

# Heberden Oration, 1974

## Human arthritis applied to animal models

### Towards a better therapy

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It is curious that today we are entrapped into a system that provides for essentially ineffective anti-inflammatory agents. Thus it was many years ago that it was noted that the willow tree flourished under damp conditions; the very conditions which were believed to provoke arthritic episodes. From these two beliefs it seemed inevitable to take an extract of the bark of the willow tree and treat arthritics. The era of the 'aspirin-like' drugs had arrived. This opening paragraph has been unashamedly stolen from an earlier lecture by Professor E. G. L. Bywaters on the development of the salicylates.

The realization of the effectiveness of the salicylates unfortunately led to a disservice to those actively engaged in the search for new therapeutic agents. All workers became caught up in a field where every new experimental model of inflammation had to fulfil the criteria of showing salicylate activity. Thus for years groups of workers have assaulted animals with a variety of irritants into various sites. As long as the subsequent reaction could be suppressed by salicylate it was seized upon as a potential new test system. Groups have exercised their ingenuity over the years in an attempt to explain the mode of action of these drugs. Each new step in the analysis of the inflammatory reaction has been used in the search for the mode of action of these 'aspirin-like' drugs. Pharmacologists have endlessly pursued the effect of salicylates upon various mediators of the inflammatory response. More recently attention has focused upon the cellular kinetics of the chronic reaction. The tests used to study the mode of action of steroids have ranged from the effect of these compounds on biochemical parameters such as oxidative phosphorylation (Adams, 1958; Dawkins, Gould, Sturman, and Smith, 1967), the enzymatic formation of pharmacologically active mediators, the effect on immunological responsiveness. A great deal of interest has lately been provoked by the work of Vane and his colleagues (see Vane, 1972) who showed clearly an inhibition of the prostaglandin-forming

system by aspirin-like drugs. It remains to be seen which of these many hypotheses will stand the test of time. Certainly the realization that the ubiquitous family of prostaglandins may modulate the inflammatory response by the process of mutual antagonism is significant (Velo, Dunn, Giroud, Timsit, and Willoughby, 1973). In addition Prostaglandin E (PGE) may provoke a rise in intracellular cyclic AMP (Weissmann, Dukor, and Zurier, 1971), which is essentially an anti-inflammatory process; this seems difficult to reconcile with the role of these fatty acids as possible mediators. Despite all these efforts it is not possible to state clearly today the mode of action of these compounds.

A further mistaken concept has opened another new pathway of anti-inflammatory therapy, namely the mode of action of penicillamine. This interesting, albeit toxic, drug appears clinically to have a specific activity directed towards rheumatoid arthritis (RA). Yet the basic rationale that it should act by lowering the titre of rheumatoid factor has not been established. A major problem with penicillamine and similar compounds is that of detecting activity in the animal model. E. C. Huskisson and others (unpublished, 1975) have recently shown the lack of correlation between the clinical effectiveness of penicillamine in RA and its ability to modify levels of rheumatoid factor (Fig. 1).

It appears that a major hold-up is occurring in the development of new anti-inflammatory therapy which is partially due to failure of communication between clinical rheumatologists and the basic scientists. A few years ago at St. Bartholomew's Hospital we developed the concept of scientists attending ward rounds and clinicians working at the bench. This integration was not easy. In the first instance two different languages were being spoken with attendant jargon. Each 'expert' was afraid to display his ignorance in the other 'expert's' field. Fortunately, with tact and patience this has been overcome; now the situation of free exchange exists.

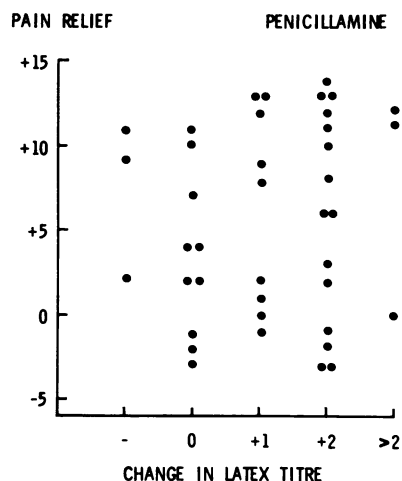


FIG. 1 *Lack of correlation between alterations in rheumatoid factor and clinical improvement of patients being treated with penicillamine (from E. C. Huskisson and others, unpublished, 1975)*

Several factors emerged almost immediately. Some of the scientific programme was outdated because a particular drug had outlived its usefulness in the clinic. Some of the parameters of measurement in the clinic did not bear close scientific scrutiny, e.g. certain scanning methodology. We drew up jointly the scheme shown in Fig. 2 and decided that what-

ever the causative mechanism these factors should be studied in animal models. In addition, a basic prerequisite was the study of the kinetics of the mononuclear phagocyte.

It seems from the work of Glynn (1968), Ziff (1973), Ruddy and Austen (1973), and others that antigen/antibody complexes capable of fixing complement might be involved in RA. Similarly, the presence of lymphokines in synovial fluid suggested the presence of a cell-mediated immune response. This meant that the basic worker should be considering complement as a proinflammatory mechanism plus the release of vasoactive and chemotactic factors released during the cell-mediated immune reactions. On the other hand, we were finding an increasing number of cases of pseudogout, some of which presented as classical clinical cases. Others seemed closer to classical RA and crystals could only be found after very close examination.

Curiously the urate crystals of gout described by van Leeuwenhoek in the 17th century and the subsequent simple string test of Garrod are easy to identify using the polarizing light microscope. Gout is not difficult to treat, and has even been described by McCarty (1973) as 'the physician's friend'. Yet many workers in their search for new anti-inflammatory drugs persist in following the course of inflammation provoked by these crystals of monosodium urate.

We found much more fascinating the array of crystals in the synovial fluid from patients with either

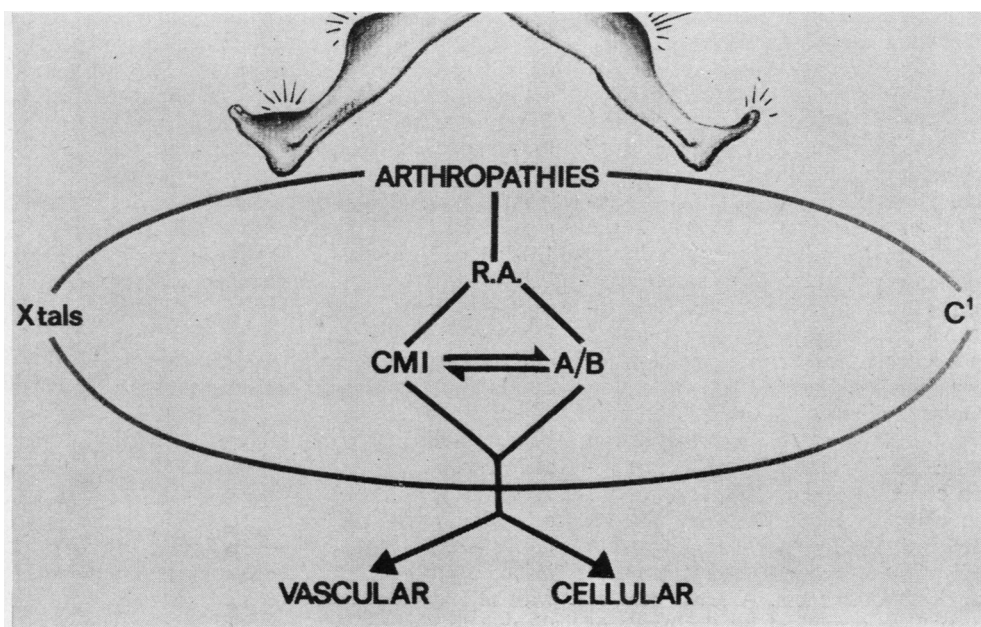


FIG. 2 *Some important factors worthy of study in the pathogenesis of the arthropathies. Xtals = crystals; RA = rheumatoid arthritis; CMI = cell mediated immunity; A/B = antibody; C¹ = complement titres*

pseudogout or RA. The identification of these crystals has proved to be a study in its own right. We have found what appears to be typical calcium pyrophosphate crystals with appropriate refringent properties (Currey, 1968). However, after *x*-ray diffraction analysis it was shown not to be identical to calcium pyrophosphate. This was then studied in greater detail by a group of workers from our department (P. Crocker, M. Archer, and P. Dieppe) who, using the Perkin-Elmer probe analysis system, found

that it did not contain calcium or phosphorus. On the contrary, sulphur, chloride, potassium, and only a trace of phosphorus were found. Fig. 3*a* and *b* show the typical probe analysis pattern of normal calcium pyrophosphate (CPPD) and the crystal which resembled CPPD under normal criteria. This curious phenomenon of crystals superficially resembling calcium pyrophosphate may represent new subclasses of joint disease requiring different therapy.

With this in mind we established a simple model to

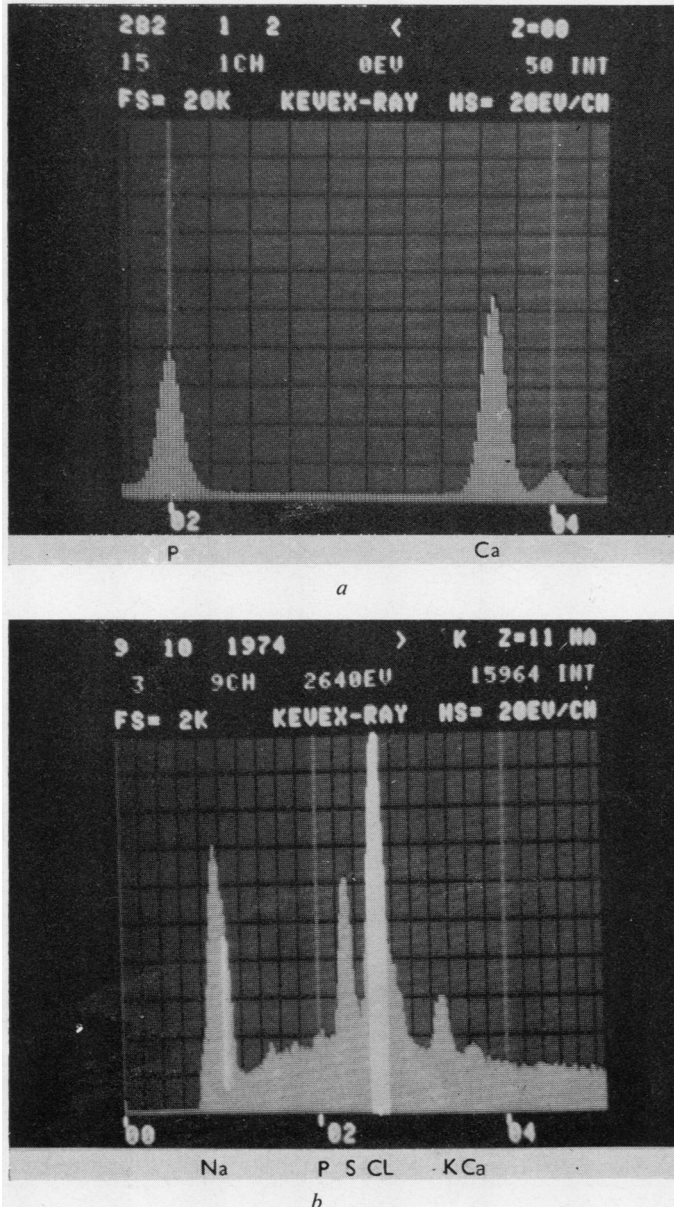


FIG. 3 Probe analysis pattern of (a) a classical calcium pyrophosphate crystal showing calcium and phosphorus as clear peaks, (b) an optically identical crystal from synovial fluid. Note the lack of calcium and phosphorus



study anti-inflammatory drugs, namely crystal-induced pleurisy (Willoughby, Dunn, Yamamoto, Capasso, Deporter, and Giroud, 1975). The reason for selecting the pleural cavity as a site of injection was the ease with which crystals and volume of exudate could be quantitatively measured. Fig. 4 shows the basic reaction to the intrapleural injection of calcium pyrophosphate. It can be seen that the injection provokes an acute reaction, reaching a peak at 6 hours, and is characterized by large numbers of polymorphonuclear cells. The reaction was found to proceed in the virtual absence of peripheral complement titres; this depletion was achieved with purified cobra venom factor, which reduced the complement titre by 96% throughout the experiments. Calcium pyrophosphate as an irritant has been used for many years by Phelps, McCarty and their colleagues, but the novel aspect in these experiments is the use of the pleural cavity.

A more standard irritant, intrapleural carrageenan, was chosen merely as a reference because previously

we had found that intrapleural injection of this irritant permitted a more precise quantitation of the inflammatory response than the conventional subcutaneous injection into the hind paw (Di Rosa, Giroud, and Willoughby, 1971; Velo and others, 1973).

Once again the reaction was maximal by 6 hours and then subsided (Fig. 5). Certain differences were apparent in the cellular sequence of events, e.g. the ultimate dominance of the mononuclear cells which contrasted with the crystal model described above. Carrageenan produces a complex inflammatory response, e.g. it causes activation of the alternate pathway of the complement system. Its long lasting effects are to produce a granuloma composed of long-lived mononuclear cells (Ryan and Spector, 1970) with a low turnover rate. For this reason Allison and Davies (1974) suggested that since carrageenan induced such a complex inflammatory reaction it had doubtful value in the understanding of the action of anti-inflammatory drugs. Depletion of the  $CH_{50}$  in this system produced a striking

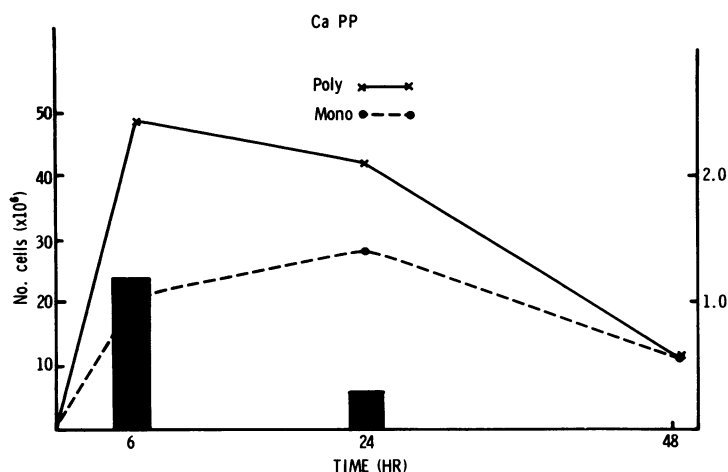


FIG. 4 The reaction to intrapleural calcium pyrophosphate. The histogram refers to volume of exudate (from Willoughby and others, 1975a)

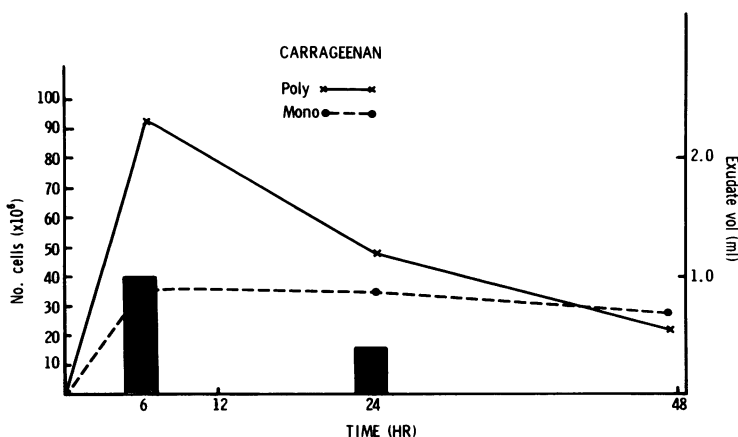


FIG. 5 The reaction to intrapleural carrageenan; histogram refers to volume of exudate (from Capasso and others, 1975)

reduction in the response, both vascular and cellular. Thus in contrast to the crystal model, this was a partially complement-dependent system (Capasso, Dunn, Yamamoto, and Willoughby, 1975).

The next model, developed in our laboratory largely by the efforts of Dr. S. Yamamoto and his colleagues (Yamamoto, Dunn, Capasso, Deporter, and Willoughby, 1975a), was cell-mediated immunity in the guinea pig. Normally this is produced as a cutaneous lesion which depends upon subjective assessment of erythema, induration, and at best an approximation of the number of cells from a histological section. There have been a few exceptions to this method of inducing delayed hypersensitivity (Apicella and Allen, 1969; Leibowitz, Kennedy, and Lessof, 1973). We decided to use the pleural cavity of the guinea pig and thus could reasonably standardize our different models. The method was to sensitize the guinea pigs with complete Freund's adjuvant and then challenge with PPD intrapleurally. In marked contrast to the other models this reaction was delayed in onset, reaching a maximum at 18–24 hours (Fig. 6). The dominant cell type was the mononuclear cell. Reduction of complement failed to effect the reaction in those guinea pigs treated with cobra venom factor. Macrophage inhibition factor was detectable in the exudates (a study shortly to be published by Yamamoto, Dunn, and Willoughby, 1976).

To study immediate hypersensitivity the reverse passive Arthus reaction was performed in the pleural cavity of rats. Once again this permitted the precise quantitation of cells and exudate. Highly purified rabbit anti-bovine serum albumin (BSA) was injected intrapleurally into rats 20 minutes after intravenous injection of BSA (Yamamoto, Dunn, Capasso, Deporter, Giroud, and Willoughby, 1975b). This reaction was again rapid in onset, becoming maximal at 6 hours, dominated by polymorphonuclear leucocytes. Depletion of complement titres reduced the inflammatory response when adjudged

by volume of exudate and numbers of cells (Fig. 7).

We were thus armed with a battery of tests which had all the appropriate characteristics expected of each model. From clinical experience the crystal arthropathy was predictably very acute. The cell-mediated immunity was dominated by mononuclear cells delayed in onset, and associated with macrophage inhibition factor activity. The Arthus reaction showed immediate hypersensitivity, was fast in onset, and at its peak was dominated by polymorphonuclear cells. The models were appropriately depen-

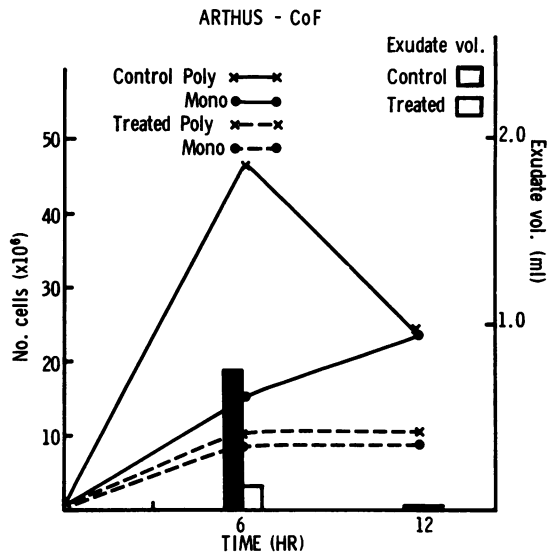


FIG. 7 The reverse passive Arthus reaction produced in the pleural cavity of rats and the effect of cobra venom factor (CoF) on the response. Histogram refers to volume of exudate. Note suppression produced by lowering the peripheral complement titres (from Yamamoto and others, 1975b)

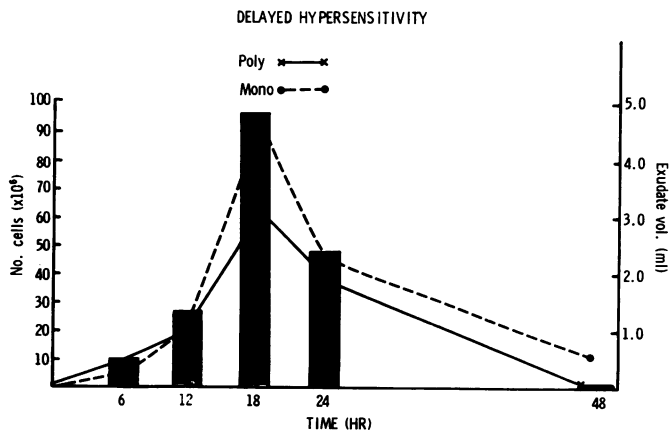


FIG. 6 Cell mediated immunity produced in the pleural cavity of guinea pigs. Note the delayed response which contrasts with the other models (Figs 6, 7, and 9) (from Yamamoto and others, 1975a)

dent or independent of complement as predicted. The main advantage of these tests was the site of inflammation, which permitted easy and accurate harvesting of the exudate and cells counted and differentiated with precision. The exudates so collected were analysed for various parameters, which are reported elsewhere (Willoughby and others, 1975; Capasso and others, 1975; Yamamoto and others, 1975a, b).

The reactions had common features in the release of the mediators (Capasso and others, 1975). There

was a sequential release of histamine, 5-hydroxytryptamine, kinin, and prostaglandins. There were variations in the time interval between the release of these mediators. The Arthus reaction which was maximal at 6 hours had a massive release of histamine throughout the early period and it was evidently an important mediator. On the other hand, in the model of delayed hypersensitivity the release of histamine was early and transient, followed by release of prostaglandins (Figs 8–11).

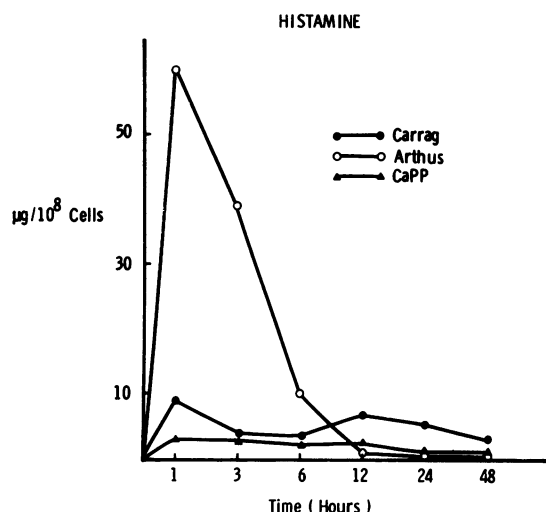


FIG. 8 Intracellular histamine concentration of exudate cells after intrapleural injection of carrageenan, calcium pyrophosphate, or the reverse Arthus reaction (from Capasso and others, 1975b)

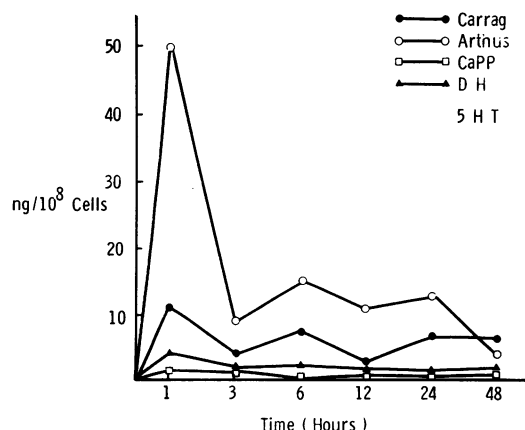


FIG. 9 Intracellular 5-hydroxytryptamine concentration in pleural exudate cells at various times after intrapleural inflammation provoked by carrageenan, Arthus, calcium pyrophosphate, or cell mediated immunity (from Capasso and others, 1975b)

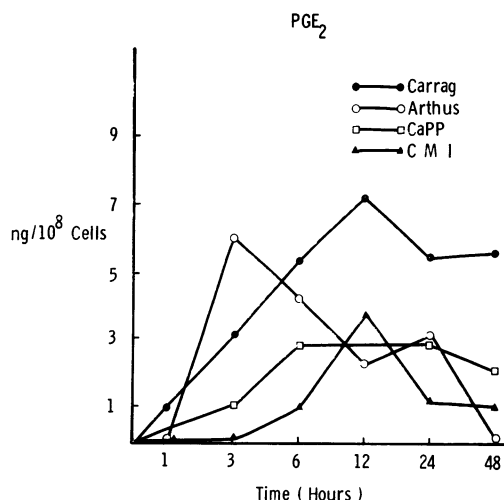


FIG. 10 Intracellular concentrations in pleural exudate cells of PGE<sub>2</sub> at various times during the inflammatory response of the models used above

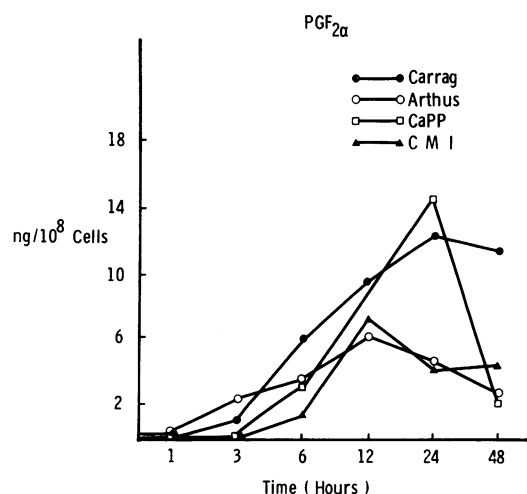


FIG. 11 As for Fig. 10, but intracellular concentrations of PGF<sub>2α</sub>. Note the delayed rise in the PGF type which could be modulating the proinflammatory effect of the PGE type shown in Fig. 10. The PGF is highest in concentration as each reaction wanes

Thus each of the models examined had different characteristics, and even the development of cyclic AMP within cells was unique unto each particular model.

The development of these different models as tests for the assay of anti-inflammatory drugs seems to be logical. The ultimate development of the crystal-induced pleurisy will be dependent upon the identification of the different crystals isolated from human synovial fluid. After their identification by the methods described above, diffraction patterns, probe analysis, etc., these crystals will then be injected into the pleural cavity of rats. It is hoped this will lead to better methods of treatment of those conditions in which crystals are observed, and is currently being pursued by P. Dieppe and his colleagues at St. Bartholomews.

Why the emphasis on the immunological models and what is their relevance in the search for new therapy? We agree completely with the hypothesis of a previous Heberden Oration by Glynn (1968), in which he proposed that inflammation could produce modified tissue proteins. These subsequently will not be recognized as 'self' and act as antigens with the perpetuation of an inflammatory response. Dumonde and Glynn's (1962) original experiments showed that an inflammatory response could be enhanced by prior sensitization with the products of an inflammatory reaction. We have extended these observations and shown that by injecting the products of an inflammatory granuloma into neonatal rats they become unresponsive to subsequent inflammatory stimuli (Giroud, Timsit, Spector and Willoughby, 1972).

The final important feature of chronic inflammation is a close study of the kinetics of the macrophage. Recently Allison and Davies (1974) proposed a scheme whereby the macrophage was envisaged as the central important cell in chronic inflammation. To include this important aspect of the inflammatory response we used a simple model of implanting glass cover slips subcutaneously into mice, described originally by Ryan and Spector (1970). The cover slips may be removed at various times after implantation and rapidly screened for the area covered by cells. In addition, closer examination using thymidine gives an indication of the rate of turnover of these cells. Normally one can count the total numbers of binucleated and giant cells. The application of this test to the assessment of anti-inflammatory drugs has recently been described in detail by Giroud and others (1973).

It is of interest that nonsteroidal anti-inflammatory drugs affect the earliest phase of the response, i.e. 2 days, but have little or no effect at 7 days. In contrast, the steroidal drugs have a more profound effect at 7 days. A novel type of anti-inflammatory agent has been shown to have activity, namely a slow-

acting type of anti-inflammatory. This requires pre-treatment with the drug to show activity. It nevertheless poses the question of how many compounds are missed because of the usual method of acute predosage being used in the screening procedure. When one considers the compounds which have gone directly to the clinic without having demonstrable activity in animals, a reappraisal seems to be justified.

Of what value is this animal data to the clinician, who is so often presented with a bewildering array of results from the laboratory with little or no explanation? The Table shows such a mixture of results that could conceivably be presented after a typical laboratory screen. It can be seen that using the test carrageenan paw oedema the product 'X' was negative, i.e. no anti-inflammatory activity. Similarly the compound 'X' had no effect on the primary lesion of adjuvant arthritis—once again not anti-inflammatory. In contrast there was marked suppression of the secondary lesions of adjuvant-induced polyarthritis. This suggests an immunosuppressant effect on cell-mediated immunity.

The investigators would then have turned to the model of Glynn arthritis and found that with constant dosing up to day 25 there was a suppression as measured by histology or thermography. If the arthritis was allowed to become established and treatment started on day 22, once again the compound would appear to be ineffective—not anti-inflammatory. The results using the implanted glass cover slip would be negative initially, but if a 5-day pre-treatment regimen was used, suppression would be observed. This mixture of activity which indeed we have seen recently should suggest a slow acting drug. Thus the clinician should not expect to see anti-inflammatory activity in too short a clinical trial.

If the drug displayed activity on, say, the delayed hypersensitivity reaction described above or in an immediate hypersensitivity reaction, the clinician should be prepared to monitor his patients for changes in responsiveness to cell-mediated immunity or to immunoglobulin levels.

There are a host of parameters easily measured in animals which if positive should be measured in man. Certainly in view of the variations existing among the

**Table** Typical profile of a drug (x) and the misleading data acquired

Treatment started (d)	Model	Result
1	Carrageenan	—ve
1	Adjuvant	1° —ve, 2° +ve
1---→	Glynn arthritis	+ve
25---→	Glynn arthritis	—ve
0	Coverslip	2 d ±, 7 d —ve
-5	Coverslip	2 d +ve, 7 d +ve
0	UV erythema	—ve

arthropathies it is important to seek correlations between the various parameters and the course of the disease. Our present philosophy is to carry out large-scale trials linked closely to laboratory screening and hopefully learn more of this baffling disease entity and why some patients respond to a new therapy and others do not.

Statistical analysis and correlations between effect of the potential new anti-inflammatory agent on various clinical parameters and laboratory findings are constantly being studied. It is hoped that this may reveal new subclasses of the arthropathies, each with distinctive pathogenic patterns requiring specific treatment.

Much of the foregoing may seem obvious but it is nevertheless amazing how neglected are some of these aspects. Certainly it is vital that scientists and clinicians work in each other's environment. Indeed

international collaboration in this type of study has proved exciting and intellectually stimulating.

In conclusion it is hoped that by extracting more information from the arthropathies in man and using this information to establish new test systems we will arrive at new therapy. This is in contrast to creating animal models which respond to the present classical nonsteroidal anti-inflammatory drugs, since these models can only lead to the development of further old-fashioned therapy.

This oration would not have been possible but for the collaboration of many colleagues. I particularly wish to thank Drs. C. J. Dunn, S. Yamamoto, E. C. Huskisson, D. A. Deporter, F. Capasso, and J. P. Giroud, and Professor W. G. Spector. Throughout this study we have received financial support from the Wellcome Trust, the Joint Research Board of St. Bartholomew's Hospital, INSERM Paris, and The European Biological Research Association.

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