NONSTEROID ANTI-INFLAMMATORY AGENTS

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INTRODUCTION

The pharmacologic study of anti-inflammatory agents is almost always a means to an end. The theorist, who uses drugs as tools to unravel the mystery of the inflammatory process, often approaches problems and interprets observations quite differently from the pragmatist whose goal is the discovery of more useful agents with which to treat inflammatory diseases. The theorist often works with simple, *in vitro* models, seeking to understand the inflammatory process one step at a time. The pragmatist also needs simple reproducible models, but he is more concerned that the model imitate the clinical situation, whether or not the mechanisms of action are understood. His models are more likely to be *in vivo* with the locus of action less well defined.

If the etiologies and mechanisms of the pathophysiology of inflammatory diseases were better understood, there would be no such dualism in the pharmacologic approach to anti-inflammatory drugs. There is little mystery to the control of inflammation in acute gonococcal arthritis treated with an appropriate antibacterial agent. But when the primary etiologic factor is unknown or unassailable, the investigator must seek the next best solution, i.e., an agent which blocks some step in the mechanism responsible for the troublesome inflammation.

Both the mechanisms and the causes of clinically important inflammatory conditions are complex and vary with the disease. The effectiveness of different drugs varies accordingly. Consequently, no one pharmacologic approach can be the *sine qua non* of anti-inflammatory research. Whenever a new agent is found to be clinically effective, each investigator rushes to study it in his favorite anti-inflammatory models, and the esteem in which each model is held rises or falls in accordance with the results. This understandable pattern tends to direct the development of anti-inflammatory drugs along a line which will select agents working by the same mechanism as previously discovered drugs, and to a collection of models which perhaps correlate too well with each other and not well enough with unsolved clinical problems. It is more of a challenge to try to combine the theorists' insight and the pragmatists' concern with clinical disease to develop novel

models, the value of which should be judged in the clinic, and not by comparison with "standard" models. Just as the efficacy of penicillin in acute gonococcal arthritis is not reflected in the many noninfectious irritant-induced models of inflammation, so the value of a novel agent which strikes at the very origin of rheumatoid disease might well be overlooked by current models which reflect the action of our present inventory of inadequate drugs. A multifaceted correlated approach to both the nature of different inflammatory diseases and the pharmacology of different agents is required.

With these thoughts as background, the current status of the pharmacologic investigation of potential anti-inflammatory agents can perhaps be more fruitfully reviewed.

EVENTS OF THE INFLAMMATORY PROCESS

At the 19th International Physiologic Congress in Montreal in 1953, Dr. H. Selye led an all-day conference of many of the world's experts on inflammation (108). The conference began in the morning with an attempt to define the term "inflammation". After three meals and many hours of discussion, the conference ended without a generally acceptable definition. Few were satisfied with the classical description of "calor, rubor, dolor, tumor". Static morphologic descriptions were inadequate because of the profound variations with different types and stages of inflammation. Fundamental mechanistic definitions, such as a shift of intracellular potassium, seemed too far removed from the general understanding of the term "inflammation". Attempts to credit one or another chemical mediator as the responsible common denominator of all inflammation were untenable.

The situation today is not much different. It is clear that inflammation is a dynamic process, which may follow a variety of pathways with some steps in common. Several recent reviews (48, 97, 124, 133, 207, 220, 225, 226, 229) present current hypotheses and schemes as to the sequence of events in inflammation and the site of action of anti-inflammatory drugs. Rather than attempt to produce another variation on these themes, we shall tabulate these events and cite recent references to developments in each area (Table I). The sequence of the events is not always readily discerned, and relationships often presented as cause and effect, may be better described as chicken and egg. For example, proteolytic activity is claimed on one hand to be the trigger which produces histamine and other amines and kinins which in turn cause inflammation, while another view pictures these latter agents as the path to the disruption of lysosomal membranes which results in the release of their inflammation-causing proteolytic enzymes. While both of these alternatives picture enzyme activation or release as critical to the development of inflammation, still another view sees such proteolytic activity as vital to the control of inflammation by clearing inflammatory "debris". Thus, both the enhancement and the inhibition of enzyme activity are proposed as the road to anti-inflammatory action. Other events, such as increased vascular permeability (5) and cell "stickiness" (65, 79, 177) are also labelled both cause and effect of some biochemical characteristics of inflammation. In-

TABLE I

EVENTS IN THE INFLAMMATORY PROCESS

Morphologic events
- Increased capillary permeability (5, 123, 138, 142, 227, 274, 284)
- Leukocyte migration and changes (21, 79, 88, 132, 143, 168, 250, 276)
- Lysosomal rupture (42, 51, 106, 235, 259, 260, 261, 262)
- Lymphocyte responses (60, 70, 143, 144)
- Lymphatic tissue responses (70, 180, 214, 276)
- Interstitial changes-healing (6, 26, 50, 62, 94–97, 117, 118, 129, 216, 276)

Chemical events
- Immunological phenomena (76, 93, 158, 182, 208, 283)
  - Serologic (8, 199, 243)
  - Tissue (7, 8, 64, 84, 85, 113, 121, 168, 231, 244, 248, 259)
- Enzymatic activation (259, 260, 262)
  - Lysosomal (acid hydrolases) (34, 41, 106, 237, 259, 260, 261)
  - "Specific" proteases (14)
    - Hyaluronidase (5, 100)
    - Plasmin (13, 110, 258, 273)
    - Other "factors" (59, 83, 86, 90, 101, 139, 145, 166, 181, 209)
- Non-protein mediators (228)
  - Histamine (183, 203, 212, 213, 228)
  - Serotonin (179, 228)
  - Other amines (227, 272)
  - Peptide "kinins" (55, 102, 103, 133, 134, 146, 204)
    - Leukotaxin (154, 155, 157)
    - Bradykinin (36, 55, 57, 58, 63, 81, 104, 131, 135, 198, 211, 252)
    - Kallidin (57, 58, 131, 198, 266)
    - Other (17, 37, 49, 205, 246)

tense endocytosis can lead to the release of acid hydrolases from newly formed secondary lysosomes into the cell sap and surrounding tissue (262). Paradoxically, lysosomes and their enzymes which normally serve a useful function in destroying engulfed debris appear to act in rheumatoid arthritis in a self-perpetuating destructive cycle (92). This view is supported by the findings of high concentrations of lysosomal enzymes in the synovial membranes and fluid of arthritic joints and in the serum of arthritic patients (45, 119).

Known anti-inflammatory compounds have been examined by the theorists for a common biochemical or physical-chemical property as a clue to the mechanism of inflammation. Among the theories derived from this approach are the uncoupling of oxidative phosphorylation (270, 271, 281), chelation of critical cations (particularly copper) (257), binding to one or another protein (39, 165, 230), activation of cyclic AMP (16, 72, 267), and the shifting of monovalent (Na^+ and K^+) or divalent (Ca^{++} and Mg^{++}) cations. In each instance there are examples of agents with great potency in influencing these mechanisms, but without significant clinical anti-inflammatory activity. By and large these approaches have been too sweeping and

nonspecific to be useful in understanding inflammation and designing anti-inflammatory drugs. They are, therefore, excluded from Table I, even though time may eventually show one or another of them to be fundamentally relevant to inflammation.

EXPERIMENTAL APPROACHES TO THE STUDY OF INFLAMMATION

The large gaps in knowledge concerning rheumatic and other inflammatory processes make it difficult but none the less important that the methodology employed to study inflammation be carefully reasoned and the strengths and limitations of each approach be considered.

The most common purpose in setting up models of inflammation is the search for and comparison of anti-inflammatory drugs (268). A review of the anti-inflammatory literature compels the unhappy conclusion that the following comments are in order: (*a*) Dose-response relationships cannot be assumed to be parallel with different agents unless studies show such parallelism; (*b*) The time course of response should be outlined to validate the significance of the points in time at which observations are made for comparative evaluation; (*c*) Methods of quantifying and comparing the effects of the variables must be defined and justified against a background of data reflecting reproducibility with positive and negative controls. The use of formal statistics per se does not necessarily solve this problem; (*d*) Conclusions should be appropriate to the model and the statistics employed. No amount of statistics applied to results in rats will improve the validity of extrapolation to other species. On the other hand, the more appropriate the experimental design, and the clearer the data, the less need is there for formal statistical evaluation to reach a valid conclusion. It takes no great degree of statistical sophistication justifiably to convince an experienced rheumatologist that standard schedules of colchicine are effective in treating acute gouty arthritis. However, it requires meticulous design and statistics to compare the relative merits of one effective treatment with that of another. Misleading conclusions are the rule rather than the exception if the parameters studied, quantitative methods employed, doses compared, and timing of events are not appropriate. The fact that a new method employs new and complex equipment, ingenious concepts, or is performed with unusual skill, is in itself no guarantee that the results are of greater significance than those derived by older simpler methods.

The following classification of anti-inflammatory models is presented for convenience of discussion. Several models combine more than one component of the classification.

In Vitro Systems

While the relative simplicity, excellent control, and large capacity of *in vitro* models makes them most attractive, the relevance of the results to the ultimate clinical objective is often in doubt.

Physical-chemical measurements.—Essentially all processes of living tissue, including inflammation, are in the last analysis physical-chemical. Were this foundation of inflammation well understood, simple *in vitro* evaluations could rationally be designed to test for anti-inflammatory activity. The following concepts represent currently fashionable attempts in this direction:

(*a*) Uncoupling oxidative phosphorylation. Since phosphorylation is critical to many energy consuming activities of living tissue, interference with it has been suggested as a mechanism for anti-inflammatory action (224, 270, 271, 281), and for a host of other drug actions as well (1, 49). The hypothesis needs to be developed much more specifically along anti-inflammatory channels (77) before *in vitro* estimation of drug effects on phosphorylation can be fruitfully employed in the search for anti-inflammatory action.

(*b*) Chelation. Since inflammation involves enzymatic and metabolic processes which are often dependent upon natural polyvalent metal complexes, it is reasonable to expect that inflammation will be influenced by agents which compete or otherwise interfere with metal binding by these natural ligands (69). At least one effective anti-inflammatory agent has evolved from an *in vitro* study of copper chelating capacity (257). Hydrocortisone itself may owe some of its activity to its capacity to bind with copper. Yet extremely potent chelating agents such as EDTA are not clinically useful anti-inflammatory drugs. Much needs to be learned about chelation, specifically in relation to inflammatory processes, before chelation chemistry can be rationally applied to anti-inflammatory screening.

(*c*) Protein binding. Many inflammatory diseases and particularly arthritis are accompanied by detectable changes in plasma or serum proteins which are reflected in a number of *in vitro* tests such as erythrocyte sedimentation rate and turbidity of plasma or serum upon heating (73, 75, 162–164, 192, 193). The courses of these diseases are often mirrored in these blood protein parameters. Essentially all clinically effective nonsteroidal broad spectrum anti-inflammatory drugs are aromatic compounds with acidic functions which bind to serum proteins and influence the turbidity of heated serum (192). Paradoxically, the altered turbidity in clinical and experimental subchronic inflammatory disease is in the same direction as that resulting from the *in vivo* or *in vitro* addition of anti-inflammatory drugs to normal serum. Yet the administration of these drugs so as to control the experimental inflammatory process does not aggravate, but actually corrects the altered turbidity (193). Although it is unlikely that these effective anti-inflammatory drugs act by virtue of their binding to serum proteins, their capacity to bind in this manner may represent a trait reflecting their significant action at some tissue site.

Since endogenous anti-inflammatory steroids bind to plasma proteins, the influence of effective nonsteroidal anti-arthritic drugs on the binding and disposition of steroids should be considered. Improved methodology makes it possible to study alterations in the bound to free steroid ratio (141).

Enzyme inhibition.—The reasoning concerning the activation as well as

the inhibition of proteolytic enzymes to cause or control inflammation has been alluded to earlier. Empirical studies of enzyme activity in human synovial fluid (119) reinforces the interest in the enzymatic approach to inflammation. εACA (ε-amino-caproic acid) and aprotinin (Trasylol®), strong inhibitors of proteolytic activity, can control experimental protease-induced inflammation (14), but have generally been unsuccessful as anti-arthritic agents clinically. Nevertheless several *in vitro* enzymatic assays are used to study and screen for anti-inflammatory agents (40, 137, 262, 265).

The carry-over of *in vitro* enzyme observations to systemic *in vivo* effects is full of pitfalls, especially if an exogenous enzyme moiety itself is expected to be absorbed and distributed to the site of inflammation (253). Enzyme therapy alleged to be systemically useful to speed the clearance of hematomas, etc., is at best, unreliable in treating arthritis.

The misnomer "collagen disease" applied to arthritic processes has led to much attention to the biochemistry of collagen-related enzymes in arthritis. Hydroxyproline, an amino acid which is almost unique to connective tissue, has been of particular interest to rheumatologists. It is formed in collagen by hydroxylation of proline already incorporated in a peptide by a specific hydroxylase enzyme (240). In the rat, cortisol, oxyphenbutazone, and indomethacin, but not acetylsalicylic acid, cause a distinct loss of cutaneous collagen associated with increased collagenolytic and other proteolytic activity in the extracellular skin compartments. Both effects are inhibited by cycloheximide pretreatment (98, 99). The action of agents in blocking the incorporation of labelled sulfur into various connective tissues has been used frequently as an index by which anti-inflammatory potential is judged (19, 20, 23, 85, 215, 242), often with confusing results.

Lathyrogens (β-aminopropionitrile and other aminonitriles) which can induce a form of osteoarthritis, have been found to inhibit the maturation of collagen by suppressing the formation in intermolecular and intramolecular cross-links. Phenylbutazone, sodium salicylate, and chloroquine prevent the manifestations of lathyrus toxicity in the rat (238). Lathyrogen-induced depression of collagen metabolism in rats is inhibited by sodium salicylate (239). The relationship of collagen chemistry to clinical arthritis and anti-arthritic drug action remains in doubt.

Cellular and subcellular systems.—(*a*) Lysosome stability. There exists dramatic cinematographic evidence that lysosomes "explode" in areas involved in the inflammatory process, releasing numerous acadic hydrolases and proteases (hyaluronidase, β-glucuronidase, acid phosphatase, etc.) which play a role in inflammation. Consequently, factors which influence the stability of the lysosomal membrane (40, 41, 259, 260, 262, 263) and perhaps other similarly constituted membranes (24, 25) may be expected to influence inflammation. Paradoxically, lysosomes and their enzymes which normally serve a useful function in destroying engulfed debris may act in rheumatoid

arthritis in a self-perpetuating destructive cycle (92) reflected in high concentrations of lysosomal enzymes in the synovial membranes and fluid of arthritic joints and in the serum of arthritic patients (45, 119).

A variety of techniques have been developed to study this membrane "stability" *in vitro* (40, 42, 44, 106, 210, 235, 264, 265). Certainly there is no one-to-one relationship between *in vitro* effects on lysosomes and clinical anti-inflammatory action (26) even when lysosomes or other membranes of human origin are studied. Nor is it proper to assume that all proteolytic activity involved in inflammation is of lysosomal origin (54).

At plasma concentrations required for anti-inflammatory efficacy in man, nonsteroidal anti-inflammatory drugs such as phenylbutazone, indomethacin, mefenamic acid, flufenamic acid, and ibufenac, have been shown to inhibit the release of hyaluronidase and β-glucuronidase from isolated rat liver lysosomes, while aspirin inhibits phosphatase and cathepsins (5). These nonsteroidal drugs may conserve the integrity of cartilage and other connective tissue chondromucoprotein *in vivo* by inhibiting the attack of lysosomal enzymes on mucopolysaccharide or protein. Gold preparations also inhibit acid phosphatase, β-glucuronidase, and cathepsin, the mechanism of action presumably being the binding with sulfhydryl groups (56, 137). Ethacrynic acid and N-ethylmaleimide, known SH binding agents, have been shown to inhibit experimental granuloma growth. The SH containing amino acid cysteine, reverses these effects as well as anti-inflammatory activity of aspirin and indomethacin (176).

Lysosomal enzymes have been studied by several techniques, including chromatographic fractionation (12). A new acid pyrophosphatase (22), two phosphodiesterases (22), and β-aspartyl glucosylamine amino hydrolase (140) have been identified in the soluble fraction of liver lysosomes. The possible role of these specific enzymes in inflammation is yet to be determined.

(*b*) Cellular cultures. The incorporation of radioactivity from labelled nucleosides into the nucleic acid fractions of human, sheep, rabbit, rat, and chicken lymphoid cells *in vitro* (66, 269) and certain cells of epithelial origin *in vitro* (269) is inhibited by anti-inflammatory anti-arthritic drugs. Chloroquine apparently causes inhibition of human lymphocyte growth *in vitro* (66) but does not inhibit lymphocyte metabolism in cultures of lymphoid cells obtained from other species (269). It also causes selective partial inhibition of thymidine incorporation into polyploid epithelial cells *in vitro* (269).

Metabolic studies of tissue cultures of normal versus arthritic human synovial cells have shown differences which may be developed into a technique for studying anti-inflammatory drugs. A small cytoplasmic protein extractable from a variety of cells, including arthritic inflammatory leukocytes, may be a key factor in the biochemical and growth differences between normal and arthritic cell cultures.

Acute *In Vivo* Experimental Inflammatory Models

In most *in vivo* models, degree of inflammation is quantified by measuring one facet of the inflammatory process, i.e., local swelling (volume or weight), temperature or redness, and possibly reactions interpreted as resulting from pain. At times other components, specific to the model, may be measured, i.e., cell count in ascitic fluid, the release or activation of an enzyme, change in collagen, other proteins, or protein fragments.

These acute nonspecific inflammatory models are commonly used to detect and compare anti-inflammatory activity. By and large, they all select the same agents as active or inactive, but with varying potencies. Short acting versus long acting agents may appear to differ widely with species, time factors, and other features of the test design. Qualitative differences may also occur, depending on the nature of the observed parameter. Thus, pure analgesics may be "positive" in pain-dependent tests, and not in tests measuring swelling. Drugs which systemically alter fluid balance may influence local swelling without being at all anti-inflammatory. There are also less well understood differences in the way classes of anti-inflammatory compounds differ in different models. Potent anti-inflammatory steroids do not influence ultraviolet-induced skin erythema in guinea pigs, while many nonsteroidal anti-inflammatory agents are consistently effective in this model.

Experimental inflammation may be induced by various agents, some of which are discussed below.

Simple non-specific irritants.—(*a*) Physical injury: Numerous tests are based on a standardiz
cotton pellet implants, burns, ultraviolet-induced skin erythema, etc.). Recent studies of clinical gout and pseudogout (*29*) have led to an arthritis model in dogs and rabbits induced by the intraarticular injection of suspensions of urate (*152, 189, 196*) or other substances with the appropriate crystal size and shape, without regard to its chemical nature. The model, based on an ingenious concept of articular inflammation initiated by the phagocytosis of such crystals, has recently been challenged by observations that intraleucocytic crystals were not surrounded by a membrane, suggesting that they were formed in the cytoplasm.

(*b*) Chemical injury: The choice among many such models is largely empirical. Carrageenin (*47*) has largely replaced formalin as the irritant in the rat paw edema test because of the more uniform degree of swelling achieved at appropriate concentrations (*277*). The foreign body reaction to a cotton pellet is often coupled with chemical irritation by soaking the pellet with carrageenin, etc. before implanting. Some test systems measure the apparent pain reaction on application of pressure to a chemically inflamed area. Another model determines the ability of a rodent to hold on to an inclined screen with chemically induced inflammation in critical joint areas (*187, 275*).

Specific mediator-induced inflammation.—The use of naturally occurring, inflammation-causing neurohumoral agents, rather than nonspecific irritants,

has long been a popular and teleologically attractive method of causing experimental inflammation presumably more closely related to the mechanism of clinical inflammatory diseases.

(*a*) Histamine. This substance is no longer considered to be the prime mediator of inflammation. The complexity of histamine pharmacology is matched by the variety of models by which its effects may be observed. The ability to block histamine-induced local inflammation does not necessarily parallel the effect on other inflammatory models. Certainly the antihistamines per se have not proved to be clinically useful in arthritis, although the ability to interfere with histamine-induced vasodilation of the isolated rabbit ear probably accurately reflected the clinical anti-arthritic activity of a series of analogues (49).

(*b*) Serotonin. The complex role of this endogenous neurohumoral transmitter, which is a vasoconstrictor under some circumstances (hence its name) and phlogistic under others, makes it difficult to evaluate its role in rheumatic diseases. While "antiserotonins" are not as commonly known and used as antihistamines, they too have not been shown to be effective in clinical inflammatory disease, and the utility of serotonin-induced inflammatory models remains in doubt.

(*c*) Kinins. These peptides, several of which have been identified in inflamed tissues (80, 167), are considered by some to be the key to the inflammatory process. They are presumably products of protein breakdown. Components of the clotting process both as enzyme (plasmin) and substrate (fibrin), and most recently Hageman Factor (234) have been associated with their generation. Reactions to several specific kinins are currently under careful study. The exquisite potency of bradykinin in producing pain in some locations but not in others is well recognized (81). The specificity of a series of these agents in causing pain, leukocyte migration, increased capillary permeability, etc. leads to the tempting hypothesis that different physical or chemical irritants each result directly or indirectly in the formation or release of its own spectrum of kinins, and the nature of each spectrum accounts for the various patterns of inflammation and differences in response to anti-inflammatory agents. This theory calls for study of the formation, identity, activity, and control of each kinin. Several steps have been reported along these lines (133, 214) since the pioneer work of Menkin (154, 155). However, the kinin models have yet to prove their superiority over less specific irritants in the search for clinically useful anti-inflammatory agents.

Immunologically-induced inflammation.—Just as an antihistaminic agent may work well against histamine-induced inflammation and not against other forms of inflammation, so an agent which interferes with the immune reaction (208) may be effective in immune-induced inflammation and not in many other models. Considering the present understanding of rheumatoid arthritis such models deserve particular attention (158). While these will be discussed below, some immune models involve relatively acute cutaneous and other cellular reactions (217).

Subchronic and Chronic Inflammation

Since most human inflammatory disease is of a more or less chronic nature, chronic inflammatory models, and particularly those involving immune mechanisms, are becoming increasingly popular, in spite of the difficulties in dealing with these prolonged, highly variable, poorly understood, complex, multifaceted systems. Immune models are nevertheless useful not only to test for anti-inflammatory action but also to study drug antigenicity which may cause reactions that limit the use of potent anti-inflammatory and other drugs.

The subchronic and chronic inflammatory models currently reported may be classified as follows:

Autoimmune models.—Numerous immunopathologic animal models have been related to specific human diseases (218A). The following are models used particularly to evaluate anti-inflammatory drugs:

(*a*) Adjuvant arthritis. The long lasting polyarthritis induced in rats by the intradermal injection of Freund's complete adjuvant is now used in many laboratories (73, 78, 114, 170, 184, 194, 249, 278). It has several features in common with human arthritis (38, 115) including the histopathology of the joints (30, 73, 184, 194) and the lack of a direct correlation in time of circulating antibody with joint lesions. A dual mechanism may be involved in that autoantibodies of a delayed hypersensitivity type may be produced to specific cellular antigens at the same time that the lymph nodes are producing circulating antibodies.

In both this model and human disease the circulating antibodies may be benign, forming harmless soluble complexes with normal antigenic determinants, or none at all, while the cellular antibodies (hypersensitivity type) may produce the arthritic process when enough antibody-carrying cells migrate into and accumulate at the target organs. The importance of the circulating lymphocytes in association with the autoimmune component of adjuvant disease is shown by studies (38) in which the secondary responses to complete adjuvant are prevented by heterologous anti-rat lymphocyte serum. This serum suppresses the onset of the immune reaction when administered at the time of adjuvant injection. It is only mildly "anti-inflammatory" when given after the experimental arthritis is established. In this respect the serum is very much like the 6-mercaptopurine type antimetabolites, which are strongly immunosuppressive and show only modest anti-inflammatory activity in some models (249).

Degradative enzymes released from lysosomes may play a role in the immune nature of this and other models in that they may "denature" the native constituents of cells or connective tissue into antigenic substances which then stimulate formation of circulating antibodies (259, 260, 262). Increases in serum lysozyme levels in adjuvant polyarthritic rats may reflect the release of enzymes from leukocytes in the initially injured or secondarily inflamed areas into the circulation. Some effective anti-inflammatory

drugs such as paramethasone, phenylbutazone, and indomethacin prevent such increases (193) possibly by stabilizing lysosomes (40), influencing cell migration (189), or inhibiting enzyme activity directly (261). Preventing the release of destructive enzymes may also influence alterations in gamma globulin seen in immune models (92, 259).

(*b*) "SI arthritis": Another chronic model which is receiving increasing attention in drug testing is the 6-Sulfanilamidoindazole (SI) induced arthritis in old rats (222). The polyarthritis following the oral administration of 6-SI may be a Herxheimer type effect resulting from drug-induced injury to or activation of an otherwise saprophytic organism, perhaps the pleuropneumonia-like organisms (PPLO) (156) which some believe to be involved in clinical arthritis (11). PPLO may produce the pre-antigens responsible for a hypersensitivity reaction resulting in connective tissue disease. Modification of such antigen development may be one mechanism by which agents interfering with hypersensitivity reactions could conceivably affect a change in tissue response. Some recent reports (35) do not support an association between PPLO and SI-induced experimental arthritis.

(*c*) NZB Lupus mice. Although the pathogenesis of the spontaneous lupus-like syndrome seen in an inbred strain of the New Zealand Black (NZB) mice and hybrid strains of NZB mice (NZB/NZW) is not established, it is believed to be of an autoimmune nature comparable to systemic lupus erythematosus in man (153). The efficacy of cyclophosphamide in this model (32) presumably by interfering with the cellular immune response, and the suppression of the hemolytic component and positive Coomb's test by anti-lymphocyte serum (43) illustrates the application of this model.

(*d*) Pig arthritis. A disease entity in swine very closely resembling human rheumatoid arthritis in its clinical course and histopathology has proved to be a most interesting model (223) but its very virtues have made it difficult to use on a large scale, i.e., chronic variable course in a large animal not readily adapted to laboratory conditions.

(*e*) Rat encephalomyelitis. The relatively acute allergic encephalomyelitis which can be induced in the Lewis rat can be useful in evaluating drug effects on cell mediated delayed hypersensitivity (201). It has been employed to determine whether the anti-inflammatory action of some agents might be mediated, at least in part, by an immunosuppressive effect.

Toxic arthritis models.—The toxic effects of the lathyerus bean and various synthetic aminonitriles includes a chronic osteoarthritic response which differs from other chronic inflammatory models in its reaction to a variety of agents. The model is used occasionally (111). It probably does not closely mimic any major form of human arthritis. Other toxic agents which influence joints have been described, particularly by Selye in his studies of calciphylaxis (218), but are not generally used as models in the search for anti-inflammatory drugs.

Nutritional models.—(*a*) Zinc deficiency in chickens results in a polyarthritis which is reported to respond to several known anti-arthritic drugs including chloroquine (171), an anti-malarial, anti-rheumatic agent which has little or no effect in the usual acute anti-inflammatory models. Experience with this model is limited.

(*b*) Hypo- and hyper-vitaminosis: Almost all vitamin disturbances involve inflammatory lesions. Vitamin A in large doses can create experimental osteoarthritis and may influence hypersensitivity (241). Hypervitaminosis D also causes calcification in joints as well as other organs. Yet it was used to treat arthritis before the steroid era (116). Its chemistry has led to attempts to relate Vitamin D action to that of the anti-inflammatory steroids. Vitamin E, with a literature proposing its relation to almost every conceivable disease process, continues to be the subject of investigations of inflammation. There is currently a flurry of interest in the effect of intra-articular Vitamin K to treat arthritis.

While scurvy resulting from Vitamin C deficiency is in a sense an arthritic inflammatory process, it is has found little use as an experimental model for exploring inflammation, although some attention is paid to its influence on healing (51). The specific lesions which are the hallmark of several of the B complex vitamins are inflammatory in nature. These have perhaps been too lightly dismissed from consideration as inflammatory models for the study of anti-inflammatory drugs.

(*c*) Obesity has recently been related to osteoarthritis in rats, and may serve as a selective model for some forms of clinical arthritis.

ANTI-INFLAMMATORY AGENTS

The above models are only some of the tools for the evaluation of potential anti-inflammatory agents. The nature of these agents dictates to a degree the type of model by which they are studied.

Types of Anti-inflammatory Agents

Specifically to attack the etiology.—This approach represents the best and most rational way to attack specific inflammatory diseases of known etiology. No nonspecific antiinflammatory agents can control acute bacterial or chronic tuberculous arthritis as well as the appropriate antibiotics. In fact, on the basis of experimental animal data, the steroids have long been considered contraindicated in the treatment of inflammation of infectious (bacterial) origin, because their immunosuppressive and possibly anti-inflammatory properties were believed to interfere with resistance to infection. Recently the applicability of this thesis to human disease has been challenged.

On the other hand, there are exciting new developments demonstrating that some generalized inflammatory diseases, including arthritis, might be

traced to a defect in lymphocytic sensitivity, resulting in impaired defenses against otherwise antigenic infectious diseases as diverse as vaccinia and candida. There have been dramatic therapeutic responses when cellular sensitivity was restored by the administration of homologous sensitive lymphocytes or even a soluble transfer factor. Macrophages also influence the process, possibly by concentrating antigen on their surfaces to form a more potent sensitizing unit than the low concentrations of antigen in solution. These instances of rampant infection related to deficiency in sensitized lymphocytes may occur in the face of high titers of circulating antibodies. Thus, alteration of immunologic competence is a double edged sword. On the one hand adjuvant arthritis can be passively transferred via sensitized lymph node or spleen cells (185) and abnormal hypersensitivity to an otherwise insignificant antigen may be the cause of generalized inflammatory reactions which can be approached therapeutically by immunosuppressive agents. On the other hand, some types of immune incompetence can allow an otherwise inconsequential invading antigen to develop into an overwhelming disease process. The therapeutic attack against this latter process calls for the restoration of immune competence, with evidence that this can be accomplished at times by as yet unidentified soluble factors which future research may be able to enhance or replace with therapeutic agents not yet conceived.

The etiology of rheumatoid arthritis and related human inflammatory diseases remains elusive. It is intriguing to consider that chloroquine, an anti-parasitic agent, gradually arrests the disease process in some rheumatoids, a phenomenon not observed with the steroids or other nonspecific anti-inflammatory agents. Might the drug be attacking an unrecognized chronic infestation which would otherwise continue to feed an antigen responsible for perpetuating the rheumatoid process? Or might it be supplementing immunocompetence in fighting off this hypothetical infestation? What differences exist between those rheumatoid patients who respond to therapy, and those who do not? Perhaps the peculiar affinity to retinal pigment (236) may be a clue to its therapeutic as well as toxic mechanism. Might the empirical discovery of an effective anti-rheumatic drug help find the cause of rheumatoid arthritis, following which its prevention would be relatively simple? There is need for more attention to this approach.

Attack a specific characteristic of a specific inflammatory disease.—Perhaps the best example in this category is the control of gout by controlling hyperuricemia. While there is no correlation between serum uric acid levels at a given time and the occurrence of acute gouty arthritis in patients with chronic tophaceous gout, there is little doubt that the long term control of hyperuricemia with uricosuric agents, and more recently with allopurinol (89) to inhibit uric acid synthesis, eventually results in a reduction in the frequency and severity of acute attacks of gouty arthritis. The interesting specificity of colchicine in controlling acute gouty arthritis without influencing uric acid levels is also intriguing, as is the recent report that colchicine

is effective in "pseudogout," a condition presumably due to intraarticular crystals of calcium phosphate rather than urate, but with similar physical crystal dimensions (147, 151). Phagocytosis of particulate matter may also be a crucial step in rheumatoid arthritis (245).

The mechanism of action of gold in rheumatoid arthritis remains a mystery. Its specificity in relation to this one kind of inflammation makes it likely that it operates at an as yet unidentified component of the mechanism of this disease. It does influence experimental immune responses (188), and chelates with SH groups.

Alterations of oxidation-reduction potential, reflected in disulfide-sulfhydryl balance have long been considered significant in the mechanism of inflammation and anti-inflammatory response. In spite of continuing efforts (4, 122, 125) the role of this mechanism remains unclear.

Nonspecific anti-inflammatory agents.—Because of the lack of satisfactory progress in attacking the specific etiologies and mechanisms of important inflammatory diseases, nonspecific drugs continue to account for the bulk of effort and bits of progress being achieved in anti-inflammatory pharmacologic research. Most of the sections to follow deal with this area.

Preclinical Drug Evaluation

To the complexities of evaluating the multitude of anti-inflammatory models described above, one must add equally complex evaluations of the potential of each agent for undesirable (toxic) effects. Achieving marked anti-inflammatory potency is a hollow victory if toxicity is equally great. Some guidelines are therefore necessary, not only for the evaluation of efficacy and potency, but for their relationship to toxicity as well.

Efficacy evaluation.—There is no simple way to grade the relative significance of the "anti-inflammatory scores" in the numerous models. Qualitative and quantitative comparisons with known compounds are made with the implication that a similar spectrum in the preclinical models will reflect similar clinical efficacy.

Widely different conclusions concerning anti-inflammatory potency may be reached depending upon criteria which are often arbitrary. Minimal effective dose comparisons may give quite different relative potencies from the dose necessary for complete suppression of inflammation or "ED_{50}" defined in a variety of ways. Efficacy quantified at a fixed time after dose can be quite different from that observed at the time of peak effect, or the summated effect over a period of time. No one rule can be laid down as the proper method of study. Experience and objective determines the best plan for each set of preclinical model data.

Toxicity evaluation.—Toxicity is no easier to evaluate. There are well-standardized techniques for determining LD_{50} with sophisticated calculations of the confidence limits. Yet the factors responsible for acute LD_{50} rarely reflect the clinical side effects which limit the use of a drug in man. Damage to a system detected in more chronic toxicity studies stands a better

chance of reflecting limiting effects in clinical therapy. The dosage creating such effects is a valuable guide, particularly if similar organ damage is seen with similar doses in more than one species. Sometimes the exaggerated therapeutic mechanism itself may be responsible for toxicity. Within the phenylbutazone series, anti-inflammatory activity seems to go hand in hand with sodium retention. It is remarkable that, of the numerous truly potent general anti-inflammatory agents, all have ulcerogenic potential. Many influence thyroid radioiodine uptake. Hepatic toxicity is a common result of chronic administration in large doses. Rare but alarming instances of blood dyscrasias are seen unpredictably in man. Specific animal models exist for estimating ulcerogenesis and hepatotoxicity with a fair correlation to clinical experience, and frank bone marrow suppression such as that seen with the potent anti-metabolites is generally detectable in experimental animals. However, no satisfactory preclinical model exists for a variety of very disturbing blood effects such as agranulocytosis and thrombocytopenia which are relatively rare but clearly attributable to several potent anti-inflammatory drugs.

It is not unusual to hear a clinician comment that he doesn't need more potent anti-inflammatory drugs—just equally potent drugs which he can use without fear of severe side effects. A breakthrough in our understanding of the mechanism of these side effects may be just as useful as the much sought after understanding of the mechanisms of inflammatory disease. This knowledge may also teach us how to select those patients who will react badly to known drugs, thus making possible fuller and safer use of these agents in the remaining patients.

Therapeutic-toxic ratio.—From the above description of the complexities of quantifying efficacy and toxicity, it is clear that numerical "toxic-therapeutic ratios" must be looked upon with reservation. A "therapeutic" effect which is evident in an animal model only at or near toxic doses cannot be looked upon as very promising. On the other hand, insisting on an arbitrarily established high therapeutic-toxic ratio is bound to eliminate compounds of potential utility. Based on past experience, one has a right to be skeptical about reports of agents claimed to be active at low doses and "nontoxic" at high doses. Sooner or later one or the other of these evaluations is found to be invalid, in fact or in significance. Certainly a toxic-therapeutic ratio should not be calculated on the basis of toxicity in one species and efficacy in another.

Drug dynamics.—So much of species differences can be explained by studying drug dynamics, that this factor needs to enter into the evaluation of candidates from the beginning.

(*a*) Evaluating absorption. Models involving oral administration can be seriously misleading if the completeness and speed of absorption is not known. The physical form, formulation, and technique of administration of a test substance can have major influences on results. While physical-chemical principles have been able to explain much about absorption (91), with

the logical assumption that these factors are independent of species, there are nevertheless very significant species differences in size, anatomy, gut motility and other factors, which make it unsafe to assume that absorption is entirely equivalent in rat, chicken, cow, and man.

Since many anti-inflammatory drugs are highly lipid soluble, it is reasonable to ask whether these drugs are absorbed with fats of dietary origin via the lymphatics into the systemic circulation by way of the thoracic duct, or whether they are absorbed via the portal venous system, passing through the liver before entering the systemic circulation. Instances of poor oral efficacy presumed to be due to poor absorption have recently been shown to be due to breakdown in the gut wall or rapid hepatic destruction after excellent absorption into the portal circulation (130). Several highly lipid soluble drugs, including oxyphenbutazone, are absorbed into the portal system rather than via the lymphatics. In the anesthetized dog, intragastric administration yielded markedly delayed absorption as compared to intraduodenal administration (255).

(*b*) Tissue distribution: Like absorption, drug distribution out of the blood stream and into extravascular structures is generally considered to be a physical-chemical passive phenomenon, which should be quite similar from one species to another. Yet there are marked species differences, only some of which can be explained by differences in drug metabolism. Even the degree of drug binding to plasma proteins can vary with species (186), and there is reason to believe that important differences in binding to tissue occur. The point is illustrated by observations concerning a new anti-inflammatory imidazole derivative, metazamide (48). This compound was found to have a plasma half-life of 14 to 19 hours in the rat, as compared to 1 to 5 hours in the dog determined in both species orally and parenterally (282). Serial studies of sacrificed rats showed a general parallelism between plasma and tissue levels after the first two hours. In the dog, sacrifice 24 hours after a single dose showed essentially no drug in plasma or tissues, consistent with the short plasma half-life. With chronic daily administration however, dog tissue levels showed remarkable accumulation of the drug 24 hours after the last dose not reflected in the plasma levels (Table II), while the rat tissue levels 24 hours after the last dose were more or less the same as after a single dose. Why dog tissue has so much greater affinity for this drug than rat tissue *in vivo* on repeated administration remains a mystery. Certainly it is not caused by a different rate of metabolism, since the drug disappears so much more quickly from dog plasma than from rat. While it is conceivable that more refined analytical techniques might some day demonstrate a species difference in metabolic pathway resulting in a presently undetected species difference in the nature of the bound materials rather than the binding substance, it nevertheless remains valid that in this instance, as in others, species differences in blood levels as detected by commonly accepted methods might not correctly reflect the relative "tissue load." The drug was more toxic to the dog than the rat on chronic administration, in

TABLE II

Drug Levels (μg/gm of tissue) in Rat and Dog 24 Hours after a Single Dose vs. the Last of 21 Daily Doses of Metazamide

	Rat (300 mg/kg)		Dog (400 mg/kg)	
Tissue	One Dose i.p.	Post 21 Daily Doses p.o.	One Dose i.p.	Post 21 Daily Doses p.o.
Liver	43	57	0	146
Heart	9	45	0	349
Fat	11	23	0	198

spite of lower blood levels, but consistent with the high tissue levels. There was no opportunity to study tissue distribution in humans, in whom both anti-inflammatory activity and side effects were demonstrated. It was of little assistance to know that the half-life of this drug in man was intermediate to that found in rat and dog.

This type of experience should not deter continuing attempts to explain species differences by studies of drug disposition, since there are several excellent examples in which pharmacologic action or toxicity occur in different species at comparable plasma levels rather than mg/kg doses. It does teach, however, that drug disposition studies are but one component in a complex pattern, and that other important variables need to be considered in parallel.

The saturation of drug binding sites on plasma proteins can lead to a situation in which increasing doses may yield proportionately less increase in plasma level, but with proportionately more unbound drug available for tissue distribution (23, 254). In such instances the modest increases seen in blood levels with higher doses may fail to reflect the considerably greater toxic potential of such doses. On the other hand, quite a few drugs show slower rates of disappearance with larger doses, so that doubling the dose more than doubles the plasma level. This is suggestive evidence that the drug is cleared from plasma in its protein bound form, rather than solely by equilibrium with the small percentage of unbound freely diffusible drug (255).

While factors which result in lower drug blood levels generally are accompanied by parallel reductions in drug effect, there are now several examples, including one involving a commonly used anti-inflammatory drug in which altered drug disposition resulted in lower blood levels and an increased drug effect (175).

(*c*) Enzyme induction and inhibition: Aside from the influence of anti-inflammatory drugs on enzymes involved in the inflammatory process, the action of these agents can be influenced by their ability to stimulate ("in-

duce") or inhibit the action of drug metabolizing enzymes. Phenylbutazone is a classical example of an inducer which, upon repeated administration, markedly enhances its own metabolism in dog and rat, but not in man. Its fate, in turn, can be influenced by other agents (256). Before attributing a drug plasma level change to enzyme induction or inhibition, one must consider influences in the gut or intestinal wall (130, 175) and tissue distribution changes, particularly in man, where direct organ assays of enzyme activity are impractical.

Clinical Pharmacology.—In the last analysis, a drug stands or falls by what it does in man with disease. Definitive evaluation in man must be preceded by cautiously selecting an appropriate dosage pattern for study. The first step is generally dose build-up within the limits of safety permitted by a full evaluation of available toxicologic data. At times this limit may be more rationally judged by blood levels than dose in mg/kg. Blood level data to confirm absorption and indicate half-life can be most helpful, but is not always feasible.

Some indication of efficacy and dose is often required before meaningful half-life and drug metabolism data can be fruitfully pursued, since these biochemical parameters can vary with dose. Presumably minor factors, such as the position of the patient and his urinary pH, can alter drug disposition, as has been shown with aspirin (71). Experimentally produced inflammation in man usually induced in skin, has not proved to be a useful intermediate step between dose build-up and evaluation in patients with systemic inflammatory diseases.

It is beyond the scope of this review to detail further the many important clinical and laboratory factors which are involved in the clinical evaluation of anti-inflammatory agents. Suffice it to say that one must define the nature and incidence of clinical toxicity or intolerance in parallel with clinical efficacy. Often this clinical ratio is quantitatively and qualitatively different from toxic-therapeutic observations in animals.

Reports of New Agents

A review of the recent literature reveals a series of new compounds classified below according to their chemistry or the nature of their action.

Acidic agents.—(*a*) Arylalkanoic acids. A number of arylacetic and arylalkanoic acids have recently been reported to have significant anti-flammatory properties. Myalex (ICI 54540), 2-4-chlorophenyl-thiazole-4-acetic acid, is effective in a variety of animal screens (carrageenin, ultraviolet erythema, pyresis, and adjuvant arthritis), and is apparently clinically effective in rheumatoid arthritis (87). Activity in the ultraviolet erythema test has been reported for 5-p-chlorophenyl-2-furanacetic acid (111). Diaryloxazole and diarylthiazole alkanoic acids have activity in the carrageenin, ultraviolet erythema, turbidity model, Whitehouse test, granuloma, and ad-

juvant arthritis anti-inflammatory screens comparable to that of phenylbutazone. Better gastric tolerance than phenylbutazone is claimed (27). 4-Isobutylphenylacetic acid (Ibufenac, Dytransin) has been withdrawn from the market because of hepatotoxicity in man (52). Clinical studies of the corresponding α-methyl acid (Ibuprofen) indicates anti-inflammatory and analgesic potency approximately seven times greater than acetylsalicylic acid (107) with no apparent hepatatoxicity.

Phenylacetic acids substituted in the 4-position by heterocyclic systems, pyrryl, phenyl, and benzoyl have been reported to have anti-inflammatory activity (190). Further absorption studies with p-(n-butoxy)-phenylacethydroxamic acid (Droxaryl) and structure-activity relationships in this series have appeared (126, 127). Clinical efficacy of Droxaryl in chronic rheumatoid arthritis has been reported (127).

Pharmacologic studies with 4-(p-biphenylyl)-3-hydroxybutyric acid (BDH 7538) indicate activity similar to that of phenylbutazone. It is particularly potent in the guinea pig ultraviolet erythema test with a prolonged duration of action (10). Structure-activity relationships in this series have also been studied (9).

The 1, 8 naphthyrid-4(1H)-ylidene derivative has been reported to be active in acute inflammatory screens (195).

(*b*) Indomethacin-like agents: Variations of the indomethacin structure (280) and a new synthesis procedure for indomethacin (279) have been reported. Related imidazopyridines show activity in the carrageenin edema test (2).

(*c*) Fenamates: N-(p-n-butylphenyl)-anthranilic acid is claimed to have anti-inflammatory activity equal to mefenamic acid (191). The tetrazole analogues of the fenamic acids show anti-inflammatory activity comparable to the corresponding carboxylic acids (109).

The pyridine analogue, Nifluril is effective in ankylosing spondylitis and gout, although 26 per cent of the patients show gastrointestinal side effects (31, 61). The related analogue Sch 10304 is claimed to have a better efficacy/ulcerogenic ratio than fenamic acids and indomethacin (251).

(*d*) Salicylates: Terpene derivatives of salicylic acid are claimed to show improved gastrointestinal tolerance but have decreased analgesic and anti-inflammatory activity (178). Some salicylic acid analogues of phenothiazine have weak anti-inflammatory activity (206).

4-Acetaminodophenyl 2-acetoxylbenzoate (WIN 11450) has been shown to be more effective than acetylsalicylic acid in antipyretic and yeast induced edema screens but less effective against ultraviolet erythema (202). Thymotic acid (2-hydroxy-4-isopropyl-6-methyl-benzoic acid) is active in acute tests such as carrageenin-induced edema but not in rat adjuvant arthritis (149). Pharmacological activity of the hexylcarbonate of salicylic acid is claimed to be similar to salicylic acid with a markedly lower gastric irritation potential (160).

All of the above reports will have to stand the test of time and reproducibility before their full significance can be evaluated.

Immunosuppressive anti-inflammatory agents.—Experience with immunosuppressive agents is too limited to draw firm conclusions regarding the utility of these toxic drugs in rheumatoid disorders. However, recent reports of clinical effectiveness (18, 33, 67, 68, 148, 233) deserve attention. Selective immunosuppressives which do not compromise valuable immunological defenses, are needed (232).

The use of azathioprine (Imuran) in rheumatoid patients has been encouraging in that no serious side effects have been reported at the doses used (247). This agent has been demonstrated to reduce the corticosteroid requirements of severe rheumatoid arthritic patients (148).

The mustard derivative cyclophosphamide (Cytoxan) has been reported slowly to bring about complete remission in some severely disabled patients (67, 68). While this drug appears to be less toxic than other immunosuppressive agents the toxic pattern is qualitatively the same, i.e., vulnerable to infection, reduced blood cells, gastrointestinal upset, and hair loss.

Podophyllinic acid ethyl hydrazide (Proresid) has also been reported to be clinically effective (33, 233).

Included as compounds reported to have both immunosuppressive and anti-inflammatory properties in animal testing are cytarabine and related cytotoxic agents (74), ICI 47776, 3-acetyl-5-p-fluorobenzylidene-2,5-dihydro-4-hydroxy-2-oxothiophen (174), and 1-aminocyclopentane-1-carboxylic acid (ACPC), a known nonmetabolizable, unnatural amino acid lacking an alpha hydrogen atom (200). Other agents with similar actions may soon be tried by rheumatologists.

Anti-lymphocyte serum (ALS) is another approach to immunosuppression. Here too, directing an immunologic attack against specific cells or sites remains a problem. There is a risk of serum sickness or anaphylaxis since the circulating antibody mechanisms are intact and foreign protein reactions can occur. The risk of enhancement of infection with viruses and malignancies associated with viruses also exists with ALS. Combined use of reduced doses of potent chemical suppressants and ALS are under study in relation to organ transplants.

Miscellaneous.—The combination of calcium D,L-aspartate and indomethacin is reported to be effective in chronic polyarthritis (172). Dialauroyl-L-lysine (169) and tranexamic acid, trans 4-aminomethyl-cyclohexame-1-carboxylic acid (221) reveal anti-inflammatory activity.

SKF 17,910-A (2-chloro-10- (β dimethylaminopropionyl)-phenothiazine-5-oxide hydrochloride) is an anti-inflammatory and diuretic agent with glucocorticoid properties (150).

Ketophenylbutazone (Kebuzone) has been reported to be clinically equivalent to phenylbutazone in rheumatoid arthritic patients with less fluid retention and less ulcerogenic potential (112, 173). These findings apparently correlate well with those of animal studies.

Clinical reports of 5-butyl-1-cyclohexyl-barbituric acid (Paramidin) from Japan claim effectiveness comparable to oxyphenbutazone with good absorption, prolonged high plasma levels and low incidence of side effects (159, 219).

Benzydamine (AF 864) found to be active against carrageenin-induced edema both orally and topically, and formalin-induced edema, is reported to be clinically useful only in nonarthritic inflammatory conditions such as post-traumatic and post-operative inflammatory edema (15, 136).

Clinical anti-inflammatory efficacy of Glyvenol (CIBA 21401-Ba) is awaited with interest in view of its unusual structure, a glucofuranoside derivative (3, 46, 53, 82, 197). Apparently the drug possesses effective analgesic and anti-edema properties.

Clinical trials of azapropazone claim anti-inflammatory efficacy (105, 120, 161).

CONCLUSIONS

There has been no truly dramatic breakthrough in our understanding of inflammation or in anti-inflammatory drugs in recent years. However, steady increases in experience, particularly in relation to immune phenomena, and the correlation of subcellular ultrastructure and biochemistry give promise that there will be major advances in the understanding and treatment of inflammatory disease in the reasonably near future.

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