Ann. rheum. Dis. (1968), **27**, 105

HEBERDEN ORATION, 1967*

THE CHRONICITY OF INFLAMMATION AND ITS SIGNIFICANCE IN RHEUMATOID ARTHRITIS

BY

L. E. GLYNN

M.R.C. Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks.

If inflammation is recognized by its classical cardinal signs, then few would deny that the rheumatoid joint is inflamed. Whether chronicity is assessed by duration in weeks, months, or years, there is equally no denying that rheumatoid arthritis is an example of chronic inflammation. To the student of chronic inflammation the problem of outstanding interest in any particular example is not necessarily what initiated the inflammatory response but the factors responsible for its maintenance.

Clues to the conditions responsible for chronicity can to some extent be discovered by a study of the factors interfering with the resolution and healing of what are usually regarded as acute, self-limiting reactions. Thus inadequate drainage of inflammatory exudates, interference with local circulatory dynamics, and the presence of necrotic tissue have long been recognized as major endogenous factors favouring chronicity. Since the joint is a closed space with limited drainage facilities, the retention of inflammatory products may therefore be an important factor in the maintenance of inflammation. The success of early synovectomy may well depend upon the removal of such products. Excessive use of an inflamed organ can also seriously impair its powers of recovery. Endogenous factors of this kind, however, take second place as causes of chronicity to those numerous micro-organisms which, by virtue of their structure or their function, or both, are able to establish a sort of symbiotic balance with their host tissues. Examples can be drawn from almost every subgroup of the microbiological world, from viruses to protozoa.

Another and distinct group of chronic inflammations is exemplified by gout with its aetiological relationship to a disturbance of uric acid metabolism. Here no exogenous agent is implicated and the various clinical manifestations are all attributable to the crystalline deposits of the excess uric acid. But even here the inflammation is at least at first intermittently acute rather than chronic, despite the inborn nature of the metabolic defect. Whether other varieties of recurrent or chronic inflammation arise on the basis of metabolic disturbances with local accumulations of metabolites not easily disposed of is a moot point. The comparable condition of ochronosis immediately springs to mind, but its pathological results are more usually regarded as degenerative than as reactive.

The chronic stages of inflammation in contrast to the acute stages are usually characterized by dense collections of mononuclear cells of either local or systemic origin. The continued presence and proliferation of these cells in the absence in many instances of any detectable cause, in rheumatoid arthritis for example, is reminiscent of the behaviour of a neoplasm. We know little of the nature of the stimuli responsible for the mobilization or proliferation of the cells so conspicuously present in chronically inflamed tissues, and it is conceivable that in some instances at least the fault may lie in the inflammatory cells themselves and the excessive ease with which they respond to sub-threshold stimuli.

Finally we come to certain immunological phenomena that may prolong inflammatory reactions. About 10 years ago Dr. Banerjee and I were studying the inflammatory response to implants of fibrin in experimental animals, and we were struck by the remarkable difference in the response to foreign fibrin as compared to the response to the homologous or autologous material. Whereas implants of the animals' own fibrin were rapidly invaded by fibroblasts and capillaries so that fragments of up to 100 mg. wet weight were completely replaced by fibrocellular scars in 1 to 4 weeks

*Delivered to the Heberden Society on November 17, 1967, at the Royal College of Physicians, London.

(Fig. 1), implants of foreign fibrin resisted such invasion and organization so that the scarcely altered material was still easily recognizable even after 16 weeks (Fig. 2). Moreover, a chronic

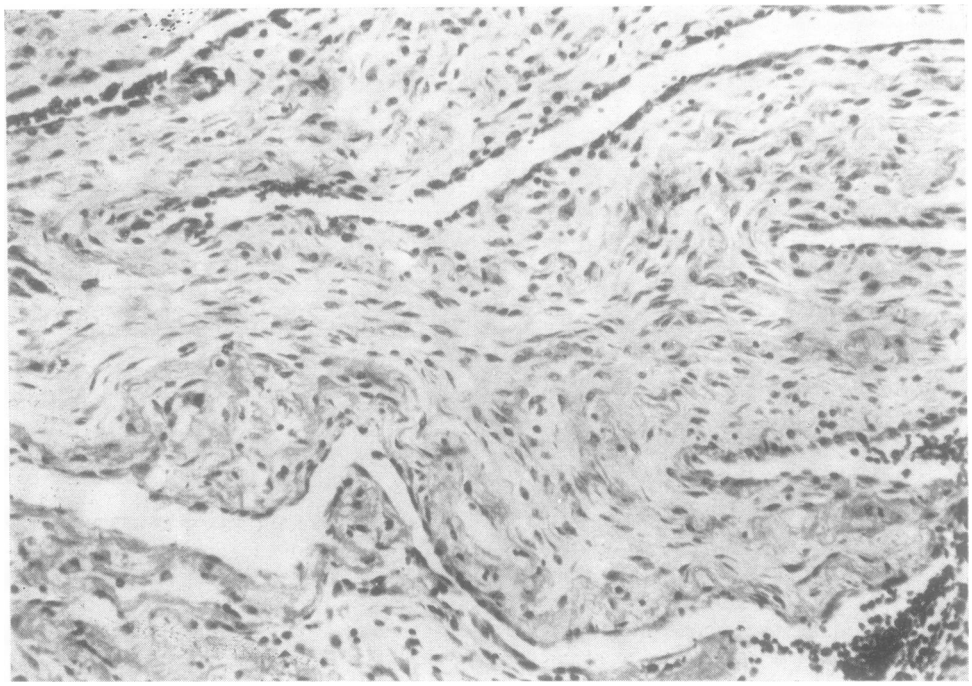

Fig. 1.—Autogenous fibrin implant in a rabbit removed after 7 days. The implant has been largely replaced by fibrocellular tissue and new capillaries. Haemotoxylin and eosin. · 70.

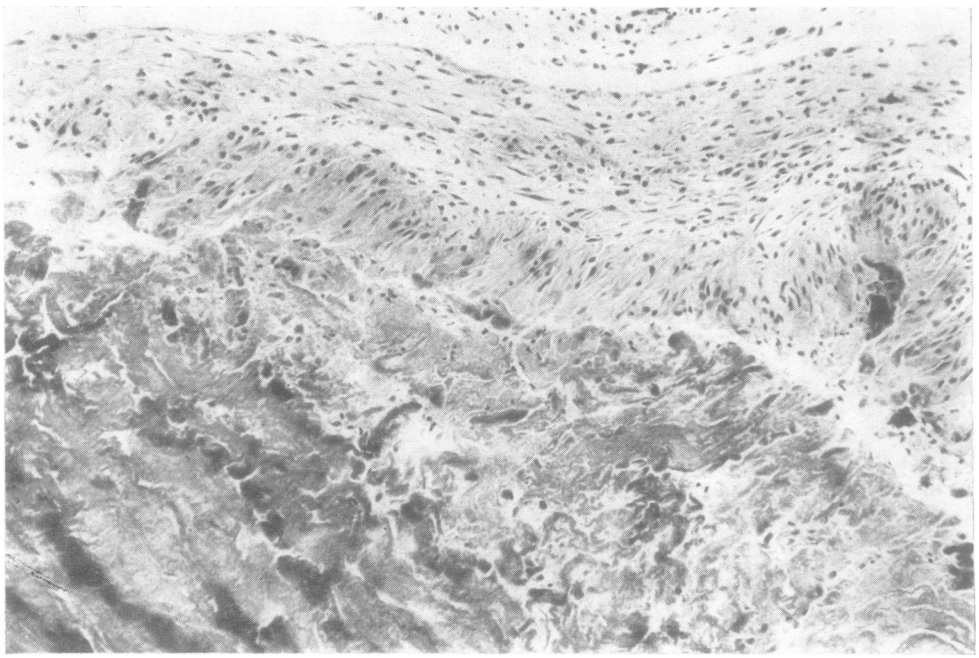

Fig. 2.—Implant of heterologous (human) fibrin after 4 weeks. The fibrin is still present but encapsulated by fibrocellular tissue with a well developed palisade at the margin of the fibrin. Haemotoxylin and eosin. · 70.
Reprinted from the *Ann. N.Y. Acad. Sci.* (1960), by courtesy of the Editors and Publishers.

inflammatory reaction was conspicuous in the adjacent tissues which showed hyperaemia, clusters of newly-formed blood vessels, and a varying degree of cellular infiltration in which lymphocytes and plasma cells frequently predominated. That the difference in the response to the two varieties of fibrin was attributable to their difference in immunological status was strongly suggested by the following observations (Banerjee and Glynn, 1960):

(*i*) No difference could be detected during the first 5 days after a first implant;

(*ii*) The reaction was accelerated when a second implant was made some weeks after the first implantation;

(*iii*) Plasma cells, known since 1948 to be mainly if not exclusively engaged in the production of immunoglobulins, appeared in large numbers in the host tissues around the foreign implant only;

(*iv*) Antibodies to fibrinogen and other serum proteins of the species providing the fibrin for implantation could be detected in the host animal's serum from about the seventh day onwards;

(*v*) Repeated implants from a single species might lead to the appearance of a necrotizing vasculitis in some of the small vessels around the implant (Fig. 3).

Some more recent experiments carried out in collaboration with Dr. D. C. Dumonde have shown that the absorption and organization of an individual animal's own fibrin can also be seriously impaired by an immunological reaction involving not the fibrin itself but other antigenic material present within the clot. In these experiments guinea-pigs received implants of their own fibrin which had been obtained by allowing plasma to clot in the presence of bovine serum albumin (BSA) thus leading to the passive incorporation of the BSA within the clot. Such implants were organized as readily as BSA-free clots unless the recipients had been previously immunized to BSA or received thrice-weekly intraperitoneal injections of guinea-pig anti-BSA antiserum prepared in other guinea-pigs. The reason for giving antiserum passively was to determine whether the impairment of organization in the actively immunized animals was due to humoral antibody or to sensitized cells. It is evident from the results that humoral antibody

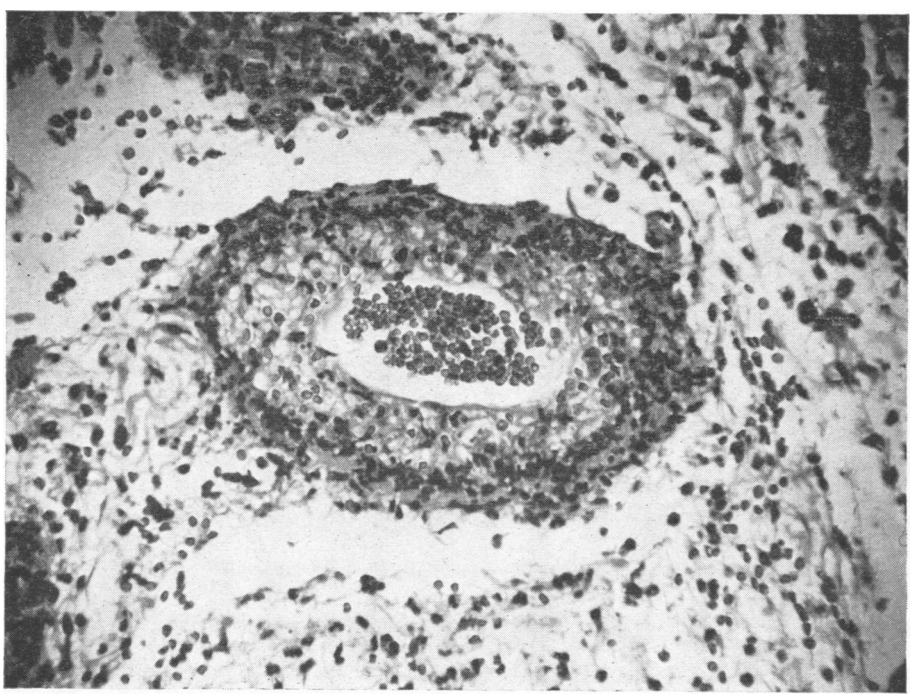

Fig. 3.—Acute necrotizing vasculitis in a rabbit in the vicinity of an implant of bovine fibrin. Two similar implants at monthly intervals had already been made into this animal. Haemotoxylin and eosin. ×275.
Reprinted from the *Ann. N.Y. Acad. Sci.* (1960), by courtesy of the Editors and Publishers.

alone is sufficient to account for the effects observed in the actively immunized animals (Figs 4 and 5).

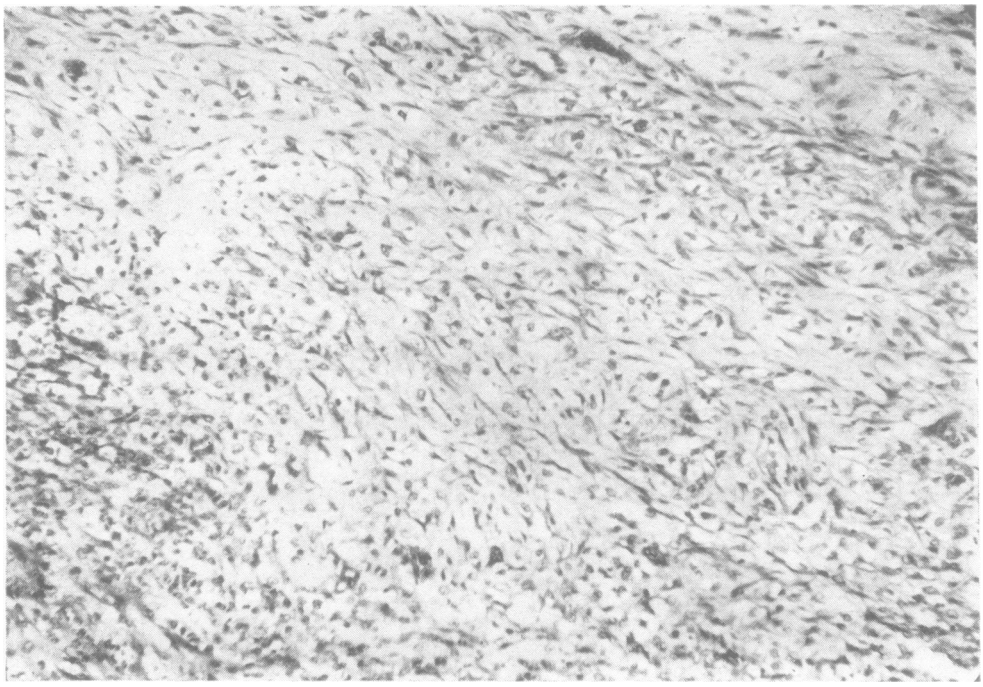

Fig. 4.—Implant of autogenous fibrin incorporating bovine serum albumin, in a guinea-pig removed after 13 days. The fibrin has been entirely replaced by richly cellular connective tissue. Haemotoxylin and eosin. × 70.

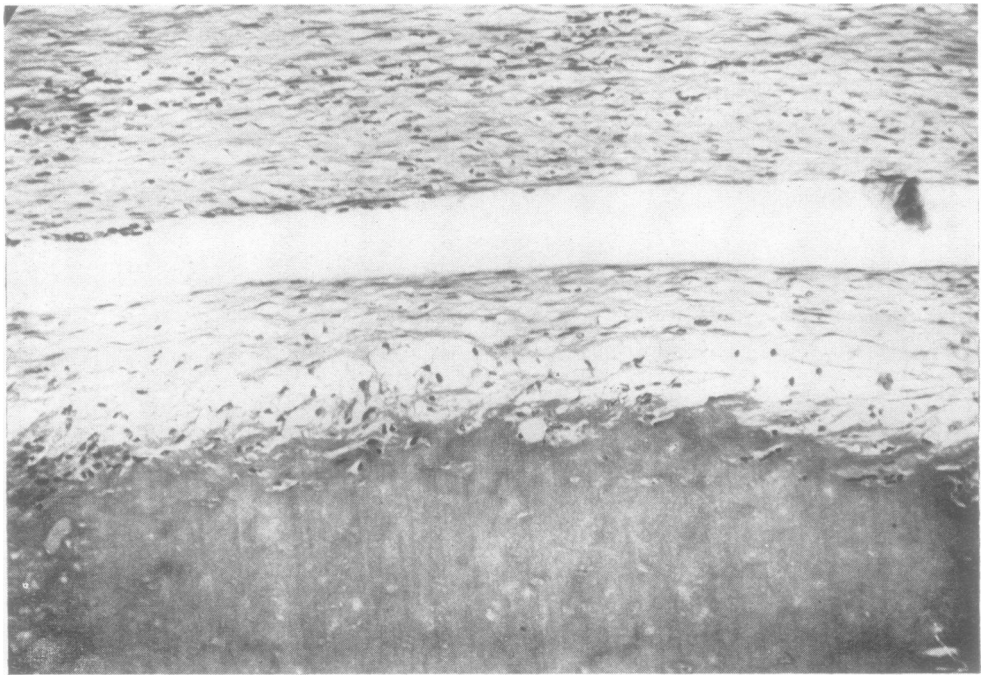

Fig. 5.—Implant similar to that in Fig. 4, but from an animal which had received repeated intraperitoneal injections of guinea-pig anti-BSA antiserum. The specimen was removed after 31 days but still shows persistence of the original implant surrounded by a thin fibrocellular capsule. Haemotoxylin and eosin. × 70.

This experimental situation in which antigen is previously introduced into the implant presumably reproduces a common natural event in pathology, since acute inflammation with its characteristic fibrinous exudate is so frequently the result of infection with highly antigenic microbial agents. Furthermore, the existence of rheumatoid factor in synovial fluid may well contribute in similar fashion to the persistence of fibrin within and upon the synovial membrane of the rheumatoid joint.

The histology of rheumatoid synovitis, although not pathognomonic, is nevertheless highly characteristic. The preponderance of lymphocytes in follicular and perivascular aggregates together with plasma cells, usually in considerable numbers, emphasizes the role of immunological reactivity in the pathogenesis of the lesion. The presence of immunoglobulins within the cytoplasm of the plasma cells, as revealed by specific fluorescent staining (Fig. 6) for the various immunoglobulin classes, confirms the immunological nature of their inflammatory response. The rare but well authenticated appearance of germinal centres in some of the follicular aggregations adds further support to this view. Their remarkable resemblance to the germinal centres that appear in lymph nodes in response to antigenic stimulation has been repeatedly commented upon. At present nothing but an antigenic stimulus is known to be capable of exciting the type of cellular response that characterizes the histological appearance of the rheumatoid synovium.

Since this same histological appearance can be present in a given site for months or even years, it must be concluded that a state of local immunological reactivity is being maintained. In our present state of knowledge such persistence implies an equally persistent antigen. This conclusion of course favours a self-reproducing agent as the cause of the disease and of its chronicity, but the continued failure to isolate such an agent with convincing consistency led us to consider alternative possibilities. It is conceivable that even in the absence of self-reproduction the insoluble residues of an infective agent could maintain a local antigenic stimulation of the degree of chronicity required. Some evidence of such persistence of bacterial residues was indeed found by us (Consden and Glynn, 1955) in the lung of patients with Caplan nodules and similar residues if present in rheumatoid synovia should also be capable of detection. Whether such insoluble

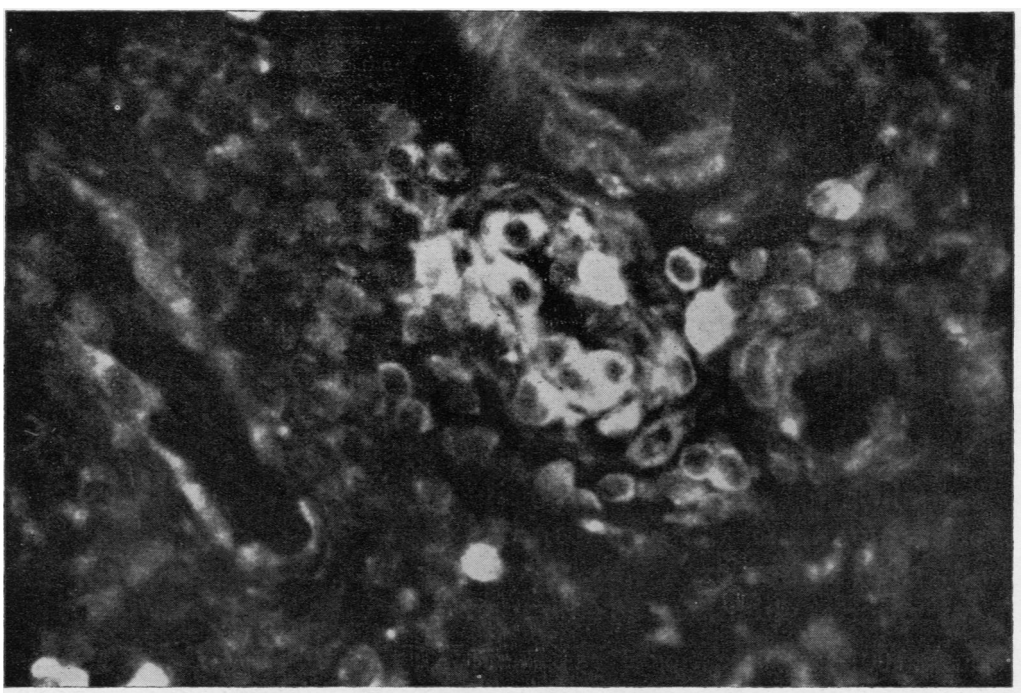

Fig. 6.—Synovial membrane from a human rheumatoid joint. The specimen was stained for rheumatoid factor by means of fluorescein-labelled heat-aggregated human γ-globulin (IgG). Note the positively stained clusters of plasma cells. Haemotoxylin and eosin. $\times 750$.

residues, if found, would retain antigenic capacity would still have to be established.

An essential part of the immune response is some processing of the antigen, probably by enzymatic breakdown within macrophages. Prolonged persistence of an antigen implies considerable resistance to such intracellular digestion so that it is doubtful whether the degree of chronicity shown by many rheumatoid joints could be accounted for on this basis.

The alternative to an exogenous antigen is, of course, an endogenous one. In this era of autoimmunity this possibility, that the antigenic stimulus is self-derived, has received wide consideration but only restricted acceptance, because of the limited nature of the evidence. Clinically, the only convincing evidence of an immune reaction against "self" antigens is the rheumatoid factor. This, as is now generally accepted, is an antibody against partly unfolded immunoglobulin G. Other autoantibodies, such as the antinuclear factor, also occur, but much less frequently. Nevertheless, the ability of a majority of rheumatoid subjects to produce autoantibodies of some sort is clearly established and can therefore be incorporated into any hypothesis as to the nature of rheumatoid arthritis without invoking the charge of baseless speculation.

This now brings us to the question of experimental arthritis. If rheumatoid synovitis is the result of an immune reaction occurring within the joint, a similar lesion should result from the experimental induction of an immunological reaction in an animal joint. This fact at least we have established unequivocally (Dumonde and Glynn, 1962). Rabbits were immunized with human fibrin and subsequently injected with similar fibrin into their knee joints. The ensuing arthritis shows how closely the experimental lesion in the rabbit resembles the natural lesion in man. The common features include:

(1) Lining cell hyperplasia (Fig. 7);
(2) Perivascular lymphocytes and follicles (Fig. 8, opposite);
(3) Diffuse plasma cell infiltration (Fig. 9, opposite);
(4) Pannus (Fig. 10, overleaf);
(5) Erosions (Fig. 11, overleaf).

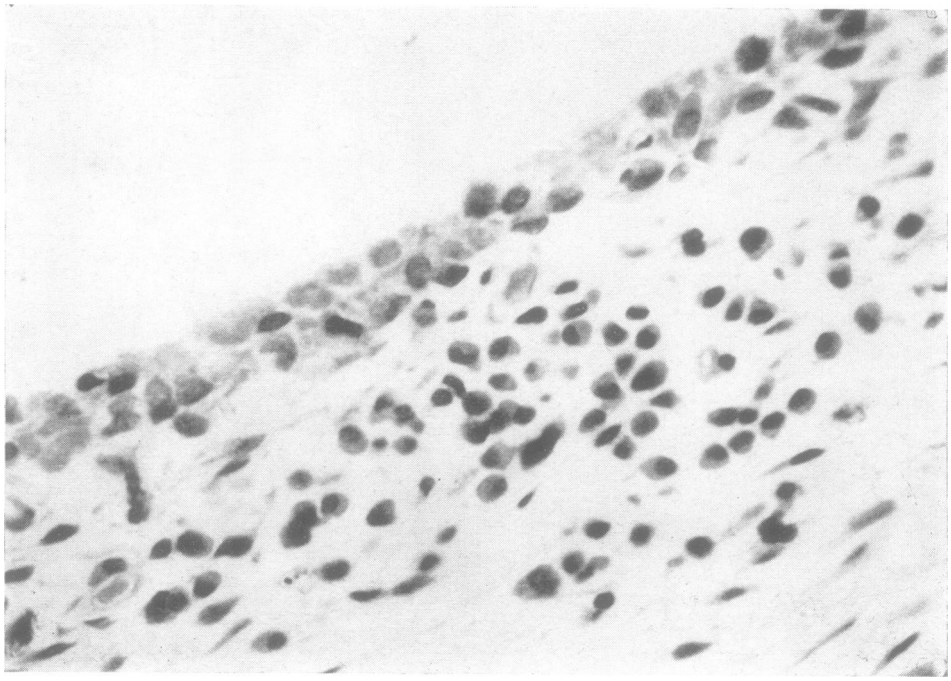

Fig. 7. Experimental immune arthritis in a rabbit. A sample of synovial membrane, showing hyperplasia and hypertrophy of the lining cells and a diffuse infiltration of plasma cells. Haemotoxylin and eosin. × 485.

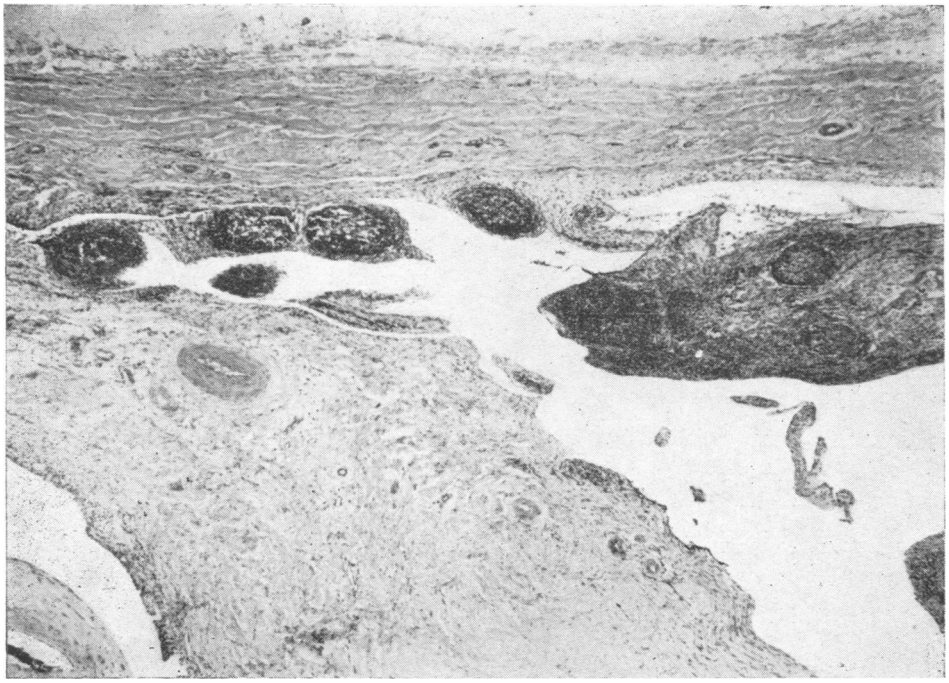

Fig. 8.—Coronal section of knee joint of a rabbit with experimental immune arthritis, showing follicular aggregations of lymphocytes in the synovial membrane. Haemotoxylin and eosin. ×70.
Reprinted from *Brit. J. exp. Path.* (1962), by courtesy of the Editor and Publishers.

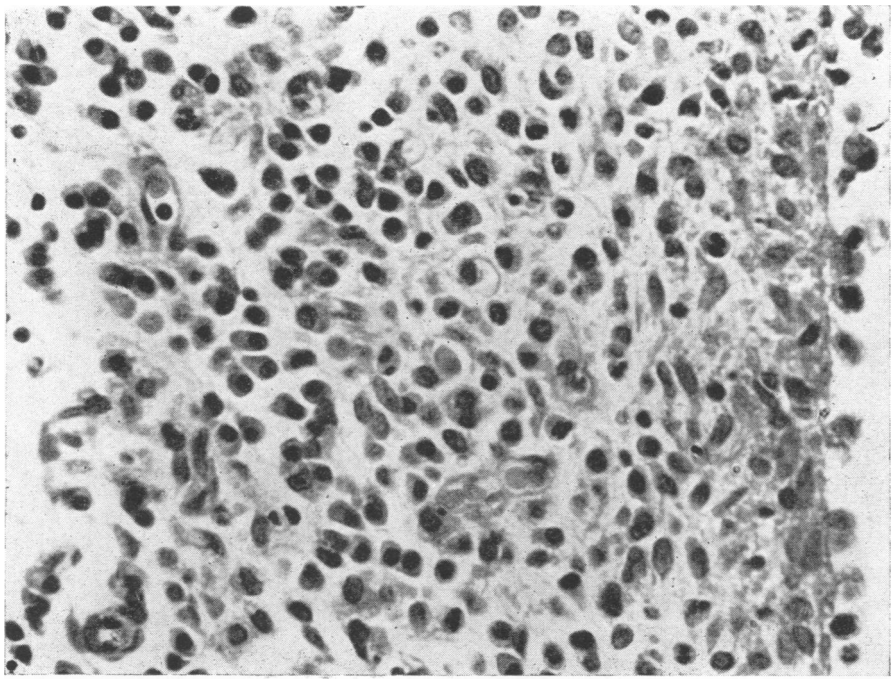

Fig. 9.—Synovial membrane of a knee joint of a rabbit with experimental immune arthritis, showing a dense and extensive cellular infiltration in which plasma cells predominate. Haemotoxylin and eosin. ×420.

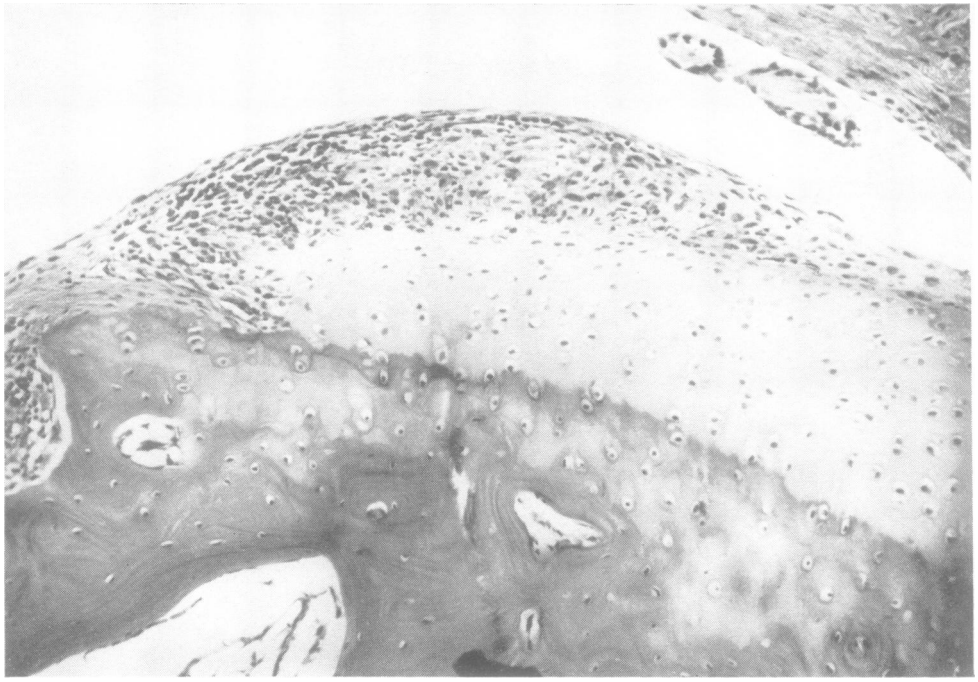

Fig. 10.—Articular surface at lower end of femur from the knee joint of a rabbit with chronic immune arthritis. A richly cellular pannus has extended across the articular cartilage. Haemotoxylin and eosin. ×120.

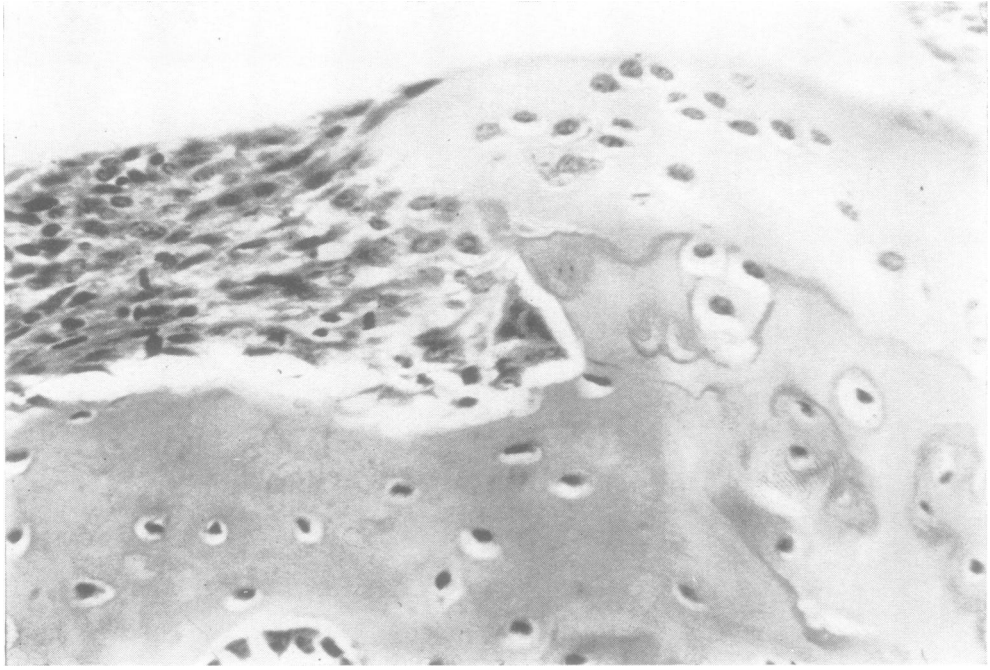

Fig. 11.—Specimen similar to that of Fig. 10 but from a different animal. Note the developing marginal erosion involving both cartilage and bone. Haemotoxylin and eosin. ×350.

Since similar experimental lesions have been produced in guinea-pigs and goats this capacity of the joints is probably general, at least amongst mammalia.

More remarkable, however, than the resemblance of the lesions to those of rheumatoid arthritis is their chronicity. After a single injection of antigen into the knee joint, the inflammation may still appear active 30 weeks later. This provides us with a unique opportunity to study the factors underlying chronicity in arthritis. The alternatives are clear. Does the activity of the inflammation continue because the injected antigen remains within the synovium? or does the activity continue after the antigen has been entirely eliminated?

Before going on to describe our attempts to answer these crucial questions, I must digress for a moment to consider the nature of the antigens capable of inducing a chronic experimental arthritis by our methods. We chose fibrin originally but it soon became apparent that serum proteins could replace fibrin without any change of result. Even non-mammalian protein, such as ovalbumin, can be used successfully, and in all our most recent experiments crystalline ovalbumin has been used because of the ease with which it can be obtained in a fairly high degree of purity.

We are at present engaged in studying the persistence of antigen when injected into a joint, but unfortunately the results are not yet available to provide a complete answer to the previously posed questions. We are using three different methods. In the first we are looking for surviving antigen by means of a fluorescein-labelled antibody. In the second the antigen is itself labelled with fluorescein. Both these methods depend upon microscopy involving a minute fraction of the whole joint. To obtain an overall assessment of the antigen remaining we propose to inject ovalbumin labelled with ^{125}I, and to estimate that remaining by determining the amount of label in an aliquot of the whole synovial membrane. Since ^{125}I has a half-life of 60 days, we should be able to detect even traces of antigen for at least that length of time. At present, by means of injecting fluorescein-labelled ovalbumin in normal rabbits knee joints, we can say that, although the antigen is readily detectable at 6 hours, it is undetectable by fluorescent microscopy at 24 hours. As we have seen from our experiments on the influence of antibodies on the removal of fibrin implants, it would be quite unjustifiable to assume that ovalbumin would disappear in 24 hours from the knee joints of an immunized animal. Nevertheless, in view of the soluble nature of the antigen (ovalbumin) and its extremely rapid disappearance from the normal joint, we feel it is highly unlikely that activity persisting for as long as 30 weeks can be attributed to persistence of the injected antigen.

Other workers have also commented upon the rapidity with which protein antigens are cleared from the knee joint. Thus Rodnan and Maclachlan (1960) found that ^{131}I-labelled serum albumin and γ-globulin were completely cleared from the normal rabbit joint in 72 hours, and Hollingsworth (1963), also employing radio-iodinated human serum albumin, found equally rapid removal from the joints of immunized as of non-immunized animals.

Those who are familiar with the older literature on experimental arthritis may well ask, as we asked ourselves, why our animals develop a chronic arthritis in response to a single intra-articular injection when all the early experimenters from Klinge (1933) onwards found that the inflammation rapidly subsided unless the injections were repeated at intervals of a few days. The answer undoubtedly lies in the difference in the technique of immunization. Earlier investigators all relied upon intravenous or subcutaneous injections without adjuvant. All our animals are immunized by intradermal injection of antigen incorporated in Freund's complete adjuvant, *i.e.* as a water-in-oil emulsion containing 2 mg./ml. dead tubercle bacilli. We therefore repeated our experiments omitting the adjuvant entirely in one group of six animals. The Table shows that the level of circulating antibody, as assessed by the tanned cell agglutination titre, was virtually identical in the group without and the group with adjuvant. Nevertheless, neither at 4 weeks nor at 8 weeks was there more than a trace of inflammation in any of the non-adjuvant group, whilst the adjuvant group all showed the usual chronic inflammatory picture (Figs 12 and 13, overleaf).

TABLE
TANNED CELL AGGLUTINATION OF IMMUNIZED RABBITS

Rabbit No.	Manner of Immunization	Tanned Cell Agglutination Titre
1	Subcutaneous in Saline	1 : 128,000
2		1 : 128,000
3		1 : 256,000
5		1 : 128,000
6		1 : 64,000
7	Intradermal with CFA	1 : 128,000
8		1 : 128,000
9		1 : 128,000
10		1 : 128,000
11		1 : 256,000
12		1 : 128,000

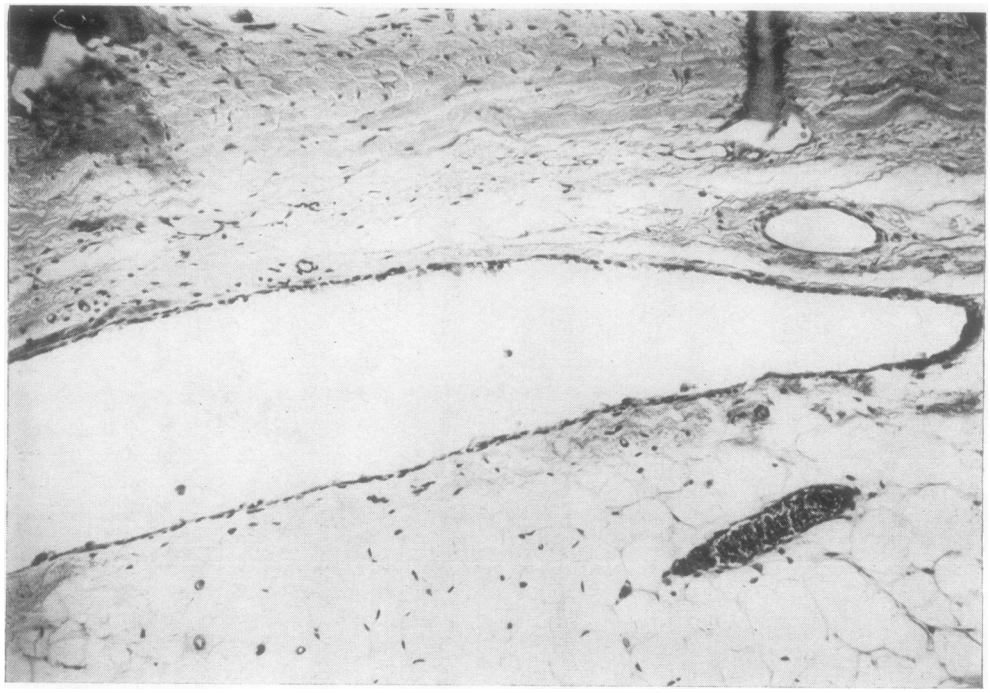

Fig. 12.—Posterior joint space of the knee joint of rabbit 179/5. This animal had been immunized with ovalbumin but without adjuvant and had then been given ovalbumin into the knee joint. 8 weeks later the joint is virtually normal. Haemotoxylin and eosin. ×70.

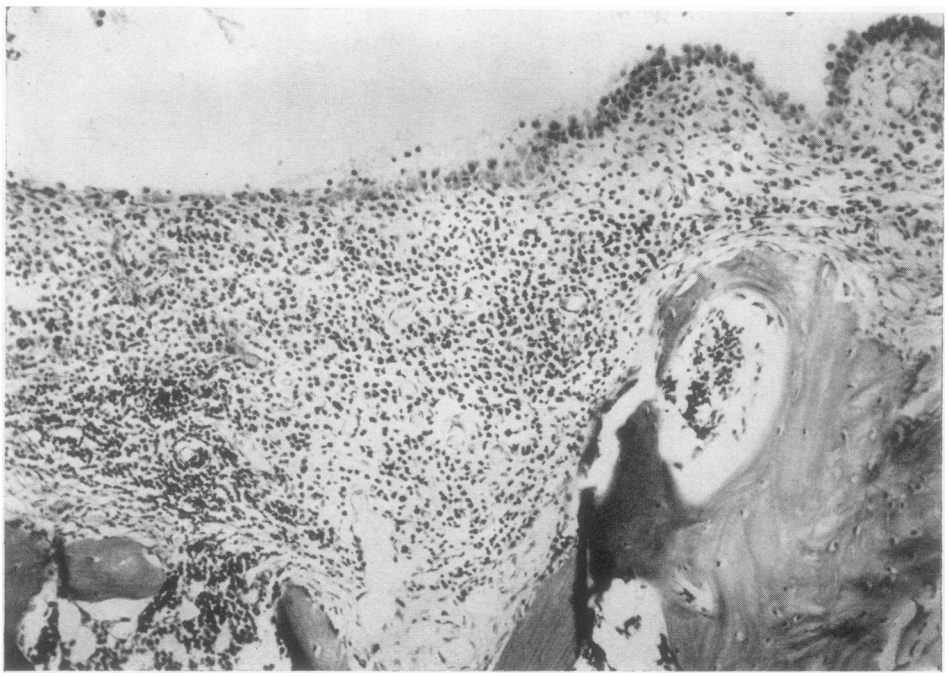

Fig. 13.—The same area of the joint as in Fig. 12, but from rabbit 179/9. This rabbit had received the same treatment as rabbit 179/5 except that the immunization included Freund's complete adjuvant. Note the severe synovitis. Haemotoxylin and eosin. ×70.

Perhaps the most important difference in the immune response to an antigen given with an adjuvant containing tubercle bacilli, as compared with the response in the absence of the bacilli, is the appearance in the former of delayed hypersensitivity to the antigen. Thus in the experiments just described, despite the equal development of humoral antibodies in the two groups, only the adjuvant group gave reactions of delayed hypersensitivity, and only this group of animals developed chronic arthritis as assessed at 4 and 8 weeks. Does this mean that delayed hypersensitivity to the intra-articular antigen is indispensable for the development of chronicity? or, if not, that the tubercle bacillus is essential for some other reason?

Evidence from other examples of experimental autoimmune disease also suggests that the sensitized cells responsible for delayed hypersensitivity play an essential role in the pathogenesis of the lesions. This has been quite clearly established for the experimental autoimmune encephalitis that follows injection of brain suspensions, the similar thyroiditis that follows injection of thyroglobulin, and the orchitis resulting from injections of antigens derived from testes. In all these experiments, lesions develop only when the immunizing schedule is such as to induce delayed hypersensitivity. As far as experimental orchitis is concerned, Dr. Brown, Dr. Holborow, and I have shown that the development of the testicular lesion requires the presence both of humoral antibody and of the sensitized cells associated with delayed hypersensitivity (Brown, Glynn, and Holborow, 1967). This was made possible by the discovery of the phenomenon of immune deviation. If an antigen, crude testis extract for example, is injected with complete Freund's adjuvant, skin testing with the antigen reveals the development of delayed hypersensitivity by about the fifth day and tests for humoral antibody in the serum become positive some 2 to 3 days later. These animals all develop testicular lesions by about the 14th day. If the tubercle bacilli are omitted from the adjuvant, the humoral response still occurs but neither delayed hypersensitivity nor testicular damage can be detected. Nor is the position altered by reimmunization with the antigen and complete adjuvant; the animals develop neither delayed hypersensitivity nor orchitis although antibody is present in readily detectable amounts. That the absence of testicular injury is due to the absence of sensitized cells in these animals was then shown by the passive transfer of sensitized cells from other guinea-pigs which had from the start received the same antigen with the complete adjuvant. Confirmation of this apparent necessity for both humoral antibody and sensitized cells was obtained by the passive transfer of humoral antibody to another group of guinea-pigs which, as a result of appropriate manipulation, showed only delayed hypersensitivity.

We have sought to answer the question whether delayed hypersensitivity is also essential for the development of chronic arthritis by the use of a polysaccharide antigen, since it is well established that such antigens, although sometimes capable of stimulating humoral antibodies, are incapable of exciting delayed hypersensitivity even when administered with tubercle bacilli. To this end we have employed a high molecular weight alginic acid (a polymannuronic acid prepared from seaweed) given with Freund's complete adjuvant in exactly the same way as we employ ovalbumin. Not only were we unable to detect any evidence of delayed hypersensitivity to the alginate, but humoral antibodies were also undetectable by the standard procedures of capillary tube precipitation, immunodiffusion, and agglutination of coated red cells. Nevertheless, five out of five animals given an intra-articular injection of alginate developed a chronic arthritis as assessed at 4 and 8 weeks. This was a highly unexpected result but was soon explained by the study of the nonimmunized controls. These clearly showed that a comparable arthritis could as readily be obtained by intra-articular injection of alginate into the nonimmunized animals.

If for the moment we put aside the results with alginate we can say that, at least as far as protein antigens are concerned, a chronic arthritis can be obtained only in those animals in which the presence of tubercle bacilli in the immunizing material has resulted in the development of delayed hypersensitivity. What we should like to know is whether these positive results are due to the delayed hypersensitivity or to some more subtle effects of the tubercle bacillus. We therefore sought to replace the tubercle bacillus by some other organism, in the hope that it, too, might result in delayed hypersensitivity to the accompanying antigen, *i.e.* to the ovalbumin in our experiments.

We have so far studied only two organisms and the results are incomplete. The first was *Haemophilus pertussis*, as this is known to exert a powerful adjuvant effect with several antigens when given intraperitoneally. With ovalbumin, however, it has proved a poor adjuvant and, although several animals developed sufficient antibody to give moderate immediate reactions of the Arthus type, none developed delayed hypersensitivity and none a chronic arthritis. The second organism tried was a diphtheroid isolated at Taplow from the synovial

fluid of a patient with rheumatoid arthritis. We were encouraged to test an organism of this group partly because Katsh, Crowle, and Katsh (1966) have shown that, of many organisms tested by them to take the place of acid-fast bacilli in the experimental production of orchitis, the most successful was one of the *Corynebacteria*, and partly because of the interest aroused in these organisms by their isolation from rheumatoid joints. Our results seemed to leave no doubt that these organisms can take the place of the acid-fast bacteria both for the induction of delayed hypersensitivity and for the development of our chronic arthritis.

I say "seemed to leave no doubt" advisedly, because there was still one control we had not studied, namely the use of Freund's incomplete adjuvant, *i.e.* a water-in-oil emulsion without bacilli. You will recall that when we used no adjuvant at all we obtained excellent titres of humoral antibody but no delayed hypersensitivity and no chronic arthritis. With the antigen given with incomplete adjuvant we likewise obtained no delayed hypersensitivity, despite the high titres of antibody. Nevertheless in each of two animals killed at 4 weeks there was unequivocal macroscopic evidence of arthritis. Twice-weekly measurements of the joints of the remaining animals, however, show very clearly (Fig. 14) that this arthritis is short-lived compared with that in the animals immunized with complete adjuvant containing either tubercle bacilli or the diphtheroid. Although this will need to be confirmed by subsequent autopsy and histology, the curves of joint swelling give a clear indication of the importance of this organism in the schedule of immunization.

Let us now return to the question of the chronicity of our experimental lesions. We have seen that persistence of the injected antigen in the knee joint, although not entirely excluded by our studies to-date, is unlikely to account for inflammation still active up to 30 weeks after a single intra-articular injection. We have considered two further alternatives:

(1) The appearance of secondary invaders or the activation of some latent infection;

(2) The development of an autoimmune reaction to one or more products of the inflammatory process or to some other local constituent released by the inflammation or local wear and tear.

We have not excluded viral or mycoplasmal agents but bacteriological studies have excluded both aerobic and anaerobic bacteria. In the absence of any demonstrable infective agent, we therefore incline to the view that the chronicity of our experimental arthritis results from the development of an autoimmune reaction following the initial phase of inflammation which develops in response to the foreign antigen.

What evidence is there that these animals are developing an autoimmune reaction, and if so, what is the auto-antigen? We have sought to answer these questions both directly and indirectly. Firstly we have established that experimental animals can be immunized with their own inflammatory exudate (Phillips, Kaklamanis, and Glynn, 1966). This was obtained by inducing a sterile inflammatory reaction in the subcutaneous tissues of the flank by injections of air followed by arachis oil containing 1 per cent. croton oil. Some 10 to 14 days later the resulting abscess was excised and the contents used for

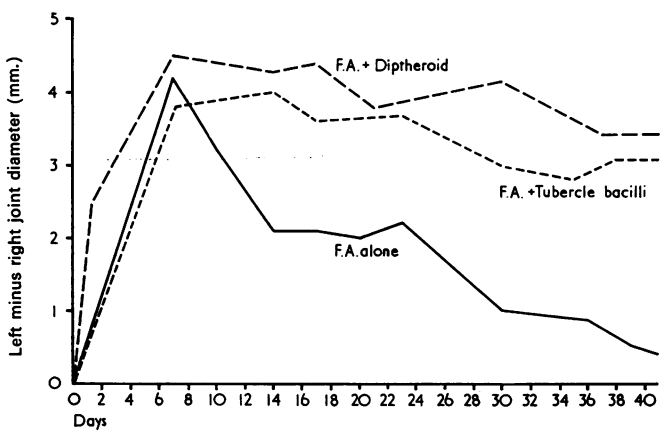

Fig. 14.—Graph showing the swelling of injected joints in three groups of animals immunized with ovalbumin in different adjuvants. The swelling shown is the difference between the transverse diameters of the injected (left) and uninjected (right) knee joints. It is evident that the arthritis rapidly subsides if the adjuvant is incomplete

immunization after incorporation in Freund's complete adjuvant. The animals so treated gave reactions of delayed hypersensitivity and several developed a chronic arthritis when the same material (without adjuvant) was injected into the knee joint. More direct evidence of autoimmunization has been sought in our animals with experimental arthritis by submitting their sera to one or other of the available tests for rheumatoid factor. Although the latex test was almost invariably negative, we have a number of animals in which a positive F.II test was obtained using tanned sheep cells coated with human F.II.

We are frequently asked, "If your experimental model is related in its pathogenesis to rheumatoid arthritis, why are the lesions confined to the injected joints?" At present we can only speculate as to the answer although clinical observations on the distribution of the lesions in the human rheumatoid subject may well provide a clue. There is no doubt that the surest and quickest way to "cure" an acutely inflamed rheumatoid joint is by its complete immobilization. Unfortunately it is equally certain that as soon as the joint is remobilized the condition will relapse. This influence of the active use of a joint upon its susceptibility to the rheumatoid process, whatever that may be, is especially well illustrated by the distribution of the lesions in patients partially paralysed before the clinical onset of their rheumatism. Whether the joint is rendered functionless by an upper or a lower motor neurone paralysis is apparently immaterial. In both instances there is a dramatic sparing of the affected limbs from the ravages of the rheumatoid process. An even more precise influence of the stresses of normal function upon the localization of rheumatic lesions is shown by the distribution of the vegetations in the cardiac involvement of rheumatic fever. Here, as in the rheumatoid joint, the cause of the damage is only vaguely understood. In both conditions, however, there can be no doubt that the individual is systemically involved and that the actual localization of the individual lesions is determined by purely secondary factors. The largely mechanical nature of these factors in rheumatic valvulitis is clearly shown by the order and severity of the valvular involvement which is directly correlated with the closing pressure of the individual valves, namely mitral > aortic > tricuspid > pulmonary. Moreover the severest cases of right-sided valvular involvement are almost invariably secondary to raised pulmonary pressure consequent upon previously established mitral stenosis.

Some recent experiments of my former collaborator Dr. P. Kaklamanis since his return to Athens also emphasize the role of joint activity in the localization of arthritis. He was concerned to discover the influence of exercise upon the arthritis that occurs in rats after a single injection of Freund's complete adjuvant. This condition, which has been extensively studied in the U.S.A. as well as in Great Britain, consists of a widespread polyarthritis involving both large and small joints; it is of a migratory nature and is frequently accompanied by nodular skin lesions. To avoid excessive variation litter mates were separated into experimental and control groups and both were injected with Freund's adjuvant. The control animals were housed in the standard rat cages, the experimental animals were allowed to run free in specially constructed runs. As far as possible all other conditions were the same for the two groups. Nevertheless the difference in the resulting lesions was striking. In speed of onset, extent, severity, and duration, the lesions of the experimental group significantly surpassed those of the controls. In a similar type of experiment in which heavy rats were compared with light rats, the severer lesions in the former again suggested the adverse effect of mechanical factors.

What has all this to do with the localization of the lesion to the injected joints in our experimental animals? Simply this. The relatively small size of our rabbit cages does not encourage our animals to take active exercise. A fairly prolonged observation of our experimental animals reveals a remarkable degree of immobile placidity. If active movement is an important factor in the localization of the rheumatoid process, then it is not surprising that our animals show little tendency for the affection to spread.

According to this view lesions should appear in the uninjected knees of our experimental animals if they could be induced to exercise them. Since we have not yet evolved a satisfactory method of exercising our animals, we have instead induced a mild non-immune inflammatory reaction in one knee and compared the result with that obtained in animals with and without immune arthritis in the contralateral knee. We first established that a single intra-articular injection of 10 mg. finely powdered silica produced a barely perceptible reaction at 4 and 8 weeks in a normal rabbit knee joint. We then studied the effect of a similar injection when the rabbit had already developed an immune arthritis in the opposite joint. The

experiment is not yet completed, but in two animals it is apparent that a greater response to the silica is occurring in the arthritic compared with the non-arthritic animals (Figs 15 and 16).

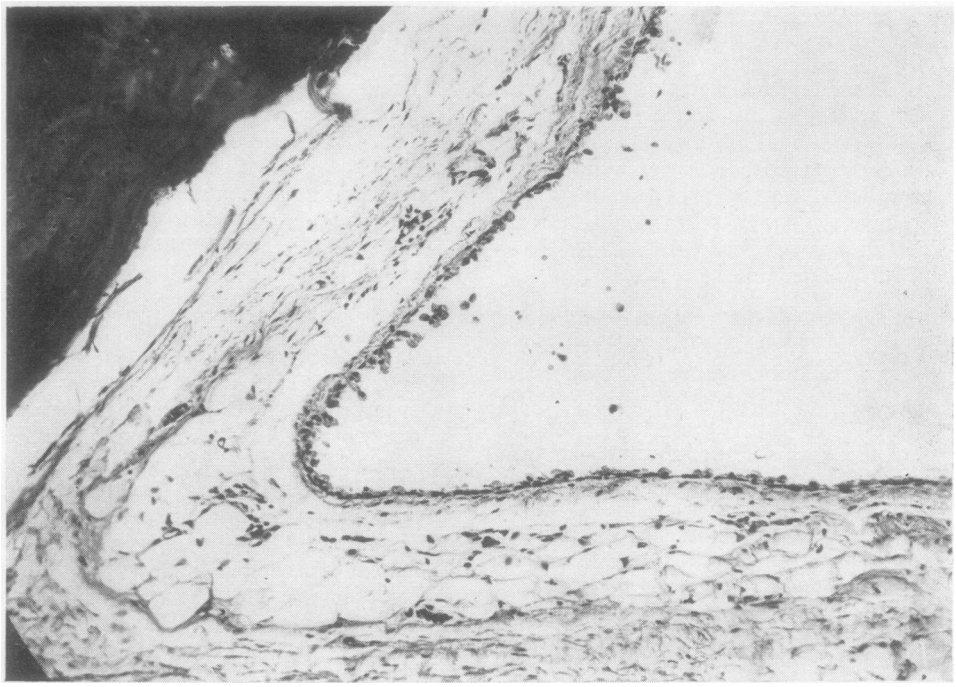

Fig. 15.—Posterior joint space of the knee joint of an unimmunized rabbit 8 weeks after the intra-articular injection of 10 mg. silica. There is some desquamation of lining cells into the cavity and a few scattered lymphocytes and histiocytes in the membrane. Haemotoxylin and eosin. ×70.

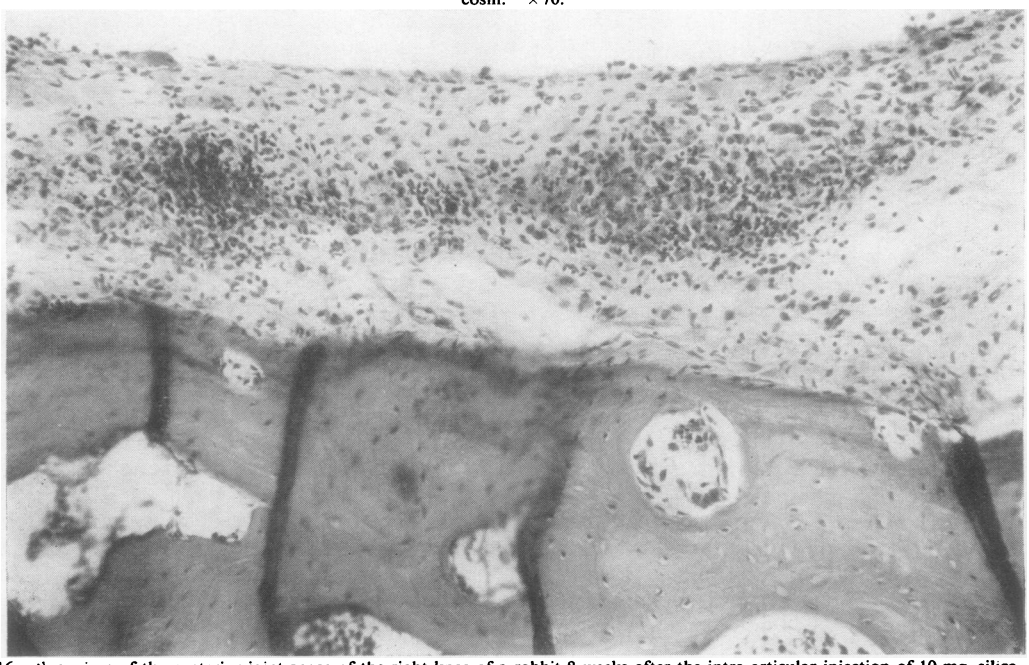

Fig. 16.—Synovium of the posterior joint space of the right knee of a rabbit 8 weeks after the intra-articular injection of 10 mg. silica. This rabbit had been immunized with ovalbumin and had an immune arthritis in the left knee of 8 weeks' duration. Note the considerable degree of inflammation with foci of macrophages surrounded by lymphocytes and plasma cells. Haemotoxylin and eosin. ×70.

The evidence, therefore, although not overwhelmingly conclusive, does support the view that, as a result of the experimentally-induced arthritis, the reaction of other joints to mild inflammatory agents is increased. In the absence of any other mechanism to account for this phenomenon, we regard it as supporting the hypothesis that some autoimmune reaction to an inflammatory product is the basis of the chronicity of our experimental arthritis.

We can now return to consider the role of the tubercle or other bacilli in the pathogenesis of our chronic arthritis. If we are correct in our conclusion that the chronicity is the result of the development of an autoimmune reaction to a product of inflammation or excessive wear and tear, then it is presumably with this autoimmune development that the bacilli are involved. What part is played by the associated delayed hypersensitivity it is not yet possible to assess. Some experiments on guinea-pigs by my colleague Dr. G. Loewi, making use of an antigen which induced delayed hypersensitivity but no humoral antibody, suggest that the cellular immunity responsible for delayed hypersensitivity is itself insufficient for the development of arthritis. Our own experiments with animals immunized without adjuvants have shown that humoral antibody alone is also inadequate for the development of a chronic lesion. This does not necessarily mean that both forms of immune response are essential. The situation is slightly different from that operating with the other experimental autoimmune lesions such as orchitis, thyroiditis, or encephalitis. In those experiments the autoimmune response is directed against the very organ incorporated in the immunizing material, namely testis, thyroid, or brain. In our experimental arthritis the postulated autoimmune component is not mediated *via* the immunizing antigen, *e.g.* the ovalbumin, but against some other component of the connective tissue or inflammatory product. In this situation at least two occasions for autoimmunization occur, the first at the immunizing site where a severe local inflammation develops, and the second in the inflamed joint itself. We have some evidence that the inflammation in the joint itself may significantly contribute to autoimmunization in those animals previously immunized with complete adjuvant. I have already mentioned that a single intra-articular injection of alginate in a non-immunized animal can produce a chronic arthritis indistinguishable, apart from the presence of the alginate, from the arthritis produced by a similar injection of ovalbumin in an immunized animal. An F.II test indicating an immune response to the rabbit's own γ-globulin is only weakly positive in the unimmunized alginate animals; even after 8 weeks of arthritis, the titre in each of four animals being only 1 : 50. Even in rabbits immunized with alginate by a method that results in specific humoral antibody but no delayed hypersensitivity, the F.II titre after intra-articular injection also did not exceed a titre of 1 : 50. In the rabbits immunized with alginate in complete Freund's adjuvant, the results were quite different. You will recall that five of these animals subsequently tested with intra-articular alginate all developed arthritis, despite the absence of any detectable immune response to this material. In the two animals killed after 4 weeks no anti γ-globulin, as detected by the F.II test, had developed. At 8 weeks, however, the titres in the three surviving animals were 1 : 200, 1600, and 6400, and in addition a positive latex-fixation test was obtained with the last serum. Although the number of animals involved is small, it is striking that titres regarded as diagnostic in the human were obtained only in the animals that had received Freund's complete adjuvant and only after 8 weeks of chronic arthritis, despite the absence of any induction of delayed hypersensitivity to the alginate itself. The conclusion, which at this stage can only be tentative, is that the importance of the adjuvant in the immunizing schedule does not lie in a capacity to induce delayed hypersensitivity but in its possibly unrelated capacity to favour the development of autoimmunization. This capacity of the organisms in the adjuvant is apparently exerted both locally and systemically. Our results with the F.II test in the alginate-injected animals imply a systemic effect, whereas in the experimentally-induced lesions of the thyroid, testis, or brain, for example, the organ antigens must be injected with the adjuvant, thus implying an essentially local effect on autoimmunization.

This postulated ability of tubercle bacilli and some other organisms to induce an autoimmune response to antigens in their vicinity can, since the work of Thewaini Ali and Oakley (1967), be regarded as an established fact. They have shown that, in rabbits infected with either *M. tuberculosis* or *P. pseudotuberculosis*, about one-quarter develop antibodies against a variety of tissue antigens present in liver, kidney, spleen, lung, and heart. There is moreover some evidence that these autoantibodies are directed against antigens modified by the infection of a particular organ, which accords precisely with what we have earlier postulated for rabbit γ-globulin.

It could reasonably be asked why, if the chronicity of the arthritis is the result of an autoimmune response to products of inflammation and tissue

destruction, the inflamed areas at the sites of immunization do not show a similar chronicity? The answer maybe is that ulceration and discharge of the necrotic and inflammatory mass removes the offending antigen, a result not unlike that produced in man by synovectomy of an affected joint.

We are still left with no adequate explanation of the development of chronic arthritis at 4 weeks in those animals in which the initial immunization was performed with incomplete adjuvant. These animals, however, do differ strikingly, as far as their immunization sites are concerned, from those immunized with no adjuvant at all, in that each immunization site becomes an area of induration with subsequent ulceration, in every way similar macroscopically to the immunization sites of animals given complete adjuvant. Two possibilities must therefore be considered:

(1) That these zones of inflammatory destruction are of major importance in the autoimmunization process.

(2) That secondary infection of the ulcerated areas provides the bacteria necessary for adjuvant activity.

We have already seen that, in the adjuvant given with ovalbumin so that delayed hypersensitivity develops, a diphtheroid can replace the tubercle bacillus. But the animals immunized without adjuvant did not show delayed hypersensitivity, so that if the subsequent chronic arthritis is to be attributed to such secondary infection of the immunization site, it is unlikely that it functions through such a mechanism. The evidence from the experiments with incomplete adjuvant therefore favours the view that the local inflammatory reaction at the immunization site is important in the subsequent development of the chronic arthritis. Since this local inflammation does not appear to enhance the antigenicity of the ovalbumin as assessed by circulating antibody titres, we conclude that it acts by enhancing the all-important autoimmune response to an inflammatory product.

This study of experimental chronic arthritis has thus led us to the conclusion that the chronicity of the lesion is most probably attributable to an autoimmune reaction, and that the process of autoimmunization begins at the site of primary immunization. It is, moreover, probable in view of the F.II results in the alginate animals that the arthritis itself can further enhance this process by producing more autoantigen. Is there any evidence that the chronicity of rheumatoid inflammation is based on a similar process of autoimmunization to some product of inflammation or local wear and tear? Against this view it has often been stated that ordinary inflammatory reactions and minor traumata heal just as well in rheumatoid subjects as in normal individuals. This may be true, although it is extremely difficult to prove. It does not refute the hypothesis because it is quite conceivable that the all-important antigen is released only from certain types of tissue and in response to certain types of injury. If we can assume, for the sake of this argument, that the subcutaneous nodule is an example of typical chronic rheumatoid inflammation, then it would seem that the most appropriate type of trauma is due to repeated minor frictional force, and that the most susceptible tissue is bursal or synovial in character.

From the now widely-confirmed observations that the subcutaneous nodules are almost exclusively confined to sero-positive cases of rheumatoid arthritis, we have grounds for assuming that the autoimmune state revealed by sero-positivity (since it is evidence of an autoantibody to denatured γ-globulin) is directly involved in the pathogenesis of the nodules. Yet another example of abnormal tissue reactivity in rheumatoid arthritis underlying the development of a lesion almost exclusively observed in sero-positive cases is the Caplan nodule. This has the typical histological features of a subcutaneous rheumatoid nodule, but of course occurs in the lung. It must, however, be emphasized that it is virtually exclusively confined to those rheumatoid individuals with an excessive exposure to dust inhalation. But what evidence is there that autoimmunity plays any part in the pathogenesis? simply this. Dust particles and especially silica dust particles are capable of adsorbing γ-globulin as a result of which the protein undergoes some change of configuration. These particles are then in essentially the same state as coated latex particles, that is they can react specifically with rheumatoid factor. It is presumably a similar reaction with the coated particles in the lungs that leads to the development of Caplan's nodules in sero-positive individuals. Finally, I should like to mention the arthritis described by Hollander and his colleagues when similar material, *i.e.* denatured γ-globulin, is injected into the knee joints of rheumatoid volunteers (Restifo, Lussier, Rawson, Rockey, and Hollander, 1965).

On the basis of our own findings in experimental animals, and the now well-established clinical separation of sero-negative from sero-positive arthritics, I should like to suggest the following hypothesis. The inflammatory lesions of rheumatoid arthritis result from an immune reaction

occurring within the synovial tissue, and for which this tissue itself provides the immune mechanism, *i.e.* the antibody-forming cells. The antigen initially is probably exogenous, a virus, a mycoplasma, or even a diphtheroid. Depending upon its nature, site of entry, and mode of dissemination, the reaction could be monoarticular or systemic. In either event, with the elimination of the exogenous agent, the disease process would come to an end. The benign polyarthritis of Lawrence (1964), with its self-limiting course, may be regarded as the form of the disease to be expected in the absence of any autoimmune contribution.

A relapse could occur because of re-activation of a few surviving organisms or because of re-infection. Some individuals, however, from predisposition inherited or otherwise, develop an autoimmune response to an antigen made available by the original inflammatory reaction. In these individuals the mechanism of a self-perpetuating inflammation is thus created, and the ultrachronicity that characterizes the sero-positive form of rheumatoid arthritis is set in motion. Sero-positivity is thus an indicator of the autoimmune reactivity responsible for chronicity.

You may well feel, not without justification, that I have allowed speculation to outrun its supporting facts. I should therefore like to conclude with a quotation from a letter written to Wallace in 1857 by Charles Darwin. He wrote "without speculation there is no good or original observation". Whether the quality or originality of our observations justify our speculations I must leave for you to judge.

REFERENCES

Banerjee, S. K., and Glynn, L. E. (1960). *Ann. N.Y. Acad. Sci.*, **86,** 1064 (Reactions to homologous and heterologous fibrin implants in experimental animals).

Brown, P. C., Glynn, L. E., and Holborow, E. J. (1967). *Immunology*, **13,** 307 (The dual necessity for delayed hypersensitivity and circulating antibody in the pathogenesis of experimental allergic orchitis in guinea-pigs).

Consden, R., and Glynn, L. E. (1955). *Lancet*, **1,** 943 (Chemically identifiable bacterial residues in lung lesions).

Dumonde, D. C., and Glynn, L. E. (1962). *Brit. J. exp. Path.*, **43,** 373 (The production of arthritis in rabbits by an immunological reaction to fibrin).

Hollingsworth, W. (1963). *Arthr. and Rheum.*, **6,** 776 (Cellular responses to foreign protein in rabbit supra-patellar bursae).

Katsh, S., Crowle, A. J., and Katsh, G. F. (1966). *Nature (Lond).*, **212,** 1486 (Non-mycobacterial adjuvant which mediates experimental aspermatogenesis).

Klinge, F. (1933). *Ergebn. allg. Path. path. Anat.*, **27,** (Der rheumatismus).

Lawrence, J. S. (1964). *In* "Textbook of the Rheumatic Diseases," ed. W. S. C. Copeman, 3rd ed., p. 95. Livingstone, Edinburgh.

Phillips, J. M., Kaklamanis, P., and Glynn, L. E. (1966). *Ann. rheum. Dis.*, **25,** 165 (Experimental arthritis associated with auto-immunization to inflammatory exudates).

Restifo, R. A., Lussier, A. J., Rawson, A. J., Rockey, J. H., and Hollander, J. L. (1965). *Ann. intern. Med.*, **62,** 285 (Studies on the pathogenesis of rheumatoid joint inflammation. III. The experimental production of arthritis by the intra-articular injection of purified 7S gamma globulin).

Rodnan, G. P., and Maclachlan, M. J. (1960). *Arthr. and Rheum.*, **3,** 152 (The absorption of serum albumin and gamma-globulin from the knee joint of man and rabbit).

Thewaini Ali, A. J., and Oakley, C. L. (1967). *J. Path. Bact.*, **93,** 413 (Auto-antibodies in experimental chronic infective disease).