Conference proceedings

First WHO/ILAR Advanced Course on Rheumatoid Arthritis and Allied Diseases *

The inflammatory connective tissue disorders have been extensively investigated over the last two decades but only a few fragmentary clues to their pathogenesis have been unearthed. Hitherto the emphasis to be placed on such information has been decided in a rather partisan spirit by the protagonists of a number of investigative disciplines. Thus for most immunologists histocompatibility antigens mean markers of immune responses, and possession of HLA B27 is assumed to imply inheritance of an immune response gene determining disease susceptibility. For many immunochemists the production of rheumatoid factor implies a disorder of immunoglobulin synthesis of primary importance in the pathogenesis of rheumatoid arthritis, and for some cellular immunologists depression of T lymphocytes or phytohaemagglutinin responsiveness has been assumed to signify a primary disorder of cell-mediated immunity. There was clearly a need to bring together exponents of a number of clinical and scientific disciplines related to the study of the inflammatory connective tissue diseases so that an agreed picture of our present knowledge could emerge and co-ordinated plans for future research could be devised. The residential course, on April 12-16, 1977, at Wall Hall College convened on the occasion of World Rheumatism Year on behalf of the World Health Organization and the International League Against Rheumatism, was an attempt to achieve the desired synthesis of ideas.

Dr. Dudley Dumonde and the Mathilda and Terence Kennedy Institute of Rheumatology assembled a group of scientists and clinicians knowledgeable in their own field of interest who might reasonably be expected to listen sympathetically to the data and opinions of their colleagues in other disciplines. Undeterred by the prospect of 2 weeks of plenary sessions, situational reports, interim reviews, and multidisciplinary workshops, the participants tackled the contributions of a number of scientific disciplines to rheumatological research, first sequentially, and then in relationship to each other in a truly international spirit, and the meeting did not deteriorate into the Tower of Babel which the uncharitable might have predicted.

MICROBIOLOGY

Rheumatologists are reluctant to relinquish the idea that rheumatoid arthritis and other inflammatory connective tissue diseases result from microbial infections whether bacterial or viral. Reports of isolations of candidate agents, electron microscopic sightings, alterations of specific immunity, and evidence for the detection of subviral components in rheumatoid arthritis and systemic lupus erythematosus were critically reviewed (Phillips, New York) and found to be negative or unconfirmed. The association of hepatitis B virus infection with polyarteritis nodosa in some patients remains the only situation where a viral aetiology for a connective tissue disease is accepted, and even this association has not been confirmed in all parts of the world. Protagonists of the viral theory draw comfort from the many mechanisms which have been recognized by which defective virus or portions of the viral genome can persist in mammalian cells. There are good animal models of chronic immune pathological damage initiated by viruses altering existing membrane antigens or inducing the formation of new antigens.

However, techniques which should possess the sensitivity needed to detect the viral genome or its products in human cells have failed to provide firm evidence of viral infection in connective tissue disorders (Marmion, Edinburgh; Phillips, New York). Particularly disappointing has been the failure to confirm earlier reports that oncoviruses* (oncornaviruses) are implicated in the pathogenesis of systemic lupus erythematosus. Investigators in this area, however, are not easily deterred and they drew some comfort from the thought that all possible methods for detecting viral genomes have not been exhausted. Enthusiasm for the potential of sophisticated new techniques for the detection of retrovirus genomes (Schnitzer and Teich, London)
was tempered however by the recognition that viruses may alter cell membranes on a 'hit and run' basis, making the detection of viral components in damaged cells impossible by any method. The possibility that failure to isolate or detect virus might be due simply to failure to examine the right cells and tissues at the right time was generally conceded.

With respect to rheumatoid arthritis the importance of obtaining material for study during the early acute stage of disease was stressed and the relevance of long-term fibroblast cultures derived from synovial membrane questioned. An ingenious method for obtaining synovial lining cells after intra-articular injection of trypsin (Muirden, Melbourne) and cogent arguments for examining lymphoreticular cells freshly obtained from blood or lymphoid tissues (Denman, London) were presented.

The concept of 'reactive arthritis' has become accepted as a form of inflammatory joint disease provoked by bacterial infections in susceptible individuals. The bacteria are gut pathogens, and polyarthritis has occurred primarily in epidemics of bacillary dysentery. Most of the patients who develop this complication possess HLA B27. Unfortunately, even this apparently secure association between inflammatory polyarthritis and a defined microbial infection was challenged on grounds that the bacteriological evidence, and particularly the serological data, do not satisfy the rigid criteria of the practising microbiologist (Rowe, London).

A withering analysis of the evidence for the postulated relationship between chlamydia infection and Reiter's syndrome (Taylor Robinson, London) suggested that though this organism can cause arthritis in animals there had been few isolations from human joints and none using the more sensitive modern techniques, while the serological data were hard to interpret. This session illustrated well the value to rheumatologists of the multidisciplinary approach. Not only were some of the wilder flights of rheumatological fancy subjected to detached and critical scrutiny, but helpful suggestions for the design of a prospective study to determine whether chlamydial infection does give rise to Reiter’s syndrome in susceptible individuals were made (Taylor Robinson, London). Given the difficulties of isolating the organisms even by the most sensitive techniques and bearing in mind that less than 10% of the population are HLA B27 positive, it was estimated that about 1000 untreated patients with urethritis would be required.

**GENETICS**

Family studies provided the first hint that ankylosing spondylitis has a genetic component and this was dramatically confirmed by the discovery of the close association with HLA B27. Studies of associations of rheumatic disorders with HLA types have become a growth industry in rheumatology yet the biological meaning of the associations are unknown and we are left with a list of unproven possibilities. Batchelor (East Grinstead) reviewed the state of current knowledge of the mapping of HLA and complement genes on human chromosome 6 and discussed the concept of linkage disequilibrium and its importance for putative immune response and other disease susceptibility genes. In analysing the groups of diseases associated with histocompatibility antigens, he emphasized the distinction between a large group of disorders associated with HLA B8 where increased autoantibody production might well be a consequence of linkage to an 1A antigen, and the HLA B27 disorders where he favoured the notion that the HLA gene itself determined disease susceptibility.

The use of HLA B27 as marker for sacroiliitis throughout the 'seronegative inflammatory spondyloarthritides' was discussed by Wright (Leeds) who felt that data from family studies favoured an extra independent, rather than a linked, disease susceptibility gene. The possibility that there might be separate immune response genes on the sex chromosomes was also considered (Batchelor, East Grinstead; Zwaifler, San Diego). The need to correlate each clinical component of a disease syndrome separately with possible histocompatibility markers was emphasized by Brewerton (London), who illustrated the point with respect to Sjögren's syndrome. This caveat is also central to the epidemiological study of connective tissue disorders where slavish adherence to artificially defined disorders may obscure important clues to their pathogenesis (Wood, Manchester).

As a unique example of a disease association with an HLA-A gene product, Eddlestone (London) discussed his work with patients with idiopathic haemachromatosis where he has found a significant association with HLA A3. His family studies appeared to identify a group of HLA A3 negative-HLA B27 positive subjects with iron overload which suggested the possibility of separate genes determining the absorption of iron from the gut and cellular uptake elsewhere in the body. The implications with regard to the importance of family studies in helping to elucidate complex genetic susceptibilities were not lost on the rheumatological investigators but one must hold strong reservations regarding the data and its physiological interpretation. Eddlestone further described his work in patients with chronic active hepatitis where there is an association with HLA B8 in HBsAg-negative but not HBsAg-positive subjects. The HLA B8 antigen appeared to be associated with higher antibody titres to measles...
and rubella but antibodies to E. coli and autoantibodies were not increased and rubella titres were not raised in HLA B8 positive first-degree relatives.

The virtues of not restricting the meeting to the study of rheumatic disorders was further illustrated by a review of the associations between multiple sclerosis and D locus antigens (Compston, London). Earlier studies linking the disease with DW2 had been extended by investigating B lymphocyte alloantigens in patients in Northern Europe and Jordan. An antigen found in English patients with multiple sclerosis differs from that in Jordanians with apparently the same disorder. Moreover, in England this antigen is also encountered in patients with primary optic neuritis in whom it indicates an increased risk of developing systemic disease, a clear indication that the course of a disease may be determined by the histocompatibility antigens that patients possess. A further association with seasonal onset of multiple sclerosis suggests the possibility of an infective aetiology.

Studies of B lymphocyte alloantigens in patients with rheumatoid arthritis reported by Panayi (London) seemed to confirm Stasny's earlier observations of an increased frequency of DW4 in rheumatoid arthritis using mixed lymphocyte cultures. Technical difficulties may have hitherto obscured the possibility that it is D locus antigens which are most closely linked to immune response genes.

**IMMUNOLOGY**

Extensive immunological investigations of the inflammatory connective tissue diseases have so far produced many hypotheses but few tangible results. The logical incentives for such studies were the discovery of autoantibodies and, in particular, rheumatoid factors and the characteristic lymphocyte infiltrations. Most attention has been given to the analysis of lymphocyte subpopulations, identified by surface markers and in vitro responses to mitogens, in the expectation that these would reveal immune defects. However, extensive studies have produced variable results and no clear evidence of any abnormality of fundamental importance. In reviewing the evidence with regard to rheumatoid arthritis (Lightfoot, Milwaukee) it was concluded that such abnormalities as have been noted appear to result from the non-specific effects of systemic disease.

Despite these disappointments, most participants were unwilling to conclude that immunological mechanisms are irrelevant to the problem of persistent inflammation in the connective tissue diseases. Clearly these contribute in some way to the continued disease process in sites such as the rheumatoid synovial membrane. In general terms three principal interpretations were considered. The first is that the antigen which provokes the immune response persists in the target organ, such as the rheumatoid synovial membrane, but in a form which has not so far been identified. The obvious candidate is some form of infectious agent, and this was the conclusion supported by some but not all the microbiologists (e.g. Phillips, New York). Thus the immune abnormality is considered to be a form of immunodeficiency which allows the infection to continue. According to the second theory, tissue components become autoantigenic after microbial infection and these autoantigens provoke the continued immune response. This concept too had its unwavering adherents (e.g. Glynn, London) who take some comfort from the findings of Doherty, Zinkernagel, and others that specifically sensitized T lymphocytes are cytotoxic for cells bearing histocompatibility antigens altered by virus or simple hapten.

In essence, the third hypothesis states that the immune response is provoked by an initial insult in the affected tissues but is not terminated by the appropriate homeostatic mechanisms. This view was propounded in very general terms (Pearson, Los Angeles) but more precise defects were proposed. Thus a physiological response might be superseded by a genetically determined proliferation of lymphocyte clones of an autonomous nature (Mackay and Whittingham, Melbourne). A variation on this theme is to propose that the same virus which induces these antigens that provoke an immune attack may grow in the lymphocytes that mediate the response; consequently the normal regulatory mechanisms may be destroyed (Marmion, Edinburgh). There is indeed preliminary evidence that a defect of suppressor T lymphocytes may be the primary abnormality in systemic lupus erythematosus, although the data used to support this claim are open to a variety of interpretations (Lightfoot, Milwaukee).

Steward (London) reviewed the factors which may affect the formation, deposition, and clearance of immune complexes, and Turk (London) discussed alterations in T and B cell interactions using leprosy as a model. In lepromatous leprosy T cell depression is associated with increased immune complex formation, uveitis, cutaneous vasculitis, and arthritis. Zwiakler (San Diego) described recent work showing that T cell helper functions and macrophage involvement in B lymphocyte responses in the mouse were mediated by soluble factors which were capable of reacting with human cells with obvious implications for human disease. Similarly, improved isotope techniques used for measuring lymphocyte traffic through different tissues in experimental animals (Ford, Manchester) might be adaptable for the study of lymphocyte recirculation in man.
Throughout all discussions of the relative importance of microbiology, genetics, and immunological mechanisms in the pathogenesis of connective tissue diseases it was apparent that many issues could be clarified by the formal demonstration that the histocompatibility antigens were acting as markers for immune response genes. A. Ebringer (London) was a strong proponent of the 'cross tolerance' hypothesis which postulates that animals produce poor antibody responses to microbial antigens which have structural similarity with their own histocompatibility antigens. Evidence was presented from the literature on immune responsiveness in laboratory animals and from experiments with a few selected antigens in inbred mice which appeared to support the co-dominant inheritance that would be predicted by the cross tolerance hypothesis. In ankylosing spondylitis antibodies to an organism in the gut or urethral tract with antigenic similarity to HLA B27 might cross-react with tissue antigens. Moreover, an impoverished immune response to the organism in question might then allow the formation and persistence of soluble complexes which would also contribute to tissue damage. Experimental evidence for cross-reactivity between human HLA B27 positive lymphocytes and Klebsiella was presented (A. Ebringer, London) and preliminary clinical studies were described (R. Ebringer, London), suggesting that Klebsiella could be isolated from the gut of individuals with ankylosing spondylitis before and during exacerbations of the disease. It was generally felt that an evaluation of the relevance of these provocative findings must await further controlled studies.

CONNECTIVE TISSUE PHYSIOLOGY
Attempts were made to survey current knowledge of the regulation of connective tissue metabolism. It is self-evident that tissue function depends on a balanced equilibrium between synthesis and degradation of its constituents, and the strong representation from the Max Planck Institute in Munich ensured that the importance of collagen in this regard was stressed. Muller reviewed the structure and synthesis of collagen and outlined how sophisticated biochemical and immunochemical techniques allowed the distribution and turnover of a number of genetically distinct collagen types to be analysed in detail. These have led to the identification of inborn errors of collagen metabolism in connective tissue disorders such as Ehlers-Danlos syndrome and osteogenesis imperfecta. Deshmukh and Nimni's controversial suggestion that there was a switch of collagen synthesis from type II to type I in osteoarthritic cartilage was alluded to, as well as Deshmukh and Muller's own experiments on the factors which regulate collagen gene expression in cultured chondrocytes. No clear picture of the mechanisms involved has yet emerged and at a time when improved biochemical techniques are delineating hitherto unsuspected collagen subunits in human and bovine cartilage it is premature to speculate on their possible relevance to arthritis and inflammatory connective tissue diseases. The notable absence of any proteoglycan chemists from the meeting left one with a rather unbalanced picture of connective tissue structure and function.

In reviewing the nature of the processes which erode cartilage and bone in rheumatoid arthritis, Saklatvala (Cambridge) emphasized how recent work at the Strangeways Laboratory had concentrated on the characterization of the serine proteinases elastase and cathepsin G which have the capacity to degrade collagen as well as the proteoglycan matrix at neutral pH. The physiological role of earlier defined acid proteinases such as cathepsin D were now thought to be limited to intracellular digestion. Work in Glasgow had shown neutral proteinase activity in rheumatoid synovial membrane but this activity is usually inhibited in synovial fluid by α₁-antitrypsin and α₂-macroglobulin inhibitors. Relatively little is known of the mechanisms of activation and inhibition of immobilized enzymes that are particle or protein bound. Wooley's recent work on the immunolocalization of collagenase to the pannus-cartilage junction was discussed and experiments were described suggesting that latent collagenase activity results from enzyme-inhibitor complexes, themselves susceptible to proteinase attack, rather than from a true proenzyme or zymogen (Murphy, Cambridge).

The potential roles of plasminogen activator, osteoclast activating factor and tumour angiogenic factor were briefly discussed and the possibility that lymphocyte infiltration might also contribute to cartilage destruction by stimulating collagenase production in rheumatoid synovial cells was raised by the experiments of Krane and his colleagues. There was scepticism about the extent to which an immune response to collagen may directly contribute to the characteristic lesions of rheumatoid arthritis. Adelmann (Munich) described some of the structural features of collagen molecules which determine their immunogenicity and stressed the methodological pitfalls encountered in studying the immunology of collagen. Clearly, data suggesting the presence of collagen antibodies and antigen-antibody complexes in serum and joints of patients with rheumatoid arthritis must be viewed with extreme caution.

A practical approach to the regulation of collagen biosynthesis in scleroderma involves the use of
INFLAMMATION AND CLINICAL PHARMACOLOGY

The sessions devoted to inflammation and clinical pharmacology did little more than act as a prelude for the more comprehensive consideration they were given in a subsequent one-day symposium on the recognition of antirheumatic drugs organized by Jasani (Horsham). The pharmacologists' view of the complex events involved in the acute inflammatory response were well reviewed (Lewis, London). Such relief as is afforded by nonsteroidal anti-inflammatory drugs is currently thought to result mainly from their ability to inhibit prostaglandin synthetase activity. The mechanisms causing chronic inflammation are more obscure but current interest in the pharmacology of prostaglandins has focused attention on the role of superoxide formation from arachidonic acid as one factor that may determine the onset of chronicity. The mechanism of activation of macrophages by lymphokines was also considered (Turk, London). There was no agreement as to whether chronic inflammation resulted from a balance of acute inflammation and repair processes (Glynn, London) or whether acute and chronic inflammation were independent processes with separate sets of mediators (Turk, London) and it was generally acknowledged that good models of chronic inflammation were urgently required.

The weakness of the pharmaceutical chemists' current approach to the development of new agents was emphasized. In a pathological process as complicated as rheumatoid arthritis it seems unlikely that therapy directed against a single mediator of inflammation would be successful (Pearson, Los Angeles). In the present state of knowledge, however, his plea for a search for agents which eradicate the primary cause, prevent initial tissue injury, and enhance repair processes rather than just moderating the acute inflammatory response is idealistic. Nevertheless, a shift in emphasis from simple nonsteroidal anti-inflammatory agents to the more slowly acting drugs was discernible among clinicians and pharmacologists alike. There was general agreement that the mode of action of gold, penicillamine, immunosuppressives, and even 'immunomodulating' drugs like levanisole were not understood. The clinical value of all the 'second line drugs' for rheumatoid arthritis remains largely empirical.

EPIDEMOLOGY AND INTERNATIONAL CO-OPERATION

Studying the distribution of connective tissue diseases has long been considered an important method of investigating these disorders. The participants at this meeting came from many parts of the world and some fascinating differences in the distribution of such diseases emerged from their reports. Two features in particular were emphasized. Firstly, there are grounds for believing that these diseases change in populations in underdeveloped countries during the process of adopting a western life-style. This trend was foreshadowed by the investigations of Greenwood in Nigeria and Brighten and colleagues in South Africa. Their results have been extensively confirmed in Nigeria (Onyewotu, Zaria). A survey by Kanyerezi (Kampala) indicates that the pattern of connective tissue diseases now encountered in East Africa resembles that familiar to rheumatologists in developed countries. In a 10-year survey substantial numbers of patients were seen with rheumatoid arthritis, juvenile chronic arthritis, and connective tissue diseases involving multiple systems. A similar experience was reported from Kenya.

The second feature of interest to emerge from these epidemiological studies was the variation in pattern of clinical and laboratory features in patients with ostensibly similar forms of inflammatory arthritis in different parts of the world. The factors which determine these differences may be fairly obvious. Thus in West Africa a high incidence of hypergammaglobulinaemia, rheumatoid factor, anti-nuclear antibody, and circulating immune complexes is observed in control subjects reflecting endemic parasitic infection (Onyewotu, Zaria). These features not only confuse the significance of the laboratory abnormalities detected in patients with arthritis but probably modify the natural history of the disease. However, not all the variations have an obvious explanation. Thus amyloidosis is a serious complication of juvenile chronic arthritis in the United Kingdom and Scandinavia (Ansell, Harrow) but is rarely seen in such patients in North America. Genetic factors are clearly relevant; thus amyloidosis more frequently accompanies familial Mediterranean fever in Sephardic than in Ashkenazi Jews (Pras, Tel Hashomer). Similarly, the joint complications of hypogammaglobulinaemia vary in different populations. Contrary to the experience of clinicians in other parts of the world, Petty (Winnipeg) reported that 25% of the patients with this disorder seen at his centre developed chronic arthritis. Moreover, this affected a limited number of large joints which were resistant even to intensive treatment with gammaglobulin. A high percentage of his patients with juvenile chronic arthritis had IgA

D-penicillamine, which it is claimed cleaves newly formed aldimine derived cross links and so presents progression of the disease (Jayson, Bristol). Unfortunately time did not allow for the critical discussion that this work deserves.
deficiency with anti-IgA antibodies, again a feature not universally recognized.

Such variations are of considerable interest since they might reflect a different aetiology in various parts of the world, or varying genetic factors in susceptible populations exposed to a common factor. For example, the relationship between acute self-limited polyarthritis in West Africa and similar syndromes in temperate zones is unknown. The unique situation of some centres in Latin America was emphasized (Alarcon-Segovia, Mexico City), where clinical expertise and laboratory facilities are available in areas where there are often large numbers of cases of untreated systemic lupus erythematosus and other connective tissue diseases. There is always the criticism that apparent regional differences in the frequency and severity of rheumatic disorders result entirely from problems of ascertainment. Thus there was general agreement that the careful collection and collation of clinical and laboratory data throughout the world has to be standardized. A new classification of the rheumatic disorders enjoying universal acceptance is shortly expected and should greatly ease this task.

BIOMEDICAL COMPUTATION
There is some difference in opinions about the extent to which disease classification will be aided by biomedical computation. Any suitable system must be sufficiently simple and flexible for it to be used accurately and routinely in busy clinics. Conversely the data must be gathered and analysed in a way that permits unequivocal conclusions without recourse to costly reappraisals. Not all observers agreed about the scope and extent of this kind of analysis. Enthusiasts such as Cats (Leyden), Hess (Cincinnati), and Mackay (Melbourne) believed that such computations allow factors predisposing to disease to be correlated in a way which transcends clinical acumen and observation. Others, notably Wood (Manchester), were more sceptical. The majority considered that such techniques are valuable, indeed indispensable, in carefully planned studies where data are accumulated to answer precise questions; specific projects relating to inflammatory disease of the spine were cited by Currey (London) and Jayson (Bristol).

In considering the possibilities for the use of computers for research, and as educational and informational tools the need for a uniform vocabulary was recognized if international co-operative systems were to make full use of national registries and data banks. Current systems such as ARAMIS (USA) and the punch card system (Belgium) function at very different levels of sophistication.

CLINICAL AND THERAPEUTIC STANDARDIZATION
The deliberations concerning therapy were sombre and realistic, reflecting the same difficulties as those discussed in relation to epidemiology. The unacceptable morbidity attached to treatment with most antirheumatic drugs is now well recognized and was emphasized by Kay (London) and Dunne (Geneva). The difficulties in deciding how such treatment acts was cogently illustrated by Scott (London) whose studies have shown that the benefits attached to the injection of yttrium 90 may result from the preliminary joint irrigation. In assessing the results of treatment the guiding principles most cogently advocated were that the methods should be simple (Huskisson, London) and even perhaps that the patient knows best (Carson Dick, Glasgow). In addition, the benefits of international collaboration apply as much to therapy as to clinical investigation. An example of such collaboration is the European trial of levamisole.

This interdisciplinary meeting did not solve any problems but it did at least induce a greater sense of realism among many investigators who had not previously had their work subjected to the detached judgement of scientists in related fields. Such realism is an indispensable foundation for progress.