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ROLE OF HYPERSENSITIVITY IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS*

BY

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William Heberden published his description of angina pectoris 192 years ago (Major, 1932). There are certain similarities between his thinking on this subject and our present approach to rheumatoid arthritis. Both are descriptive with glimmerings of possible pathogenic mechanisms. To-day, I propose to consider one such mechanism and I hope that my humility will be as obvious as was Heberden's.

This discussion will pose a question, present the available evidence, and offer a tentative conclusion. It will be obvious that further data must be obtained before a final satisfactory conclusion can be reached.

The question to be raised is "What is the pathogenesis of peripheral joint rheumatoid arthritis?" We shall, of course, be limited to pathogenic mechanisms that might explain a self-perpetuating disease characterized by inflammation associated with the formation of granulomata. Hill (1958), in discussing the aetiology of this disease, has pointed out that no hypothesis rests on more than a few loosely connected pieces of evidence. He has summarized in excellent fashion the four chief theories. These are infection and focal sepsis, metabolic abnormality, endocrine abnormality, and hypersensitivity. The last is currently the most attractive hypothesis and will be the subject of this discussion.

We are concerned solely with peripheral joint rheumatoid arthritis, excluding all variants such as spondylitis, the arthritis associated with psoriasis, ulcerative colitis, and Reiter's syndrome. Clinically, the disease covers a wide spectrum of severity and variability. It is a disease which, as far as we know, appears only in human beings. Although a female preponderance has been noted in most hospital studies (Ragan, 1949; Short, Bauer, and Reynolds, 1957), recent epidemiological work (Kellgren and

Lawrence, 1956) involving whole populations has shown that the sex incidence of sero-positive and x-ray proven rheumatoid arthritis approaches a 1:1 ratio. Patients with clinical manifestations alone, however, show the same female preponderance. Age distribution is wide, with peaks in females appearing around the menopause in some series (Short and others, 1957) and at the ages of 35 and 45 in others (Clemmensen and Arnsø, 1952). Isolated instances have been described with onset at the age of 80 and, at the other extreme, in the first year of life. This is in contrast to rheumatic fever where the onset before the age of 3 or 4 years is extremely rare. No adequate data are available concerning geographic distribution. However, it may be noted that a group of our Columbia students (Malawista, to be published) made an incomplete epidemiological study of the native population of Liberia last year and found no example of rheumatoid arthritis in a fairly extensive survey of the native population. There was, however, a large sample—about 15 per cent.—of false positive serologic reactions for rheumatoid arthritis as measured by the F II tanned sheep cell agglutination reaction. In contrast to these observations, the frequency of rheumatoid arthritis in Negro populations in the United States is similar to that seen in white populations. The role played by hereditary factors in this disease is not clear. Apparently, the frequency with which positive serologic reactions are seen in families of patients with rheumatoid-factor-positive rheumatoid arthritis is higher than in the general population, but this does not seem to apply to rheumatoid patients in whom the factor is not demonstrable (Lawrence and Ball, 1958). Most observers have seen instances of families in which the incidence of the disease is high, but these are infrequent and numerous examples are available in which a single case of rheumatoid arthritis appears in a large family.

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It is no longer fashionable in psychiatric circles to talk about personality patterns, but it has seemed to us after rather extensive observation that the patient with rheumatoid arthritis may be characterized as an individual with deep-seated anxieties which are probably justified by the chronicity and discomfort of the disease process. In summary, the average host may be described as an individual in middle life but with a wide age range, living in a temperate climate, of varied racial extraction, and occasionally with an impressive family history of rheumatoid arthritis.

The course of the disease emphasizes the problems that one must face. Short and others (1957) stressed two types of clinical course: episodic, *i.e.* of less than 6 months duration and sustained. These may merge and an episodic onset is not uncommon. In sustained disease, a remission may occur at any time. This variable life history has been a major factor in the present-day uneasiness regarding therapeutic evaluations. The disease is often progressive with ever-increasing increments of deformity and restriction of function as time passes; indeed, not uncommonly patients followed for 30 years have had no periods of freedom from rheumatoid manifestations.

In a study of the reaction of the host to this disease, the first point of attack should be the tissues involved. The involvement of connective tissues in rheumatoid arthritis had led to its designation as primarily a disease of connective tissue. This may be true, yet one can say that almost every disease involves the connective tissue, including even such a well-established and understood phenomenon as pneumococcal pneumonia, where the primary locus is the connective tissues of the lung. Nevertheless, the varied and widespread participation of connective tissue demands the most serious consideration in a discussion of pathogenesis in rheumatoid arthritis.

May I present, therefore, as a working hypothesis, the suggestion that the primary lesion of rheumatoid arthritis is a vasculitis. Following the vasculitis comes granuloma formation, leading ultimately to scarring with resulting fibrosis. Sokoloff, McCluskey, and Bunim (1953) suggested—on limited number of cases, the authors concede—that the rheumatoid nodule develops from a necrotizing vasculitis in its centre which leads to granuloma formation and eventually to the necrosis seen in the late stages. Furthermore, Sokoloff, Wilens and, Bunim (1951), and Cruickshank (1954) have shown that vasculitis is present to a minor degree in other areas, although, in skeletal muscle, there is little necrosis, and it must be emphasized that the

necrotizing character of the arteritis is its important feature. Since the advent of steroids, the appearance of widespread necrotizing vasculitis in rheumatoid arthritis has been increasingly noted (Bevan, Nadell, Demartini, and Ragan, 1954; Sokoloff and Bunim, 1957), although examples of this were seen infrequently before steroids came into use (Southwood, Willkens, and McLendon, 1958). It seems reasonable to suggest that this complication is an augmentation of the minor vasculitis seen in the muscle and of the necrotizing vasculitis which seen in the genesis of the rheumatoid nodule, but the role of steroids in this development is not clear at this time. Thus, in summary, this is a disease of variable course, often sustained, characterized by chronic granulomatous inflammation with an element of arteritis which, in the early nodule, clearly necrotizing. The characteristic feature is sustained inflammation, primarily vascular, with the granulomatous and sclerotic elements resulting therefrom.

The relatively frequent occurrence of amyloid in association with rheumatoid arthritis has suggested to some investigators by analogy a hypersensitivity pathogenesis. Experimentally, amyloid may be produced by chronic infection with a viable agent (Gellhorn, Van Dyke, Pyles, and Tupikova, 1949) or by hyperimmunization, with its locus in the connective tissue. Thus it is not unreasonable to infer that, in the absence of a viable agent in rheumatoid arthritis, the appearance of amyloid might by analogy suggest the participation of hypersensitivity in its pathogenesis.

Serum sickness demonstrates the best experimental model of joint inflammation and vasculitis on the basis of hypersensitivity presently available to us. The outstanding difference between this model and human rheumatoid arthritis is that no sustained serum sickness has yet been described in man or in an experimental animal. This applies to the joint inflammation studied (Boots and Swift, 1923) in the 20s as well as to the vasculitis described by Rich (1947) and further studied by Germuth, Flanagan, and Montenegro (1957). In all these models, the lesions appear over a brief period of time and then subside. To account for the sustained nature of rheumatoid arthritis, one must therefore invoke an element of autosensitization.

In most instances, protein hypersensitivity of the serum sickness type is characterized by association with circulating antibody, and, in rheumatoid arthritis, the only evidence we have for a circulating antibody at this time is derived from the rheumatoid factor (Ziff, 1957).

The history of the rheumatoid factor affords

interesting panorama of medical thought concerning the pathogenesis of rheumatoid arthritis. Cecil, Nicholls, and Stainsby (1929), and Nicholls and Stainsby (1931) introduced the factor when they described the agglutination of haemolytic streptococci in this disease. Because they found that a particular streptococcus was agglutinated by rheumatoid serum, they felt strongly that the disease was an infectious one with this streptococcus as the aetiological agent. Dawson, Olmstead, and Boots (1932) disproved this hypothesis by showing that the agglutination applied to most Group A haemolytic streptococci as well as to a certain number of pneumococci and staphylococci. The belief remained, nevertheless, that the streptococcus might have something to do with the disease. A technician working in Rose's laboratory—Elizabeth Pearce—was a clinic patient of ours with rheumatoid arthritis. While working on rickettsial pox, she contracted that disease as well. When her serum was set up for complement-fixation studies with the *Rickettsiae*, it was noted that her serum would agglutinate sensitized sheep cells in high titre. Thus, the sensitized sheep cell reaction which had been described by Waaler (1940) was unwittingly resurrected, and not until the phenomenon had been published (Rose, Ragan, Pearce, and Lipman, 1948) did one of the authors recall that she had heard Waaler's original communication at the 2nd International Congress of Microbiology. Heller, Jacobson, Kolodny, and Kammerer (1954) noted that the human gamma globulin would inhibit the sensitized sheep cell reaction, used tanned sheep cells to absorb the gamma globulin, and described the tanned sheep cell F II agglutination reaction. Latex particles (Singer and Plotz, 1956) apparently adsorb gamma globulin as well and afford a much more readily available supply of particulate matter.

Lamont-Havers (1955) showed that two factors were necessary for the development of the streptococcus agglutination—a specific rheumatoid one and a non-specific one which presumably sensitized the suspended streptococci. The implication was that this was an antibody to the streptococcus—a gamma globulin. At about this time, suspicions were expressed (Epstein and Ragan, 1956) that we were dealing with a factor which reacted with gamma globulin in some fashion and the F II precipitin reaction was described (Epstein, Johnson, and Ragan, 1956) which has many of the characteristics of an antigen-antibody reaction. This served to place the phenomenon of the rheumatoid factor on a plane where quantitative immuno-chemical techniques could be employed. Franklin, Holman, Müller-Eberhard, and Kunkel (1957) characterized

the factor as a 19S gamma globulin, probably circulating as a soluble complex with a Svedberg constant of 22S which can be dissociated by various means into two components, a 19S (the active factor) and a 7S gamma (Table I). Christian (1958) characterized the reactant gamma globulin in the F II tanned sheep cell reaction and the F II precipitin reaction as an aggregated gamma globulin which can be produced artificially by heating. When antibody combines with antigen, the antibody is in an aggregated form. Christian's findings indicate that the gamma globulin which reacts with the rheumatoid factor is also in an aggregated form. In this reaction, therefore, gamma globulin is in a physical state similar to that in which it exists when in combination with antigen, as in immune precipitates. His observations apply to the F II tanned sheep cell and precipitin reactions where antigen is not available. In all other reactions where rheumatoid factor combines with gamma globulin, the globulin is combined with antigen before reacting with rheumatoid factor in the usual antigen-antibody complex: sensitized sheep cell, brucella-antibrucella (Foz and Batalla, 1955), human Rh-anti Rh (Waller and Vaughan, 1956), eggwhite-anti-eggwhite (Vaughan, 1956). The latex-fixation technique is the exception and it is assumed that the charge on the latex may cause aggregation when the gamma globulin becomes adsorbed to the particle. The rheumatoid factor can combine with its own naturally existent circulating aggregated 7S gamma—in *vitro* at 4° C.—as a spontaneous precipitate. The possibility of an auto-immune reaction thus becomes a tenable hypothesis.

TABLE I
RHEUMATOID FACTOR

Rheumatoid Factor	Gamma Globulin	Reaction
19 S +	Non-aggregated gamma globulin ($S_{20} = 7$)	→ Soluble complex ($S_{20} = 22$)
19 S +	Aggregated gamma globulin ($S_{20} = > 7$)	→ Insoluble complex (precipitate)
19 S +	Aggregated gamma globulin ($S_{20} = > 7$) coated on tanned sheep erythrocytes	→ F II agglutination
19 S +	Aggregated gamma globulin as antibody in combination with antigen*	→ Agglutination of particles or adsorption of rheumatoid factor on immune complexes

* Systems studied:

- (1) Sheep cells—Rabbit amboceptor
- (2) Brucella—Antibrucella serum
- (3) Human Rh cell—Anti Rh serum
- (4) Eggwhite—Anti-eggwhite serum

It is entertaining to recall that during the period of popularity of the infectious aetiology of rheumatoid arthritis, the rheumatoid factor appeared in the guise of an infectious agent. At present, when hypersensitivity is the vogue, the factor may be invoked to support the role of hypersensitivity with an element of autosensitization. If speculation is at all proper, it might be suggested that the rheumatoid factor represents an antibody to an antibody when the latter exists as an antigen-antibody complex. It possesses many of the characteristics of an antibody, but it is species non-specific. It will react with rabbit gamma globulin in combination with sheep cell antigen as well as with bovine, guinea-pig, or human gamma globulin. This definite species non-specificity leads immuno-chemists to doubt that the factor is a true antibody. It should be noted, however, that in human thyroiditis, the sensitization is not species specific, for these patients have antibodies to thyroid of other species, as well as antibodies to human thyroid (Rose and Witebsky, 1955).

Vaughan and Harris (1958) injected rheumatoid factor of high titre into volunteers with no significant results. The disappearance curve of the factor in these volunteers, as measured by titration in an appropriate agglutinating system, was similar to that observed with other types of homologous serum protein, indicating that the recipient did not treat the factor as a foreign protein. Recently Christian has injected aggregated gamma globulin in large amounts intramuscularly into two patients with severe rheumatoid arthritis, again without clinical or serological effect. When administered to the guinea-pig intravenously, aggregated gamma globulin removes complement.

Many investigators have noted that individuals with agammaglobulinaemia may develop joint findings resembling those of rheumatoid arthritis (Janeway, Apt, and Gitlin, 1953). In rebuttal, it may be mentioned that these patients do not develop the rheumatoid factor and that a few of the patients with this syndrome have the capacity to make fixed tissue antibody. Thus, to date, the attractive concept that the rheumatoid factor may represent a circulating antibody, thereby providing a tool for desensitization of the patient with rheumatoid arthritis, has not borne fruit. Moreover, transmission of the factor to volunteers does not produce the disease; in the so-called false positive individuals, the factor is present without the disease, as is also the case in some unaffected siblings of patients with rheumatoid factor-positive rheumatoid arthritis. It remains, however, our only definite link between the clinical disease, rheumatoid

arthritis, and the nebulous hypothesis of auto-sensitization. If it develops that the factor plays no role in this mechanism, we are deprived of a most hopeful lead. We must then search for an antigen in a disease which offers no animal experimental model, a disease involving the arterial walls and probably the whole connective tissue.

The absence of definitive evidence of an infectious agent and the sustained nature of the disease strongly suggest an element of autosensitization. Thyroiditis (Rose and Witebsky, 1955), and allergic encephalitis (Kabat, Wolf, and Bezer, 1947) are prototypes of such a mechanism. The need is to find the antigen. If this be the fixed tissue or delayed type of hypersensitivity, the presence of a circulating antibody would be unlikely. There would, therefore, be no readily available technique by which to identify the antigen such as is possible when circulating antibody is present. But there is evidence which points to a localization of the process in the connective tissue and possibly in the blood vessel wall. Zweifach (1954), however, suggested that while the first visible lesion is in the blood vessel wall, the actual primary insult may be in the loose areolar tissue close to but not necessarily directly contiguous to the blood vessel wall. This is mentioned solely to underscore the point that the first visible lesion may not be the locus of the proposed antigen. It must also be emphasized that microbial agents, such as rickettsiae and even viruses (Jones, Doll, and Bryans, 1957) have been shown to produce a disseminated vasculitis. Hypersensitivity provides only one of several mechanisms for the development of this pattern.

Search for a pure antigen involves us directly in the chemical complexity of connective tissues. Study of the chemical composition of the connective tissue has long lagged behind the chemistry of other body constituents. Its importance has been recognized, but the technical difficulties are enormous, a fact easily apparent as one works in close proximity to Karl Meyer's laboratory. In citing Karl Meyer for a Lasker Award in 1956, it was noted that he and F. O. Schmitt had opened an area which had been largely overlooked. When I first entered this field, in 1941, hyaluronic acid had only recently been described. The isolation of identical material in vitreous humour (Meyer and Palmer, 1936), synovial fluid (Meyer, Smyth, and Dawson, 1938) and in the capsule of Group A haemolytic streptococci (Kendall, Heidelberger, and Dawson, 1937) led to a unifying and simplifying concept of the nature of connective tissue.

Subsequent events have not borne out this hope. With each succeeding year, the number of new

polysaccharides, their relationship to tissue proteins, the native physical state of the materials, their state of polymerization, of binding with weak or strong bonds, has forced one to suspect that the possible relationship of these materials to disease states as we know them will be established in our time only by chance. In order to prove that a disease is due to hypersensitivity, some chemical purity of the antigen is necessary, though not to the degree required by the immuno-chemist working with gel diffusion techniques. The material must be somewhat refined and those who have run a Waring blender making a brei of tendon as a first step toward isolation of one of the chondroitin sulphates quickly realize, at the end of the first step in isolation, that the product has little relationship to the native material. If the antigen requires subtle forces such as hydrogen bonds or Wandervall's forces to develop its antigenic potency, isolation of a pure antigen by the techniques presently available seems unlikely. The known components of the connective tissue are outlined in Table II. The greatest chemical purity obtained so far is in the acid mucopolysaccharides. Their distribution as shown in Table II demonstrates the complexity of the problem at a glance.

In the chemical isolation of connective tissue components, two major difficulties present themselves. The first lies in the fact that isolation procedures practically always modify the native state of the material, either by decreasing the chain length of the polysaccharides so that they are less polymerized in the isolated than in the native state or

by breaking certain weak bonds between a polysaccharide and a protein thus possibly changing the biological significance of the material. The second difficulty is due to the ease with which small amounts of material can be overlooked. A classic example of this is the discovery of keratosulphate in various tissues by Meyer, Hoffman, and Linker (1958). The complexity of the chemistry of these materials is overwhelming, and they require extremely special knowledge and a great deal of hard work for their isolation. Undoubtedly further research will increase our overall knowledge of connective tissue, but I must confess that in the past 15 years the augmentation of this overall knowledge has added few concepts as regards the pathogenesis of rheumatoid arthritis. There is no easy road to the understanding either of the composition of the connective tissue or of its relationship to the problem of rheumatoid arthritis.

All materials which have now been isolated in pure form have not yet been proved antigenic. The work of Hill and Cruickshank (1953) in the antigenicity of basement membrane and reticulin and of Scott (1957) on the differentiation of the two are certainly landmarks in this field. The antigenicity described by Hill, and later defined by Scott, as localizing in the reticular structures of the body, could possibly be related to an antigen to reticulin itself but at present these experiments cannot be construed as demonstrating the antigenicity of reticulin. Though the chemical structure of this material is only vaguely understood at this time,

TABLE II
COMPONENTS OF CONNECTIVE TISSUE

Blood and Lymph	Lipid	Pigment	Scleroproteins			Polysaccharides
			Elastin	Reticulin	Collagens	
Proteins Lipids Polysaccharides Mucoids Sialic acid	Fatty tissue Fat depots	Water soluble Lipid soluble	Pigments	Lipid Carbohydrate	Tropocollagen Procollagen Acid soluble Neutral salt soluble Insoluble carbo- hydrate	Neutral Acid

ACID MUCOPOLYSACCHARIDES OF CONNECTIVE TISSUE—DISTRIBUTION

Heparitin Sulphate	Kerato-sulphate	Hyaluronate	Chondroitin	Chondroitin Sulphate		
				A	B	C
Aorta All blood vessels (?)	Cornea Nucleus pulposus Bone Adult cartilage	Synovial fluid Umbilical cord Skin Ligaments Aorta Tendon Almost all con- nective tissue	Cornea	Cartilage Bone Aorta Cornea	Skin Tendon Heart valves Aorta Yellow liga- ments	Nucleus pulposus Cartilage Bone Tendon Embryonal tissue

there is undoubtedly a carbohydrate closely associated with it. In the human synovium, used by Scott to prepare his antigen, there may have been at least fifty or sixty different chemical substances. It is possible that this same number of substances may be in the material which is stained by fluorescent antibody although they were still distinct from the others present in glomeruli.

None of the mixtures or fractions of connective tissue, either autologous or homologous, which have been injected into the usual laboratory animal, has produced anything resembling rheumatoid arthritis. These materials have been injected by various routes employing the usual techniques for augmenting responses such as alum precipitate and Freund's adjuvant.

If the particles of material are large, granulomatous lesions in the lungs have been produced—probably a foreign body reaction at the site where the particles are screened out in the pulmonary capillary bed. If the particles are small, the animals have shown nothing. Various isolated materials from Karl Meyer's laboratory, including hyaluronate and chondroitin A, B, and C, as well as aggregated gamma globulin, have failed to produce a skin reaction in patients with rheumatoid arthritis which differed in any way from that seen in controls. Let me emphasize again that the materials which have so far been made available to us have been non-reactive in patients with rheumatoid arthritis. We have no alternative but to continue such studies, but it is disturbing to realize that the presently available isolation techniques may destroy important linkages and that, moreover, we may be dealing with a type of hypersensitivity in which there is no skin response.

Lastly, we may be investigating a mechanism which requires man for its development. If this assumption be true, further advances will continue to be difficult, for most of the great forward steps in medicine have been assisted immeasurably by the employment of experimental animals. One experimental model which would be of inestimable value in this field would be an animal which would develop a self-perpetuating reaction of hypersensitivity that involved the connective tissue or the blood vessel walls. This we do not now have. A model which deserves further study is the parabiotic rat which, rarely, develops in one partner a relatively sustained vasculitis, arthritis, and haemolytic anaemia (Hall and Hall, 1951).

In contrast to the rapidly growing knowledge of liver cells and the glomeruli, we know relatively little about fibroblasts. These cells are dispersed, one cannot readily study them in slices or in a brei,

and a method has not yet been developed for isolating fibroblasts as is done by the differential centrifugation of glomeruli. The life history of the fibroblast—its origin and its half-life—is the subject of considerable dispute, as is its potential for transforming itself into other types of cell under appropriate stimuli. Tissue culture methods have been used in an attempt to solve some of these problems, and they have proved difficult, though we have been able to show that fibroblasts in culture produce hyaluronic acid and a chondroitin sulphate (Grossfeld, Meyer, Godman, and Linker, 1957). One main source of disappointment has been the dedifferentiation of fibroblasts from various sources on prolonged culture. Only recently, methods have been developed which permit accurate quantitation of polysaccharide production in relation to growth as measured by DNA content. These will provide us with a tool for studying the metabolism of the fibroblast and may possibly help in exploring the antigenicity of the cell itself and of the materials it produces when grown in a relatively simple medium free of blood vessels and circulating materials.

Suggestions have been made that microbial hypersensitivity may play a part in the development of rheumatoid arthritis. Evidence presented in favour of this is indirect and difficult to interpret. The role of this mechanism may be important but cannot be evaluated at this time.

As a last possible speculation, the observation of Beiser, Erlanger, Agate and Lieberman (in the press), concerning the antigenicity of albumin conjugated steroids and the inhibition of the specific precipitin reaction by a particular steroid, suggests the possibility that the adrenal steroids may in themselves be the offending antigen in some instances, and that the anti-inflammatory effect of the 11-oxysteroids may perhaps represent an excess of antigen preventing the combination of antigen and antibody in the tissues.

In summary, failure to discover a circulating antibody would discourage the hope of an early solution to the pathogenesis of rheumatoid arthritis. A continuing characterization of the chemical and physical composition of the connective tissue is necessary coupled with a willingness to determine whether newly discovered compounds have an antigenic role. If a circulating antibody does exist, the most readily available one is the rheumatoid factor, which resides in the fraction containing known antibodies, appears in the circulation as a soluble complex, and reacts with autologous material. Until the possible pathogenic role of this factor is thoroughly and completely disproved, it provides the only support for the implication of

hypersensitivity in the pathogenesis of rheumatoid arthritis. If its role is disproved, we face a long, hard struggle which can be solved in the foreseeable future only through great luck with a dash of serendipity.

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