The in vivo methods

A number of animal models are employed for the evaluation of NSAIDs for both
acute and chronic inflammation. The appropriate use of animal models for the study of
NSAIDs requires detailed examination of the models from several perspectives. Use of
standard drugs is particularly important in evaluating both mechanisms and methodology. Among the commonly used methods the carrageenan induced rat paw edema model and adjuvant induced arthritis model are being dealt with in some detail.

**Models for Acute Inflammation**

I. Carrageenan Paw Edema Assay

Since swelling is one of the cardinal symptoms of inflammation, edema tests are among the most prominent models used to assess the efficacy of drugs for treating inflammatory diseases such as arthritis.

Many irritants were used earlier to produce edema in rat hind paw. Irritants such as egg white, kaolin, dextran, formalin and yeast were once commonly used agents. However, these irritants were found to be unsuitable for the proper assessment of antiinflammatory activity, where the edema would be inhibited by NSAIDs in a dose-dependent manner.

Carrageenan, a sulphated mucopolysaccharide derived from Irish sea mass, chondrus, was first used by Winter *et al.* (1962) as a phlogistic agent. The irritant is devoid of the drawbacks of the others. It produces reproducible edema, which can be inhibited by therapeutic doses of NSAIDs in a dose-responsive manner. The carrageenan edema model has a fairly high specificity. The activity and potency characteristic of most NSAIDs roughly parallel to those observed in man, and this is one reason for which the test is most widely used, although some agents without known antiarthritic activity in man may inhibit carrageenan-induced edema. These and other similar false-positive agents can be excluded by further testing in other assay systems for arthritis.

The test also works with mice, but not with guinea pigs, and rats are generally the animals of choice. Stress of any kind should be minimised in the rats, since adrenal output in response to stress inhibits the swelling. The time of the day for the assay should be standardized, because rats, like man have a circadian rhythm of adrenal output. The degree of foot swelling depends significantly on the carrageenan preparation, and in particular on the amount of carrageenan in the preparation.
The time course of edema development is generally represented by a biphasic curve. The first phase occurs within an hour of injection and is partly due to trauma of injection and also due to serotonin component. However, some investigators showed that certain classical serotonin antagonists have failed to show any suppressant effect. Later studies also suggested the release of histamine and kinins. Prostaglandins play a major role in the development of the second phase of reaction, which is measured around 3-4 hours time. The presence of prostaglandin E2 (PGE2) in the inflammatory exudates from the injected foot can be demonstrated at three hours time period and thereafter. Hence, the carrageenan edema can be inhibited by inhibition of the prostaglandin amplification mechanism.

Over the years a number of drugs have been screened for the edema inhibitory effect. There has been a good correlation between edema inhibitory effect and clinical anti-inflammatory activity. This model has given consistent dose-response curves for the major anti-inflammatory drugs, aspirin, phenylbutazone, and indomethacin. The data obtained through extensive screening by this method suggest that carrageenan edema test closely predicts the human potency for NSAIDs and thus provides a very good method for the determination of structure activity relationships (S.A.R.).

2. Ultraviolet light-induced Erythema test

One of the major consequences of inflammation is increased blood flow through the inflamed tissue causing local redness (erythema) and an increase in skin temperature (local hyperthermia).

For UV erythema test, the animal of choice is an albino guinea pig. The hair in the abdominal area are removed and the animals are dosed an hour prior to the exposure to ultraviolet light. A quartz lamp with a high-pressure mercury burner is used with a heat filter to avoid non-specific burning. The irradiation is done for one minute at a distance of 15 cm from the light source and the degree of erythema is scored after two hours. The scoring of erythema is done by Winder's method as follows:

A lack of erythema is scored as 0, a full circle as 1 unit and a partial circle as 0.5 units. With this system the maximum score can be 3 units. However, the correlation with
human clinical activity is poorer than that with the carrageenan model. Poor correlation with human clinical activity, necessity of a large number of animals and the cumbersome nature of the test gives it a secondary role, i.e. as a confirmatory test.

3. Models involving pain as the parameter

Many methods are available for evaluation of drugs for analgesic effect. In all these methods, one or other type of stimulus is applied to produce a pain reaction. The methods can be classified based on the type of stimulus used.

i. Thermal stimulus
   a. Hot plate test
   b. Radiant heat methods using analgesiometer or an electric bulb as the source of heat.

ii. Mechanical stimulus
   a. Tail clip
   b. Randall Selitto assay

iii. Chemical stimulus

    Writhing by acetic acid or phenylquinone

iv. Electrical stimulus
   a. Pododolorimeter
   b. Rectodolorimeter

Of these methods available, the Randall Selitto test and the chemical induced writhing test are used for the evaluation of the analgesic activities of peripherally acting drugs. The other methods enumerated are mainly meant to evaluate the drugs that act by central mechanisms. However, the tail clip, tail flick and hot plate methods could be employed for screening non-narcotic drugs.
Randall Selitto Assay

The test involves the injection of brewer's yeast (0.1 ml of 20% suspension) at time zero to develop inflammation, administration of NSAIDs subcutaneously and measuring the pressure sensitivity of the inflamed paw to elicit escape response at 1, 2 and 4 hr time period. The major drawback of this test for NSAIDs is that it is not possible to distinguish between the phenomenon of hyperalgia and edema.

The Writhing Test

This technique and its modification were first developed by Siegmund et al. Writhing is defined as intermittent contractions of the abdomen, twisting and turning of the trunk and extension of the hind limbs. Any writhing is considered as a positive analgesic response. The writhing or stretching phenomenon can be induced by treatment with phenylbenzoquinone, acetic acid, acetyl choline or 4% NaCl. In this study, acetic acid induced writhing as described by Kostel et al. was used with a slight modification. The method is sensitive, simple and reproducible for screening of weak analgesics. The main disadvantage however, is the lack of specificity. Tranquilizers and antihistaminic show activity by this test.

4. Exudative Models of Inflammation

Animal models of acute inflammation such as the carrageenan foot edema test and the UV light-induced erythema test monitor only gross changes such as edema or erythema. In contrast, the subcutaneous implantation of sponges or the intrapleural injection of carrageenan in rodents provides inflammatory exudates that are easily sampled. It thus permits the numbers of infiltrating cells and their functional capacity. The mediators of inflammatory response can also be identified and measured in the exudate fluid.

Chronic Arthritis Models

Adjuvant-induced Arthritis

This is the most widely used chronic model for studying the antiinflammatory and immunosuppressant properties of drugs. The model has many similarities, but does not
possess all the features of human rheumatoid arthritis. The disease is induced by an injection of *Mycobacterium tuberculosis* or *Mycobacterium butyricum*, in mineral oil as an emulsion, either in the hind foot pad or at the base tail of rats. The active moiety responsible for the induction of the disease is the peptidoglycans of the bacterial cell wall.

The disease is restricted only in rats. The attempts to produce this disease in other species, such as rabbit, hamster, guinea pig, chick, dog etc. have not been successful. After immunization, the acute inflammatory response (Primary response) becomes evident in the injected hind paw within 24 hours.

The paw swelling increases for about 4 days and plateaus thereafter until day 12. The secondary or immune mediated response starts at around day 12. As a result, the uninjected paw exhibits swelling which continues to increase and peaks at about day 18 or 21 post immunization. The injected hind paw shows the chronic phase of the biphasic response and further increases in volume, which also plateaus at days 18-21. The swelling of both the paws may subside to some extent after 35-40 days post immunization, but permanent point deformity persists.

The injection of mycobacteria elicits a classical immune response involving antigen-presenting cells, T-cells and B-cells of the immune cascade. A prior intramuscular or intravenous injection of mycobacteria induces tolerance to the development of arthritis. It is believed that cell mediated hypersensitivity primarily accounts for the disease, although the importance of antibody component cannot be completely ruled out. A possibility of the involvement of a second antigen response and the chronicity of the disease being the result of autoimmunity to this second antigen exists. Among various proteins serving as the second antigen, the major protein of cartilage i.e. type II collagen stands out as a reasonable candidate, as cartilage is the primary target of destruction in an arthritic joint.

Many steroidal and nonsteroidal anti-inflammatory agents can inhibit the adjuvant arthritis in rat and the effect correlates with the clinical effects in man. Classical immunosuppressive drugs also inhibit adjuvant arthritis. However, the antirheumatic
drugs, such as levamisol, D-penicillamine etc. that are successfully used to treat human rheumatoid arthritis enhance the severity of rat adjuvant arthritis.\textsuperscript{106}

Most of the anti-inflammatory and immunosuppressant drugs suppress the development of arthritis when given daily throughout the disease process. Generally, when NSAIDs are administered after the first 14 days when chronic inflammatory response is already established, they reduce the swelling to some extent, but do not have considerable effect on joint deformities.

In general, it can be said that adjuvant arthritis is a model suitable to detect cyclooxygenase inhibitors. Thus far, the compounds with high lipoxygenase inhibiting activity with little or no cyclooxygenase inhibiting property have not been successfully detected in this test.

Other animal models of rheumatoid arthritis are collagen-induced arthritis,\textsuperscript{107} Granuloma Pouch\textsuperscript{108} and cotton pellet granuloma\textsuperscript{109} methods.

**Acute Immune Complex-Induced Inflammation**

There is a component of immune complex-induced inflammation in most autoimmune diseases. The Arthus reaction involves immune complex generated inflammation and could therefore be appropriate model for the discovery of novel anti-inflammatory drugs.

There are several variants of the Arthus reaction. The passive Arthus reaction has the advantage that a large batch of antibody can be prepared and a measured amount of antibody injected to elicit a reproducible reaction. The reaction can be carried out by injecting antigen intravenously and then antibody intradermally, that is, the reversed passive Arthus (RPA) reaction.\textsuperscript{110} Alternatively the antibody is injected intravenously and then antigen intradermally that is, the direct passive Arthus reaction. The RPA reaction in the rat provides a more practical and widely used alternative for drug testing.

**In vitro Models for Inflammation**

Many *in vitro* assays involving specific biochemical or cellular mechanism have been developed for the screening of anti-inflammatory compounds. In recent years,
biochemical approaches based on the regulation of the arachidonic acid cascade and
leucocyte function have received greater attention. These assays in conjunction with
animal models provide very useful information about the mechanism of action of
antiinflammatory drugs.

The *in vitro* assays commonly employed are as follows:

1. **Prostaglandin and Leukotriene Biosynthesis**

   The major pathway of arachidonic acid metabolism that is inhibited by most of
the NSAIDs is the cyclooxygenase pathway, which leads to the production of
prostaglandins. Many inhibitors of lipoxygenase pathway i.e. leukotriene inhibitors and
inhibitors of phospholipases, which block both cyclooxygenase and lipoxygenase
enzymes are identified. To test various drugs for their inhibitory properties, they are
added at various concentrations to the isolated enzymes, or to the activated mouse
macrophages or rat neutrophils and their release of enzymes is studied. The activities
and IC₅₀ levels of most of the NSAIDs have been established based on assays.

Other *in vitro* models are

2. *In vitro* chemotaxis of neutrophils or macrophages.

3. Assay for production of Interleukins

2.1.4. **CHEMISTRY OF NSAIDs**

   Generally these are weakly acidic compounds. Their acidic nature is of some
importance because the relatively acidic environment of inflamed tissue, and also of the
gastric mucosa and renal medulla, predisposes them to being concentrated at these sites.
They are highly protein bound such that only a very small proportion of the drug is
believed to be available for diffusion and equilibration across membrane barriers.
Another chemical characteristic of these drugs is that many are asymmetric. They are
often administered as racemates. This is of interest for two reasons. Firstly, their action
on prostaglandin synthetase is highly stereoselective, favouring the S-configuration. Secondly, while R-enantiomers are intrinsically inactive in this respect, some of them are inverted \textit{in vivo} to yield their active S-counter parts.\textsuperscript{113,114}

The commonly used NSAIDs are

\textbf{Salicylates:} Aspirin, Sodium salicylate, Diflunisal

\textbf{2-Arylacetic acids:} Indomethacin, Sulindac, Tolmetin, Dichlofenac

\textbf{2-Arylpropionic acids:} Ibuprofen, Flurbiprofen, Ketoprofen, Naproxen

\textbf{Fenamates:} Mefenamic acid, Meclofenamic acid

\textbf{Enolic Acids:} Phenylbutazone, azapropazone

\textbf{Oxicams:} Piroxicam

\textbf{Salicylates}

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\textbf{1a}
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Sodium salicylate (1a) is a weak, reversible inhibitor of cyclooxygenase and hence prostaglandin concentrations, notably \textit{PGE}_{2}, in inflammatory exudates.\textsuperscript{115}

\begin{center}
\textbf{1b}
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Aspirin (Ib) is a prodrug of Salicylic acid. Aspirin acetylates cyclooxygenase leading to irreversible inhibition of its action.116 The clinical use of aspirin is accompanied by many undesirable side effects, which include dyspepsia, gastrointestinal bleeding, tinnitus, and rashes.

\[ \text{COOH} \]
\[ \text{F} \quad \text{OH} \]
\[ \text{F} \]

Ic

A thorough investigation of the 5-phenylsalicylic acids showed that the most active analogs were the ones in which the 5-phenyl ring was substituted with either chlorine or fluorine. Diflunisal (Ic) is a difluorophenyl derivative of salicylic acid. It is more potent than aspirin with less gastric irritation and has a prolonged duration of analgesic activity.119

It was found that some 4- and 5-heteroarylsalicylic acids possessed better anti-inflammatory activity and caused less gastric irritation in animals.120,121 One of the most soluble acids, 5-(N-pyrrol) salicylic acid was the most potent member of the series. Presumably, solubility of organic compound in the almost completely aqueous environment of the gastrointestinal tract is essential for good absorption. An indole salicylic acid derivative, Fendosal is superior to aspirin in its anti-inflammatory and analgesic properties, and its gastric irritation properties are very low in animal studies.122
Arylacetic acids

Indomethacin (IIa) is among the earliest nonsalicylate compounds to be discovered and developed for use as an antiinflammatory agent. This compound was the result of a systematic study to examine indole and indene derivatives as potential antiinflammatory agents. It is a potent inhibitor of cyclooxygenase. Like many other aspirin like drugs, indomethacin produces severe irritation.

Sulindac (IIb), an indene derivative is closely related to indomethacin. Sulindac may be considered electronically isosteric with the N-acyl indole structure of indomethacin. It is a prodrug that is almost devoid of intrinsic pharmacological activity but is metabolised into the sulfide,\textsuperscript{123} which is the pharmacophore. Sulindac is less than half as potent as indomethacin and causes less gastric irritation.

In the structure-activity relationships in this series it was observed that the substitution pattern for optimum activity is similar for the N-benzoyl, N-benzyl and benzylidene series, which suggests a common site of action for these. Shen\textsuperscript{124} proposed an
active site for indomethacin analogs that involved a cationic center and two nonplanar hydrophobic regions.

\[
\begin{align*}
\text{IIc} \\
\begin{array}{c}
\text{CH}_3 \\
\text{C} \\
\text{CH}_3 \\
\text{CH}_2\text{COOH}
\end{array}
\end{align*}
\]

Tolmetin (IIc): Replacement of the indole ring system of indomethacin by a pyrrole ring produces the pyrrole acetic acid, Tolmetin. It is an effective anti-inflammatory agent that also exerts antipyretic and analgesic effects.

\[
\begin{align*}
\text{II d} \\
\begin{array}{c}
\text{HO-} \\
\text{CH}_2
\end{array}
\end{align*}
\]

Diclofenac (IIId): It is a member of a series of anilinophenylacetic acids that was made to test a hypothesis that two structural features are essential for good antiinflammatory activity; an acidic function giving a pH of 6 and two aromatic nuclei whose substitution inhibits coplanarity.\(^{125}\) Diclofenac possesses pronounced anti-inflammatory, analgesic and antipyretic properties and is a potent competitive and reversible inhibitor of prostaglandin synthesis.\(^{126}\)

2-Arylpropionic Acids

The 2-arylpropionates (2-APAs) have been the backbone of anti-inflammatory drug treatment for the arthropathies since the prototype, ibuprofen was first marketed in the 1970s. There are three key structural components required for anti-inflammatory
activity of this group of drugs. They require the carboxylic acid function, an aromatic hydrophobic region and most importantly, the stereochemistry at the α-carbon must be of the S-configuration. The anti-inflammatory action of 2-APAs has been primarily attributed to the reversible inhibition of cyclooxygenase and their activity in this respect generally parallels their in vivo inflammatory activity. These drugs produce gastrointestinal side effects, although less severe than with aspirin.

![Chemical structure of ibuprofen](image)

Ibuprofen: There are data suggesting that ibuprofen (IIIa) inhibits the 5-lipoxygenase pathway of neutrophils and PMN leukocytes, leading to reduction in the chemo attractant LTB₄. No difference in biological-activity was detected between the two optically active enantiomers of ibuprofen. On administration of the racemic ibuprofen, conversion of the levorotatory isomer to the dextrorotatory isomer takes place, and it is the activity of latter isomer that is observed in vivo. Ibuprofen is a well tolerated analgesic agent.

![Chemical structure of flurbiprofen](image)

Flurbiprofen (IIIb) is structurally related to ibuprofen. It is ten times as potent as ibuprofen. It is one of the most potent inhibitors of PG synthesis.
Ketoprofen: Addition of 3-benzoyl functions to phenylpropionic acid produces ketoprofen (IIIc). It is as active as indomethacin in several animal models of inflammation.

Naproxen (IIIc) is a naphthylpropionic acid derivative. The d-isomer of naproxen possesses good antiinflammatory, analgesic and antipyretic properties than the enantiomeric 1-isomer.\textsuperscript{133}

Fenamates

\[ \text{IV} \]
Fenamates are a family of N-arylanthranilic acids, which include mefenamic acid (IVa), and meclofenamic acid (IVb). These compounds possess significant anti-inflammatory, analgesic and antipyretic properties. Structure-activity relations in this series showed that the position of the carboxyl group relative to the second phenyl ring is critical since the m- and p-carboxy compounds were inactive. Substitution in the carboxyl-bearing ring, generally reduced the activity, substituents in the other aromatic ring enhance the activity, and the m- trifluoromethylphenyl compounds were among the most active monosubstituted compounds in this series. N- alkylation or N-acylation of the active anthranilic acids either markedly reduce or abolish antiinflammatory activity.

Enolic Acids

Phenylbutazone (Va) was discovered in 1940s and was the first acidic, non-carboxylic acid utilized for the treatment of inflammatory diseases. Oxyphenbutazone, one of the metabolites of phenylbutazone is also active. It was realized at an early date that an acidic proton is required in the pyrazolidinedione ring to preserve antiinflammatory activity. A large number of analogs of phenylbutazone have been made in an attempt to increase activity and reduce toxicity, but only a few have reached the market place.
Condensation of pyrazolidine ring with benzotriazines gave azapropazone (Vb) and related analogs. Azapropazone is effective in the treatment of chronic arthritic disorders in patients and possesses a low incidence of side effects.\textsuperscript{136}

\textbf{Oxicams}

Piroxicam (VIa) is a member of a novel class of enolic acids. It is the most potent nonsteroidal drug available for the treatment of human inflammatory diseases. A single daily dose of 20 mg is normally sufficient to maintain adequate therapeutic plasma levels of the drug. This compound stems from a study of the anti-inflammatory properties of a series of N-aryl and N-alkylcarboxamide derivatives of 2-substituted-4-hydroxy-2H-1,2-benzothiazine-3-carboxylic acid-1,1-dioxides.

The best substituent in the 2-position was methyl, and the N-arylamides produced more active compounds than the N-alkyl amides. The N-heteroarylcarboxamides were also made, and these generally possessed more potent anti-inflammatory activity than the
best N-arylcarboxamides. Sudoxicam a close relative of piroxicam, and another analog isoxicam were also reported to be effective in rheumatoid arthritis.

In addition to the above classes of compounds, large numbers of other classes of compounds have also been reported to possess anti-inflammatory activity. Several combinations of three aryl groups, such as two phenyl and a five membered heterocycle, in a fused or angular stereochemical configuration have been found to possess anti-inflammatory activities. Further examination of this type of heteroaryls led to the synthesis of 2-substituted phenyl oxazolopyridines. Several members in this series are comparable to indomethacin. The antiinflammatory activity of quinazolinones was observed early. The nicotinylindole, nictindole exhibits antiinflammatory activity comparable to phenylbutazone.

2.1.5. TOXICITIES OF NSAIDs

Toxicity is dose limiting for the NSAIDs. The most serious adverse effects, gastrointestinal and renal, are intimately associated with the primary mechanism of action of these drugs, viz. inhibition of prostaglandin synthesis. Although there are no clear differences in efficacy between the various NSAIDs, there is some evidence for differences in tolerance between individual NSAIDs. The newer NSAIDs are less directly toxic on the gastric mucosa than the older drugs aspirin, indomethacin and phenylbutazone.

The chronic use of NSAIDs carries with it an increased risk of gastrointestinal disturbances, notably dyspepsia and much less commonly complications of peptic ulceration. This appears to be correlated with inhibition of the biosynthesis of gastric prostaglandins, especially PGI₂ and PGE₂. These eicosanoids inhibit acid secretion by the stomach and promote the secretion or cytoprotective mucus in the intestine. Inhibition of their synthesis may render the stomach more susceptible to damage. The mechanism of the dyspepsia is unknown and does not correlate with gastrointestinal damage.
The next most important adverse effect of NSAIDs is renal toxicity. This appears to be related to the inhibition of prostaglandins which regulate renal blood flow and glomerular filtration\(^{142}\) and which also modulate tubular transport of ions and water.\(^{143}\) Decrease in glomerular filtration rate which is generally reversible, is probably very common. This type of renal toxicity is most likely in elderly patients and those with other cases of compromised renal function, e.g. low cardiac output, or intrinsic renal disease.

The NSAIDs are relatively non-polar and well able to partition across membranes. The central adverse effects account for up to 10% of all adverse events, although these are generally mild. They include visual disturbance, tinnitus, headache, depression and depersonalisation.\(^{144}\)

### 2.2 PHARMACOLOGICAL ACTIVITIES OF COPPER COMPLEXES.

#### 2.2.1. INTRODUCTION.

Copper is an essential metalloelement. Essential metalloelements, just as essential amino acids, fatty acids, and co-factors (Vitamins), are required by all cells for normal metabolic processes but cannot be synthesized \textit{in vivo} and dietary intake and absorption are required to obtain them.

Amounts of this essential metalloelement found in body tissue\(^{145,146}\) and fluids correlate with the number and kind of metabolic processes requiring them. All measurable amounts of this metalloelement exist in tissues and biological systems as complexes.

#### 2.2.2. INGESTION, ABSORPTION, DISTRIBUTION, UTILIZATION AND EXCRETION OF COPPER COMPLEXES.

Ingested foods and beverages contain Cu complexes which, following digestion, can give rise to other complexes as a result of ligand exchange in the digest or form other binary or ternary complexes. Some of the originally ingested complexes may survive as
binary or ternary complexes and undergo absorption at pH values found in the stomach.\textsuperscript{147,148}

Copper complexes found in the digest or instilled into an empty stomach may also undergo ligand exchange or ternary complex formation with ligands found in blood following absorption.\textsuperscript{149} However, some of the absorbed complexes may exist in blood as the original complexes or as ternary complexes. Absorbed complexes undergo systemic circulation to all tissues and the copper is utilized by cells and tissues following ligand exchange with apoproteins and apoenzymes to form Cu-dependent proteins and enzymes, stored in the appropriate tissue as Cu-thionein, or excreted in the event tissue needs have been met and stores replenished. These have been summarised in Fig.II b.

Stored copper is released via ligand exchange to meet metabolic needs. This homeostatic release of relatively small amounts of Cu complexes meets normal physiological requirements. Release of large quantities and pronounced mobilization of copper is a feature of acute and chronic responses to many disease states. Acute-phase response\textsuperscript{150} to many disease involves a release of copper-thionein-stored copper from the liver as ceruloplasmin, copper amino-acid complex and a copper albumin complex to meet increased metabolic needs for copper. This exceeds normal needs, and plasma copper concentration increases 200-300\%, above normal. The appropriate increase coupled with an ability to sustain this response may lead to spontaneous or facilitated therapy-induced remission of disease within a relatively short period. However, when liver stores are inadequate and this copper-dependent response cannot be maintained, remission cannot occur, and the disease persists as chronic disease.
CuL₂ (in food and beverages)

Ingestion and digestion ($L^a$, $L^b$)

CuL₂ + CuL$^a_2$ + CuL$^b_2$ etc.

Absorption

($L^c$, $L^d$, albumin, transcuprein)

CuL₂ + CuL$^a_2$ + CuL$^b_2$ + CuL$^d_2$ + Cu – Transcuprein, Cu-Albumin, etc.

Systemic circulation

Liver storage as Cu-thionein (CuL$^a_2$ + metallothionein)

or apoprotein

Activated enzyme or metalloprotein

Blood serum or Plasma

Ceruloplasmin

Cu-amino acid complexes

Cu-albumin complex

(extracellular superoxide dismutases)

1. Homeostasis

2. Interleukin-1 mediated acute phase response to many disease states.

Fig:-II b. Ingestion, absorption, distribution, utilization, and excretion of copper complexes.
It is suggested that this redistribution of copper has a general role in responding to physiological, disease, or injury stresses. These responses are suggested to account for de novo synthesis of Cu-dependent regulatory proteins and enzymes required for biochemical response to stress and enable replacement of cells and repair of tissues.

2.2.3. COPPER-DEPENDENT MAMMALIAN ENZYMES.

Copper-dependent mammalian enzymes include Cytochrome-c oxidase, extra cellular and cytosolic Cu-dependent and Zn-modulated superoxide dismutases (Cu2Zn2SOD), tyrosinase, dopamine-β-mono-oxygenase, the acidic Cu-containing protein (neurocuprein) which provides reducing equivalents for dopamine-β-mono-oxygenase, amine oxidases, blood clotting Factor V and Factor VIII, ceruloplasmin, the α-amidating mono-oxygenases, required for synthesis of a large group of neuroendocrine hormones including: gastrin, cholecystokinin, α-melanocyte stimulating hormone, calcitonin, vasopressin, secretin, and some enkephalins, and procollagen and proelastin peptidyllysyl oxidases required for collagen and elastin cross linking. Other possible Cu-dependent enzymes include: adenylate cyclase, guanylcyyclase, a lipolytic protein, and metallothionein gene transcription regulatory protein ACE-1.

Copper is deeply involved in a number of biochemical pathways that are connected with inflammation. For instance, copper participates in prostaglandin biosynthesis, stimulating PGF2α production and it is known that copper complexing agents can inhibit overall PG-biosynthesis. Copper is also involved in connective-tissue metabolism. In vitro studies by McCord have shown that purified hyaluronic acids as well as synovial fluid were depolymerized by superoxide-generated hydroxyl radicals, and that the addition of copper-zinc SOD to the system could actively prevent such degradation.

Superoxide anions (O2−) and hydroxyl radicals (OH) are widespread, as they are produced by many biochemically relevant oxidations. Those very reactive radicals are released in considerable amounts following the activation of Phagocytes, so that they may be seen as a significant components of many, if not all, types of cell mediated
inflammation. Oyanagui, claimed that superoxide anions are capable of stimulating prostaglandin biosynthesis interacting with Phagocyte cell membranes, and Kuhel et al., proposed the hydroxyl radical as the major inflammatory component liberated during the enzymatic oxidation of arachidonic acid. Besides the scavenger activity of copper-zinc SOD copper itself is able to catalyse the dismutation of superoxide anions in vitro thereby preventing their direct acting on the tissues and largely impeding the production of the more dangerous hydroxyl radicals.

De novo synthesis of Cu-dependent enzymes required for utilization of oxygen and prevention of \( O_2^- \) accumulation as well as tissue repair processes, are key to the hypothesis that Cu complexes prevent the facilitate recovery from oxyl-radical mediated pathology. A widely held understanding is that \( O_2^- \) accumulation due to the lack of normal concentrations of Cu-dependent SODs, \( O_2^- \) production in bursts by polymorphonuclear leukocytes leading to relatively large steady concentrations of \( O_2^- \), or inappropriate release of \( O_2^- \) from oxygen activation centres, leads to the formation of much more reactive oxyl-radicals including singlet oxygen (\( ^1O_2 \)), hydroxy radical (HO), and hydroperoxyl radical (HOO\(^-\)) as well as hydrogen peroxide (\( H_2O_2 \)).

These oxyl-radical and \( H_2O_2 \) are suggested to account for pathological changes associated with many disease states. Support for this understanding comes from observations that SOD-mimetic Cu complexes are effective in preventing and/or treating many animal models of disease states including arthritides, fever, gastrointestinal ulcers, wound healing pain, epilepsies, cancer, carcinogenesis, diabetes, radiation death (immunodeficiency), and isocherms reperfusion injured. Copper complexes have also been used to successfully treat patients with arthritic and other chronic degenerative disease. These Cu complexes are also more likely to be effective as a result of their facilitation of de novo synthases of other Cu-dependent enzymes required for normal oxygen utilization and tissue repair processes.
As summarized in Fig IIc copper and the copper-zinc SOD are acting as scavengers of the superoxide anions produced by phagocytes after anti-inflammatory stimulus. This action can prevent damage to the cells and to the tissue, and simultaneously can interfere with the amplification of prostaglandin biosynthesis, which is also capable of generating free radicals.\(^{177}\)

2.2.4. PHARMACOLOGICAL ACTIVITIES OF COPPER COMPLEXES.

2.2.4.1 ANTI-INFLAMMATORY ACTIVITY.

In 1969, Bonta\(^{178}\) and Laroche\(^{179}\) reported that cupric carbonate, [Cu(OH)\(_2\)CuCO\(_3\)] and cupric complexes of acetic, lauric, oleic, caprylic, butyric, sebasic, lipoic and cinnamic acids\(^{179}\) were effective in animal models of inflammation.

Later it was suggested that copper complexes of clinically used anti-arthritis drugs were responsible for the beneficial effect.\(^{180-182}\) This suggestion was supported by observations that copper complexes of many non-antiinflammatory complexing agents, including amino acids, heterocyclic carboxylic acids, amines, and other classes of
chemical compounds, had anti-inflammatory activity in recognized animal models of inflammation. In addition, copper complexes of antiarthritic drugs, including salicylic acid, acetyl salicylic acid, 1-phenyl-5-aminotetrazole, 2-(3-trifluoromethyl phenyl) amino nicotinic acid, penicillamine and several corticoids, were found to be more active than their parent drugs. All of these copper complexes were found to be more active than either inorganic copper salts or their parent complexing agents, regardless of whether or not the parent complexing agent had anti-inflammatory activity. Cupric chloride had no activity in any of the models of inflammation. Complexed copper is a more active anti-inflammatory form of copper and led to the suggestion that copper complexes of active anti-inflammatory agents might be more active than their parent anti-inflammatory drugs.

Comparison of Cu (II)2(aspirinate)4 with aspirin showed that it was eight-times as effective as aspirin in the carrageenan paw edema model of inflammation and greater than five-times as effective as aspirin in the polyarthritis model of inflammation. Acute toxicity studies of copper complexes demonstrated that anti-inflammatory copper complexes were less toxic than inorganic forms of copper as well as their parent anti-arthritis drugs.181

There are many observations consistent with the hypothesis that an elevation of blood Cu-containing components associated with various inflammatory diseases and physiological stresses facilitate the elevation of Cu2Zn2SOD concentrations in affected tissue and play a role in bringing about remission.152, 183-186 It is plausible that inflammatory diseases of man are in part due to decreased dietary intake of copper.187, 188 Feeding diets low in Cu or treating with penicillamine reduces tissue Cu2Zn2SOD activity, serum SOD-mimetic activity, and/or ceruloplasmin (CP) concentration in carrageenan paw edema (CPE)189 turpentine inflammation,190-192 endotoxin-induced ocular inflammation,193-196 and decreases endotoxin-induced gene expression of CP mRNA.197 The pharmacological usefulness of Cu2Zn2SOD in CPE, ischemic paw edema, and poly-arthritis (PA) is limited and varies from no activity to marginal short-term activity due to its antigenicity and rapid excretion (T1/2= 5 to 10 minutes).198-200 Treatment with Cu2Zn2SOD reduced schistosome egg-induced pulmonary granulomas201 and Feldman et.al.,202 found marked inhibition of this inflammation in mice treated with
Cu(II)$_2$(3,5-DIPS)$_4$. Derivatives of Cu$_2$Zn$_2$SOD and extracellular SOD have anti-inflammatory activity in animal models of inflammation$^{203,204}$ and antiulcer activity.$^{205}$

Following the report that Cu-complexes of antiarthritic drugs are more active and less toxic anti-inflammatory agents than their parent drugs, Sorenson,$^{181}$ De Alvare et al.$^{206}$ and many others$^{149}$ suggested that O$_2^-$ disproportionation accounted for this activity. This continues to be the subject of many reports addressing mechanistic aspects of anti-inflammatory and antiulcer Cu complexes.$^{214-231}$

Comparative studies on the effective molar concentrations of parent ligands with the effective molar concentrations of their complexes given subcutaneously, orally, or topically have provided additional evidence for increased activity of Cu(II) complexes with a variety of ligands in various animal models of inflammation, pain and gastrointestinal injury.$^{225-231}$

Copper complexes have been successfully used to treat rheumatoid and other chronic degenerative diseases.$^{147}$ Possible alternative structures of one of these, Cupralene, have recently been suggested to be 1:1 and 1:4 complexes.$^{232}$ Patents have been issued for the treatment of acne with Cu complexes of lianolin fatty acids$^{233}$ and for the use of Cu-complexes as antiarthritic and anti-inflammatory drugs with antiulcer activity.$^{234}$

All of the anti-inflammatory activities reported for compounds listed in Table II a were obtained following oral or parenteral administration. Even mixtures of copper salts and ligands or copper complexes in aqueous ethanol or glycerol-dimethylsulphoxide solutions have been shown to have anti-inflammatory activity in a variety of animal models of inflammation following topical application.$^{254-259,265}$
### TABLE: II a

COPPER COMPLEXES STUDIED AS ANTI-INFLAMMATORY AGENTS

<table>
<thead>
<tr>
<th>INORGANIC COPPER COMPOUNDS</th>
<th>Copper Complexes Studied as Anti-Inflammatory Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metallic Cu</td>
<td>Cu(II)(D- and L-histidinate) &lt;sup&gt;262,263&lt;/sup&gt;, Cu(II)(D- and L-cystinate) &lt;sup&gt;262,263&lt;/sup&gt;, Cu(II)(alaninate) &lt;sup&gt;268&lt;/sup&gt;, Cu(II)(phenylalaninate) &lt;sup&gt;271&lt;/sup&gt;, Cu(II)-(glutamate) &lt;sup&gt;271&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cu(I)I</td>
<td></td>
</tr>
<tr>
<td>Cu(II)Cl</td>
<td></td>
</tr>
<tr>
<td>Cu(II)O</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(OH) &lt;sub&gt;2&lt;/sub&gt;CuCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Cu(I)Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Cu(II)O&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(pyridine)&lt;sub&gt;2&lt;/sub&gt;(Cl) &lt;sub&gt;2&lt;/sub&gt;</td>
<td>Cu(II)(morpholine)&lt;sub&gt;2&lt;/sub&gt;(HCl) &lt;sub&gt;2&lt;/sub&gt;, Cu(I)(thioacetamide)&lt;sub&gt;2&lt;/sub&gt;Cl &lt;sup&gt;240&lt;/sup&gt;, Cu(I)(MeCN)&lt;sub&gt;2&lt;/sub&gt;(CO) &lt;sub&gt;4&lt;/sub&gt;, Cu(I)(Cl)(dimethylsulphoxide) &lt;sup&gt;240&lt;/sup&gt;, Cu(II)(thiourea)&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; &lt;sup&gt;253&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALIPHATIC CARBOXYLIC ACIDS</td>
<td></td>
</tr>
<tr>
<td>Cu(II)&lt;sub&gt;2&lt;/sub&gt;(acetate) &lt;sub&gt;4&lt;/sub&gt;</td>
<td>Cu(II)&lt;sub&gt;2&lt;/sub&gt;(acetate)&lt;sub&gt;4&lt;/sub&gt;(pyridine) &lt;sup&gt;253&lt;/sup&gt;, Cu(II)-(citrate) &lt;sup&gt;239&lt;/sup&gt;, Cu(II)-(butyrate) &lt;sup&gt;238,265&lt;/sup&gt;, Cu(II)-(cinnamate) &lt;sup&gt;238&lt;/sup&gt;, Cu(II)-(caprylate) &lt;sup&gt;238&lt;/sup&gt;, Cu(II)-(laurate) &lt;sup&gt;265&lt;/sup&gt;, Cu(II)-(lactate) &lt;sup&gt;265&lt;/sup&gt;, Cu(II)-(oleate) &lt;sup&gt;238&lt;/sup&gt;, Cu(II)-(sebasate) &lt;sup&gt;238&lt;/sup&gt;, Cu(II)-(glycyrhrzic acid) &lt;sup&gt;257&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARYL ACETIC ACIDS</td>
<td></td>
</tr>
<tr>
<td>Cu(II)&lt;sub&gt;2&lt;/sub&gt;[1-(4-chlorophenyl)-&lt;sup&gt;-2,5&lt;/sup&gt;5-dimensional-&lt;sup&gt;-1H&lt;/sup&gt;-pyrrole-3-acetate] &lt;sup&gt;243&lt;/sup&gt;, Cu(II)&lt;sub&gt;2&lt;/sub&gt;[1-(p-chlorobenzoyl)-5-methoxy-2-Methyl-3-indolylacetate]&lt;sup&gt;243,264,273&lt;/sup&gt;, Cu(II)(4-cyclopropylmethylhexoxyl-3-Chlorophenylacetate) &lt;sup&gt;251&lt;/sup&gt;, Cu(II)&lt;sub&gt;2&lt;/sub&gt;(3-p-chlorophenol)-1-phenylpyrrole-4-acetate) &lt;sup&gt;253&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>ARYL PROPIONIC ACIDS</td>
<td></td>
</tr>
<tr>
<td>Cu(II)2[2-(3-benzoylephenyl) Propionate] &lt;sup&gt;243&lt;/sup&gt;, Cu(II).2.2-[6-methoxynaphthalene)] Propionate &lt;sup&gt;248&lt;/sup&gt;, Cu(II)-[2-(4-isobutylphenyl) Propionate] &lt;sup&gt;249,250,279&lt;/sup&gt;, Cu(II)-[2-(3-benzophenone)propionate] &lt;sup&gt;251&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

AMINO ACIDS

Cu(II)(L-tryptophanate)<sub>2</sub> <sup>181,262,263,271</sup>, Cu(II)(D-tryptophanate)<sub>2</sub> <sup>181,262,263</sup>, Cu(I)<sub>n</sub>(D-penicillamine)<sub>2</sub> <sup>181,240,253,276</sup>, Cu(II)<sub>2</sub>(D-penicillaminedisulphide)<sub>2</sub> <sup>181,240</sup>, Cu-D-penicillamine <sup>257</sup>, Cu(II)(L-histidine)<sub>2</sub>NO<sub>3</sub> <sup>253</sup>
<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_3$Cu(I)$_6$Cu(II)$_6$(D-penicillamine)$_2$Cl</td>
<td>240</td>
</tr>
<tr>
<td>Cu(I)-(N-acetylpenicillamine)</td>
<td>265</td>
</tr>
<tr>
<td>Cu(II)(D-aspartate)</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)(L-aspartate)</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)(L-lysinate)(Cl)$_2$</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)(glycinate)$_2$</td>
<td>239,240,276</td>
</tr>
</tbody>
</table>

**BENZOIC ACIDS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)-(N-acetylpenicillamine)</td>
<td>265</td>
</tr>
<tr>
<td>Cu(II)-(3-hydroxybenzoate)</td>
<td>265</td>
</tr>
<tr>
<td>Cu(II)-(4-hydroxybenzoate)</td>
<td>265</td>
</tr>
<tr>
<td>Cu(II)-2-chloro-4-(o-carboxyphenylamino)pyrimidine</td>
<td>265</td>
</tr>
<tr>
<td>Cu(II)(2-selenobenzoyl)</td>
<td>256</td>
</tr>
<tr>
<td>Cu(II)-2,4-di(p-carboxyphenylamino)pyrimidine</td>
<td>246</td>
</tr>
<tr>
<td>Cu(II)-2,4-di(m-carboxyphenylamino)pyrimidine</td>
<td>246</td>
</tr>
<tr>
<td>Cu(II)-2,4-di(o-carboxyphenylamino)pyrimidine</td>
<td>246</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-carboxyphenyl)amino-4-(2-carboxyphenyl)aminopyrimidine</td>
<td>258</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-carboxyphenyl)amino-4-(2,3-dicarboxyphenyl)aminopyrimidine</td>
<td>258</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-carboxyphenyl)amino-4-(2,4-dicarboxyphenyl)aminopyrimidine</td>
<td>258</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-thienylglyoxylate)</td>
<td>270</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-methoxybenzoate)</td>
<td>264</td>
</tr>
<tr>
<td>Cu(II)$_2$(4-methoxybenzoate)</td>
<td>264</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-furanoate)</td>
<td>264</td>
</tr>
<tr>
<td>Cu(I)-(2-mercaptobenzoate)</td>
<td>265</td>
</tr>
</tbody>
</table>

**CORTICOIDS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)$_3$(hydrocortisone-21-phosphate)$_2$</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)$_2$(hydrocortisone-21-Hemisuccinate)</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)$_2$(dexamethasone-21-phosphate)</td>
<td>181</td>
</tr>
</tbody>
</table>

**ETHYLENEDIAMINES**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)(H$_2$-ethylenediaminetetraacetate)</td>
<td>252</td>
</tr>
<tr>
<td>K$_2$Cu(II)(ethylenediaminetetraacetate)</td>
<td>252</td>
</tr>
<tr>
<td>Cu(II)(ethylenediamine)$_2$(Cl)$_2$</td>
<td>252</td>
</tr>
<tr>
<td>Cu(II)(ethylenediamine)$_2$(NO$_3$)$_2$</td>
<td>252</td>
</tr>
</tbody>
</table>

**THIOLS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$Cu(I)thiotalate</td>
<td>240</td>
</tr>
<tr>
<td>Cu(I)dithiodiglycol</td>
<td>240</td>
</tr>
<tr>
<td>NaCu(I)(3-N-allylthiouridobenzoate)</td>
<td>235,236</td>
</tr>
<tr>
<td>Cu(I)-(2-mercaptoethanol)</td>
<td>265</td>
</tr>
</tbody>
</table>

**HETEROCYCLIC CARBOXYLIC ACIDS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)(1-carboxyisoquinoline)</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)$_2$(2-carboxyindole)$_3$(acetate)</td>
<td>181</td>
</tr>
</tbody>
</table>

**SALICYLATES**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)$_2$(3,5-diisopropylsalicylate)$_2$</td>
<td>181</td>
</tr>
<tr>
<td>Cu(II)$_2$(acetyl salicylate)$_4$</td>
<td>181,241-245,252,268,269</td>
</tr>
<tr>
<td>Cu(II)$_2$(salicylate)$_2$</td>
<td>182,242,252,268,273</td>
</tr>
<tr>
<td>Cu(II)$_2$(salicylate)$_2$(ethanol-glycerol) or Dimethylsulphoxide</td>
<td>254,259,265</td>
</tr>
<tr>
<td>Cu(II)$_2$(pyridine)$_2$ acetylsalicylate</td>
<td>252</td>
</tr>
<tr>
<td>Cu(II)$_2$(4-methylsalicylate)</td>
<td>252</td>
</tr>
<tr>
<td>Cu(II)$_2$(5-methylsalicylate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(4,5-dimethylsalicylate)</td>
<td>182</td>
</tr>
<tr>
<td>Cu(II)$_2$(O-salicylsalicylate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(ethylsalicylate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(4-acetamidosalicylate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(salicylurate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(dimethylsalicylate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(I-hydroxy-2-naphthoate)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(salicylaldehyde)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(salicylaldoxime)</td>
<td>183</td>
</tr>
<tr>
<td>Cu(II)$_2$(salicylhydroxamate)</td>
<td>183</td>
</tr>
</tbody>
</table>

**SULFONAMIDES**

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)$_2$(N-[(2-phenyl-2-hydroxyethyl)amino]-p-toluene Sulphonamide)</td>
<td>252</td>
</tr>
<tr>
<td>Cu(II)$_2$(N-[(1-phenyl-2-hydroxyethyl)aminoethyl]-p-TolueneSulphonamide)</td>
<td>252</td>
</tr>
<tr>
<td>Cu(II)$_2$(N-[(1-phenyl-2-(2-iminothiazolidin-3-yl) ethyl]-p-tolueneSulphonamide)</td>
<td>252</td>
</tr>
</tbody>
</table>
Cu(II)(ethylenediamine)$_2$SO$_4$ $^{252}$
Cu(II)-(ethylenediamine)-(malonate) $^{265}$
Cu(II)-(ethylenediamine)-(thiocyanate)$_2$ $^{265}$
Cu(II)-(1,2-glycylethane) $^{271}$
Cu(II)-(1,2-valylethane) $^{271}$
Cu(II)-(1,2-leucylethane) $^{271}$
Cu(II)-(1,2-tryptophanylethane) $^{271}$

**TETRAZOLES**

Cu(II)$_2$(1-phenyl-5-aminotetrazole)$_2$(acetate)$_4$ $^{181}$
Cu(II)$_2$(1-phenyl-5-aminotetrazole)$_4$(Cl)$_2$ $^{181}$
Cu(II)-(1-phenyl-5-aminotetrazole) $^{265}$

**TRIPHENYLPHOSPHINES**

Cu(I)(triphenylphosphine)$_2$Cl $^{252}$
Cu(I)(triphenylphosphine)$_2$(diethyldithiocarbamate) $^{252}$
Cu(I)(triphenylphosphine)(acetylsalicylate) $^{253}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)(N-2-amino-2-deoxy-x,β-D-Glucopyranose salicylaldimine)</td>
<td>$^{270}$</td>
</tr>
</tbody>
</table>

**MISCELLANEOUS**

Cu-ascorbate $^{240}$
Cu(II)(aminophenazol)$_2$(ClO$_4$)$_2$ $^{277}$
Cu(II)(antipyrene)(ClO$_4$)$_2$ $^{264,277}$
NaCu(II)-(chlorophylline) $^{266}$
Cu(II)-(3,4-dimethoxycinnamyl Hydroxamate) $^{269}$
Cu(II)(azopropazone)$_2$ $^{275}$
Cu(I)(D-maldeleate)$_2$ $^{270}$
Cu(I)(L-mandelate)$_2$ $^{270}$
Cu(I)(D,L-mandelate)$_2$ $^{270}$
Cu(II)((-)-2,3,5,6-tetrahydro-6-Phenylimidazo(2,1-b)thiazole)$_2$Cl$_2$ $^{247,253}$
Cu(II)(diethyldithiocarbamate) $^{253}$
Cu(II)(2-amino-2-thiazoline)$_4$Cl$_2$ $^{253}$
Cu(II)(4-hydroxy-3-(5-methyl-3-Isoxazolocarbamyl-2-methyl-2H-1,2-Benzothiazine-1,1-dioxide)$_2$ $^{275}$
Cu(II)((5-dimethylamino)-9-methyl-2-Propyl-1H-pyrazolo[1,2-a] [1,2,4]benzotriazine-1,3(2H)-dione)$_2$ $^{275}$
Cu(II)-(α-tropolone) $^{265}$
Cu(II)-(2-hydroxyacetophenone) $^{265}$
Cu(II)-(trifluoroacetylacetone) $^{265}$
Cu(II)-(hexafluoroacetylacetone) $^{265}$
Cu(II)-(β-oxo-2-thiophenepropionitrile) $^{261}$
Cu(II)-(phenylbutazone) $^{260,265}$

2.2.4.2. ANTIPYRETIC ACTIVITIES

In 1960's, Schubert reported that Cu(II)(salicylate)$_2$ was more effective than either Cu(II)Cl$_2$ or salicylic acid in lowering yeast-induced fever in rats $^{280,281}$ Hac and Gagalo $^{282}$ reported that Cu(II)(salicylate)$_2$ is much more antipyrogenic than sodium salicylate in a
rabbit model of endotoxin-induced fever produced by the injection of a lipopolysaccharide pyrogen derived from Escherichia coli.

Pifferi reported that \( \text{Cu(II)}_2(4\text{-cyclopropylmethyleneoxy-3-chlorophenylacetate})_4 \) was four times as effective, on a molar basis, as an antipyretic agent as its parent ligand in bactopeptone-injected rats.\(^{251}\) \( \text{Cu(II)}_2(2\text{-methoxybenzoate})_4 \), \( \text{Cu(II)}_2(2\text{-furanoate})_4 \) and \( \text{Cu(II)}-(\text{antipyrine}) \) were also found to have greater antipyretic activity than either antipyrine or phenylbutazone in endotoxin-treated rabbits.\(^{264,283}\) Prolonged antipyretic activity has also been reported for \( \text{Cu(II)}_2(\text{N-3, 4-dimethylphenylanthranilate})_4 \).\(^{272}\)

2.2.4.3. ANALGESIC ACTIVITIES.

Pifferi\(^{251}\) observed that \( \text{Cu(II)}_2(4\text{-cyclopropylmethyleneoxy-3-chlorophenylacetate})_4 \) had nearly 10-times the analgesic activity of its parent ligand in a yeast-induced paw edema model of pain (ED\(_{50}\) value 0.018 mmol/kg i.p. and i.g.) and the phenylquinone writhing pain model (ED\(_{50}\) value 0.023 m mol/kg i.g.) and that the copper complex was more effective than phenylbutazone in both pain models.\(^{284}\) A subsequent studies have also shown that Cu complexes of some ligands are more effective analgesics than their parent ligands.\(^{269}\)

2.2.4.4. ANTIULCER ACTIVITIES.

There are historical reports of the use of copper compounds in the treatment of ulcers.\(^{285}\) Copper complexes/ compounds listed in Table II b have been reported to have antiulcer activity.
### Table II b

**Copper Compounds Found to be Effective Antiulcer Agents in Animals**

<table>
<thead>
<tr>
<th>Inorganic Copper Compounds and Salts</th>
<th>Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(I)Cl</td>
<td>Cu(II)(L-alaninate)(L-histidinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)Cl₂</td>
<td>Cu(II)(L-methioninate)₂Cl²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)O</td>
<td>Cu(II)(L-histidinate)(L-serinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(acetate)₄</td>
<td>Cu(II)(L-leucinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(pyridine)₂(acetate)₄</td>
<td>Cu(II)(L-isoleucinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(H)₂(pyridine)₂Cl₂</td>
<td>Cu(II)(L-cystinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(imidazole)₂Cl₂</td>
<td>Cu(II)(L-seroninate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(MeCN)₄ClO₄</td>
<td>Cu(II)(L-alaninate)salicylidene²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>Cu(II)(anthranilate)(L-phenylalaninate)²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>Cu(II)(anthranilate)(L-tryptophanate)²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>NaCu(II)(L-alanine)(salicylate)²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>Cu(II)(anthranilate)(L-methioninate)²⁹⁴</td>
</tr>
<tr>
<td>Aromatic Carboxylic Acids</td>
<td>Aryl Acetic Acids</td>
</tr>
<tr>
<td>Cu(II)(anthranilate)₂</td>
<td>Cu(II)₂[1-(4-chlorophenyl)-2,5-dimethyl-1H-pyrrrole-3-acctate]⁴²⁴³</td>
</tr>
<tr>
<td>Cu(II)₂(nicotinate)₄</td>
<td>Cu(II)₂[1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetate]⁴¹⁸¹³⁰³</td>
</tr>
<tr>
<td>Cu(II)(1-carboxyisoquinoline)₂</td>
<td>Cu(II)(17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)(2-carboxyindole)₂(acctate)</td>
<td>Cu(II)₂(hydrocortisone-21-hemisuccinate)⁴¹⁸¹³⁰³</td>
</tr>
<tr>
<td></td>
<td>Cu(II)₃(hydrocortisone-21-phosphate)²³⁰³</td>
</tr>
</tbody>
</table>

**Inorganic Copper Compounds and Salts**

<table>
<thead>
<tr>
<th>Inorganic Copper Compounds and Salts</th>
<th>Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(I)Cl</td>
<td>Cu(II)(L-alaninate)(L-histidinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)Cl₂</td>
<td>Cu(II)(L-methioninate)₂Cl²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)O</td>
<td>Cu(II)(L-histidinate)(L-serinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(acetate)₄</td>
<td>Cu(II)(L-leucinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(pyridine)₂(acetate)₄</td>
<td>Cu(II)(L-isoleucinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(H)₂(pyridine)₂Cl₂</td>
<td>Cu(II)(L-cystinate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(imidazole)₂Cl₂</td>
<td>Cu(II)(L-seroninate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)₂(MeCN)₄ClO₄</td>
<td>Cu(II)(L-alaninate)salicylidene²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>Cu(II)(anthranilate)(L-phenylalaninate)²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>Cu(II)(anthranilate)(L-tryptophanate)²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>NaCu(II)(L-alanine)(salicylate)²⁹⁴</td>
</tr>
<tr>
<td></td>
<td>Cu(II)(anthranilate)(L-methioninate)²⁹⁴</td>
</tr>
</tbody>
</table>

**Aromatic Carboxylic Acids**

<table>
<thead>
<tr>
<th>Aromatic Carboxylic Acids</th>
<th>Aryl Acetic Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)(anthranilate)₂</td>
<td>Cu(II)₂[1-(4-chlorophenyl)-2,5-dimethyl-1H-pyrrrole-3-acctate]⁴²⁴³</td>
</tr>
<tr>
<td>Cu(II)₂(nicotinate)₄</td>
<td>Cu(II)₂[1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetate]⁴¹⁸¹³⁰³</td>
</tr>
<tr>
<td>Cu(II)(1-carboxyisoquinoline)₂</td>
<td>Cu(II)(17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate)²⁹⁴</td>
</tr>
<tr>
<td>Cu(II)(2-carboxyindole)₂(acctate)</td>
<td>Cu(II)₂(hydrocortisone-21-hemisuccinate)⁴¹⁸¹³⁰³</td>
</tr>
<tr>
<td>Cu(II)(2-phenyl-4-carboxyisoquinoline)₂</td>
<td>Cu(II)₃(hydrocortisone-21-phosphate)²³⁰³</td>
</tr>
</tbody>
</table>
### HISTAMINES

Cu(II)(histamine)(Cl)₂(HCl)₂ \(^{181,294,303}\)
Cu(II)(histaminesalicylidene)(Cl)₂ \(^{294}\)

### TETRAZOLES

Cu(II)₂(l-phenyl-5-aminotetrazole)₂(acetate)₄ \(^{181,303}\)
Cu(II)₂(l-phenyl-5-aminotetrazole)₄ \(^{181,303}\)

### PENICILLAMINES

Cu(II)ₙ(D-penicillamine)ₙ \(^{293,299}\)
Cu(II)₂(D-penicillamine Disulphide)₂ \(^{181,293,303}\)
Na₈Cu(I)₈Cu(II)₆(D-pencillamine)₁₂Cl \(^{293}\)

### MISCELLANEOUS

Cu(II)₄(D-pemcillamine) \(^{299}\)
Cu(II)₄(D-pemcillamine Cu(II)(4-n-butyl-1,2-diphenvl-3,5-pyrazolidinedione)₂ DisuIphide)₂ \(^{299}\)

### SALICYLATES

Cu(II)(salicylate)₂ \(^{181,242,290-292,299,303}\)
Cu(II)(3,5-diisopropyl Salicylate)₂ \(^{181,297,300,303}\)
Cu(II)₂(acetylsalicylate)₄ \(^{297,299,301,303}\)

### MISCELLANEOUS

Cu(II)(4-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione)₂ \(^{181,303}\)
Cu(II)-(carnosine) \(^{299}\)
Cu(II)-(TRIEN) \(^{299}\)
Cu(II)-(Gly-His-Gly) \(^{299}\)
Cu(II)-(Gly-Ile-Gly) \(^{299}\)
Cu(II)-(nitritriacetate) \(^{299}\)
Cu(II)(thiourea)₂Cl \(^{299}\)
Cu(I)-(Na₂thiomalate) \(^{299}\)
Cu-Tamrabhasma \(^{298}\)
Cu(II)(cimetidine)₂ \(^{304,305}\)
Cu(I)(cimetidine) \(^{305}\)

---

### 2.2.4.5. PREVENTION OF ISCHEMIA-REPERFUSION AND PERFUSION INJURY WITH COPPER COMPLEXES

It is understood that reperfusion injury is inflammation mediated. Reperfusion injury is generally accepted as being due to O₂⁻ and other oxyl-radical mediated pathological changes (associated with tissue anoxia, the subsequent catabolism of ATP, and concomitant conversion of xanthine dehydrogenese to xanthine oxidase) which utilize oxygen as an electron acceptor when oxygenated blood reperfuses tissues producing O₂⁻ in amounts that exceed the removal capacity of existing Cu₂Zn₂SOD. Superoxide inactivation of creatine phosphokinase during reperfusion further exacerbates the anoxia-induced energy deficit and offers a further accounting of the etiology of ischemia-reperfusion injury. \(^{306}\)

Hernandez et al.\(^ {307}\) demonstrated that the lipophilic SOD-mimetic, Cu(II)₂(3,5-DIPS)₄, is as effective as Cu₂Zn₂SOD in preventing reperfusion injury in a cat mesenteric model of ischemia-reperfusion injury. Since Cu(II)₂(3,5-DIPS)₄ also has catalase-mimetic
and peroxidase-mimetic activities, it is possible that this approach can be used to improve the outcome of ischemia-reperfusion injury of all tissues.

SOD-mimetic Cu (II) cryptand complexes inhibit sodium-calcium exchange across cardiac muscle cell membranes and were suggested to be a new class of cardioprotective agents.

2.2.4.6. WOUND HEALING ACTIVITIES.

Townsend's rat model of surgically induced ulcer is also viewed as a gastric wound-healing model. Data provided by Townsend demonstrated that Cu(II)(tryptophanate)2 and Cu(II)2(aspirinate)4 markedly increased healing-rate and prevented liver-spleen-pancreas adhesions, a constant feature of this model. Complex-treated gastric wounds healed at a rate five days faster than vehicle-treated wounds and replacement of submucosal connective tissue components could not be distinguished from non-operated rats. This increase in rate was due to rapid re-epithelization of the gastric mucosa, possibly due to facilitation of lysyl oxidase activity required for repair of connective tissue components and either SOD-mimetic activity of administered Cu complexes or their facilitation of Cu2Zn2SOD synthesis.

Non-steroidal antiinflammatory drugs suppress wound healing, which may be due to inhibition of Cu2Zn2SOD and/or connective tissue replacement. In an effort to eliminate this suppressant activity, Rao et al. studied Cu complexes of ibuprofen and enefenamic acid and found promotion of normal wound healing with retention of antiinflammatory activity using: sutured skin incision, circular skin excision, and cotton wad granuloma models of wound healing. These results are consistent with induction of lysyl oxidase activity and formation of superior wound closures and granuloma as originally reported for Cu complexes in the treatment of the cotton wad granuloma model of inflammation.

All of these data support the use of Cu complexes as a physiological approach to promoting wound healing in man.
2.2.4.7. ANTICONVULSANT ACTIVITIES.

Brain contains more copper than any other non-storage tissue of the human body. There are many reports of seizures in animals and humans following the consumption of Cu-deficient diets. Correction of dietary deficiency and treatment with Cu complexes bring about remission of seizures in man.

To pursue the possibility that treatment with SOD-mimetic Cu complexes might prevent seizures, they were tested for anticonvulsant activity. It was found that Cu complexes of ligands that did not have anticonvulsant activity were potent anticonvulsants. It was subsequently found that Cu complexes of all existing anti-epileptic drugs were more effective than their parent drugs. Based upon these observations, it was suggested that these complexes, which can be formed in vivo, were active forms of antiepileptic drugs and accounted for the beneficial effects of these drugs.

It has been estimated that 25% of brain copper is contained in Cu$_2$Zn$_2$SOD. Idiopathic seizures have been suggested to be due to accumulation of O$_2^-$ as a result of less than normal levels of Cu$_2$Zn$_2$SOD and O$_2^-$ is thought to account for seizures due to brain tumor, trauma, and infection, which are all associated with inflammation. Anticonvulsant Cu complexes have all been found to have SOD-mimetic activity. Mimetic activity or facilitation of de novo synthesis of Cu$_2$Zn$_2$SOD and/or other Cu-dependent enzymes may account for anticonvulsant activities of Cu complexes. Specific mechanistic possibilities support the use of Cu complexes as a physiological approach to treating epileptic seizures.
<table>
<thead>
<tr>
<th>AMINO ACIDS</th>
<th>COPPER COMPLEXES OF VARIOUS CLASSES OF COMPOUNDS REPORTED TO HAVE ANTICONVULSANT ACTIVITY [313-316]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)(L-threoninate)(L-serinate)</td>
<td>Cu(II)(3,5-diisopropylsalicylate)₂</td>
</tr>
<tr>
<td>Cu(II)(L-threoninate)(L-alaninate)</td>
<td>Cu(II)₂(adamantylsalicylate)₄</td>
</tr>
<tr>
<td>Cu(II)(L-valinate)₂</td>
<td>Cu(II)₂(acetysalicylate)₄</td>
</tr>
<tr>
<td>Cu(II)(L-threoninate)₂</td>
<td>Cu(II)(acetysalicylate)₂(pyridine)₂</td>
</tr>
<tr>
<td>Cu(II)(L-alaninate)₂</td>
<td>Cu(II)(acetysalicylate)₂(dimethylsulphoxide)₂</td>
</tr>
<tr>
<td>Cu(II)(L-phenylalaninate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(L-cystinate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(L-serinate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(L-glutamate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(L-tryptophanate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(L-leucinate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(L-isoleucinate)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(salicylidene-L-valinate)</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(salicylidene-L-histidinate)</td>
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<tr>
<td>SALICYLIC ACIDS</td>
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<tr>
<td>Cu(II)(salicylate)₂</td>
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<tr>
<td>Cu(II)(4-aminosalicylate)₂</td>
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<tr>
<td>Cu(II)(4-tertiarybutylsalicylate)₂</td>
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</tr>
<tr>
<td>Cu(II)(salicylatoethyleneimine)₂</td>
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<tr>
<td>ANTIEPILEPTIC DRUGS</td>
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<tr>
<td>Cu(II)(dilantin)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)₂(valproate)₄</td>
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<tr>
<td>Cu(II)(Phenobarbital)₂(H₂O)₃</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(phenobarbital)₂(pyridine)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(phenobarbital)₂(imidazole)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(amobarbital)₂(H₂O)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(amobarbital)₂(pyridine)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(amobarbital)₂(imidazole)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(lorazepam)₂(Cl)₂H₂O</td>
<td></td>
</tr>
<tr>
<td>MISCELLANEOUS</td>
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</tr>
<tr>
<td>Cu(II)₂(acetate)₄</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(acetylacetoneethyleneimine)₂</td>
<td></td>
</tr>
<tr>
<td>Cu(II)(salicylatoethyleneimine)₂</td>
<td></td>
</tr>
</tbody>
</table>

2.2.4.8. ANTICANCER ACTIVITIES.

Oberley et al.¹⁴⁹ originally observed that Cu₂Zn₂SOD had anticancer activity in a mouse model of solid tumor. This observation along with the report by De Alvare et al.²⁰⁶ that Cu salicylate complexes have SOD-mimetic activity lead to a number of reports that these small molar mass Cu complexes have anticancer activity.¹⁴⁹ Result of these studies demonstrated that treatment of solid tumors with SOD-mimetic Cu complexes markedly decreased tumor growth and metastasis, increased survival of tumor-bearing mice, and caused differentiation of transformed Ehrlich and neuroblastoma cells. The complex that appeared to be most effective was Cu(II)₂(3,5-DIPS)₄.
Some studies of Cu(II)$_2$(3,5-DIPS)$_4$ have demonstrated that this binuclear complex is in equilibrium with its mononuclear complex, Cu(II)(3,5-DIPS)$_2$ in polar solutions.\textsuperscript{317} Electron spin resonance studies revealed that the mononuclear complex interacts with Ehrlich cell membranes and appears to be rapidly absorbed with less impedance due to strong lipophilic bonding interactions with cell membrane macromolecules.\textsuperscript{318}

New Cu complexes have been found to possess anticancer activity. A very stable Cu (II) complex of daunorubicin, which does not stimulate oxyl-radical production as daunorubicin does, was suggested to be a promising approach to treating neoplastic diseases without cardiotoxic effects.\textsuperscript{319} It is most likely that re-establishment of Cu-dependent enzymes and proteins are the principal mechanistic parameters accounting for anticancer activities of Cu complexes.

All of these data support the use of Cu complexes as a physiological approach to preventing and treating human cancers.

2.2.4.9. ANTIMUTAGENIC ACTIVITIES.

Kensler and his colleagues\textsuperscript{320} reported anticarcinogenic activity of Cu(II)$_2$(3,5-DIPS)$_4$. Solanki et al.,\textsuperscript{321} confirmed and extended this observation with the demonstration that Cu(II)$_2$(3,5-DIPS)$_4$ also has antimutagenic activity.

The inhibition of mutagenesis by Cu(II)$_2$(3,5-DIPS)$_4$ was due to inhibition of cytochrome P-450 NADPH-dependent reductase required for metabolic activation of benzo [a] pyrene.\textsuperscript{322} Antimutagenic activity of Cu complexes makes them strong candidates in a physiological approach to treatment or prevention of cancers.

2.2.4.10. ANTIDIABETIC ACTIVITIES.

Rosalie Crouch et al. reported that streptozotocin decreased pancreatic $\beta$-cell Cu$_2$Zn$_2$SOD in causing diabetes and that treatment of the streptozotocin-induced diabetic rat with Cu$_2$Zn$_2$SOD decreased this diabetic response. Both Cu(II)$_2$(salicylate)$_4$ and
Cu(II)₂(3,5-DIPS)₄ were found to have antidiabetic activity in this model of diabetes and markedly improved glucose utilization and decreased urinary glucose excretion.³²³,¹⁴⁹

In addition to SOD-mimetic activity of Cu complexes and facilitated de novo synthesis of Cu₂Zn₂SOD, other mechanistic possibilities merit consideration in accounting for antidiabetic activities of Cu complexes. Since diabetes can be produced with Cu deficiency, which may decrease pancreatic β-cell Cu₂Zn₂SOD activity, it is plausible that Cu complexes may be useful in preventing or treating this and perhaps other models of diabetes. It is also likely that Cu complexes can be used in a physiological approach to treating human diabetic diseases.

2.2.4.11. RADIATION PROTECTION AND RADIATION RECOVERY ACTIVITIES.

Petkau et al. reported that Cu₂Zn₂SOD treatment produced 17% survival in lethally irradiated mice; Cu(II)₂(3,5-DIPS)₄ was studied as a radioprotectant.¹⁴⁹

Weiss et al.,³²⁴,³²⁵ also studied the influence of Cu(II)SO₄ administration on the toxicity and radioprotectant activity of WR-2721, the thiophosphate ester of N(3-aminopropyl)₂- aminoethanethiol, which is one of the most effective radioprotectants known. The toxicity of WR-2721 was reduced while radioprotectant activity (survival) was increased with Cu (II) SO₄ and WR-2721 combination treatment.

Both the historical and current research results support the hypothesis that treatment with Cu complexes offers a new approach to overcoming the pathological effects of ionizing radiation and suggest their use as a physiological approach to treating radiation injury.³²⁶

2.2.4.12. ANTIMICROBIAL ACTIVITIES

Various Cu complexes have been reported to inhibit bacterial, fungal, yeast, algal, mycoplasmal, and viral growth as well as cause death of these microorganisms.¹⁴⁹ It was suspected that SOD-mimetic activity of Cu complexes would impede PMNL and
macrophage microbiocidal and phagocytic activities. However, it has been demonstrated that microbiocidal activity is potentiated by Cu complexes and lysosomal, residence time is decreased in Cu(II)₂(aspirinate)₄ versus aspirin-treated PMNLs, which may be due to myeloperoxidase-mimetic activity of Cu complexes.³²⁷

It has been reported that antiviral activity of a series of triazines was increased by Cu complex formation and SOD-mimetic activity correlated with their antiviral activity.³²⁸-³³⁰

Immunological properties of 3-morpholine-2-hydroxypropyl ether of dextran³³¹ led to the discovery of superior immunostimulant properties of the Cu(II) complex of 3-mercaptop-2-hydroxypropyl ether of dextran³³² which afforded nearly 100% survival of mice infected with Shigella sonnei dysentery bacilli,³³³ attenuated tumor growth and increased survival of sarcoma 180 implanted mice, suppressed spontaneous leukemia and prolonged survival of AKR-mice,³³⁴ and has radioprotectant activity.³³⁵

Reported antimicrobial activities of Cu complexes support their use as a physiological approach to prevention and treatment of infections.