BRITISH MEDICAL JOURNAL

LONDON SATURDAY MAY 24 1952

SOME CONCEPTS OF RHEUMATIC DISEASE*

BY

J. H. KELLGREN, M.B., F.R.C.S., M.R.C.P.

Reader in Rheumatic Diseases and Clinical Director, Rheumatism Research Centre, University of Manchester

Although joint disease and podagra were known to the ancients, it was not until after 1676, when Sydenham described rheumatic fever, that rheumatism began to be thought of as a separate entity. During the following centuries of careful description and classification of disease various clinico-pathological entities, such as gout, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, osteochondritis, and many less common syndromes were described and separated from joint disease due to bacterial infection, trauma, and new growths, and we owe much to the brilliant work of Heberden (1802), Fagge (1877), Goldthwait (1904), Garrod (1907), and others in this field. Although in gout and certain rare conditions, such as alkaptonuric arthritis, an underlying metabolic aberration could be demonstrated, most of the rheumatic diseases remained only clinico-pathological syndromes, so that precise definition and aetiological classification still eluded us.

Perhaps because of this failure and because certain histological connective-tissue lesions, such as the fibrinoid change, were found in more than one of these syndromes (Klinge, 1933, 1934), there has been a recurring tendency to return to a unitarian concept of an all-embracing "rheumatic process," and many valiant attempts have been made to explain "rheumatism" in terms of known pathological mechanisms, such as infection with bacteria or virus, allergic reactions, nutritional deficiencies, and autonomic or psychosomatic disorder; but these efforts have not as yet proved particularly rewarding. This is perhaps not surprising, because the assumption that there is a single all-embracing "rheumatic process" is most unlikely to be correct.

The rheumatic diseases have been grouped together because they have certain clinical and pathological features in common, such as widespread changes in the supporting tissues of the body. They are essentially diseases of the connective tissues; but no one to-day would seriously search for a single cause and cure for diseases of the nervous system; and it is equally unreasonable to search for a single explanation for diseases of the supporting system of the body. It would thus seem more profitable to abandon the unitarian concept and to return to the careful description and classification of clinico-pathological entities on the one hand and a more thorough study of the nature and behaviour of connective tissue on the other. As our knowledge of the connective tissues increases and the various clinico-

pathological entities become more clearly defined, investigation into their aetiology and pathogenesis can be undertaken with greater prospect of success, leading eventually to more specific and efficient methods of prevention and treatment.

The introduction of cortisone through the brilliant discoveries of Hench and his colleagues (1949, 1950) temporarily revived the unitarian concept; but, although cortisone has illumined certain aspects of the rheumatic diseases and provided us with a new and potent therapeutic weapon, increasing experience suggests that the pituitary adrenocortical system has only a regulatory function, damping down reactions to injury and disease of all kinds. Thus in spite of the elaborate theories of Selye (1950) we can more usefully look elsewhere for the fundamental processes and the aetiological factors causing the rheumatic diseases. In this connexion it is well to remember that the largely unknown mechanisms responsible for the production and maintenance of the supporting tissues of the body may be disturbed by pathological processes which have yet to be discovered.

Structure and Function of the Connective Tissues

The supporting system of the body is composed of connective tissue in various degrees of organization, forming structures such as bone, cartilage, tendon, and synovia. All these tissues arise from primitive mesenchyme, which in the adult persists to some extent in the areolar connective tissues and in the newly formed granulation tissue that follows injury of any kind. The fact that the various supporting tissues are to some extent interchangeable is clearly demonstrated by the transformation of ligament into bone and the development of pseudoarthroses; and when we consider their intrinsic nature the similarity of the various connective tissues will be even more apparent.

The connective tissues perform at least two important functions. First, they provide a tough framework for the body, giving it cohesion and form, and also a system of levers which allow the exertion of considerable mechanical force; and, secondly, they provide the lubricating materials which enable the various parts of the body to move smoothly and efficiently upon each other. It is also possible that they may play an important part in metabolism, but this possibility still remains unexplored.

The connective tissues are composed of cells and intercellular material, the latter being made of varying mixtures of formed fibrous elements and unformed

*The first Goulstonian Lecture, given at the Royal College of Physicians of London on January 15.

mucoïd. In primitive mesenchyme and in granulation tissue the cells are relatively numerous, large, and active, and the intercellular material is relatively scanty and largely mucoïd in nature; but in the well-differentiated adult tissues the cells are relatively few, small, and inactive, and tend to be separated from each other by large masses of intercellular material.

Where tensile strength is required the material is predominantly fibrous, but where lubrication is needed the mucoïd predominates. Where rigidity is essential, as in bone, the fibrous material is impregnated with mineral salts, and this places bone in a separate category, since its content of calcium and phosphorus renders it peculiarly susceptible to disturbances of electrolyte metabolism; but this aspect of bone disease is outside the scope of these lectures.

Although the cells presumably play an important part in the formation and maintenance of the connective tissues, the functional efficiency of these tissues at any one time depends almost entirely upon the physical properties of the intercellular fibrous and mucoïd material. Its nature and behaviour will therefore be considered in some detail. The intercellular material has been studied by histologists, chemists, and biophysicists, and we must therefore discuss its nature in terms of information obtained at more than one level of investigation.

Fibres

At the histological level, connective-tissue fibres are of three types—elastic, collagenous, and reticulin. Elastic fibres are characteristically branched and smooth, and can be demonstrated readily by special stains like Weigert's elastic-tissue stain. They are found in large numbers only in certain situations, such as the ligamenta flava and the walls of arteries. Collagenous fibres form the bulk of bone, cartilage, tendon, and fibrous tissue generally. They are the largest of the three fibre types, and, although they stain pink with eosin, they are better demonstrated by one of the more or less specific stains, such as Van Gieson's or Mallory's triple stain. The reticulin fibres form the deli-

cate supporting network of organs like the spleen; they are also found in developing connective tissue, and may often be seen where it is undergoing pathological change. The reticulin fibres are the finest of the three types. They stain but faintly with eosin, but are readily demonstrated by impregnation with silver. Although reticulin fibres are thus distinguishable from collagenous fibres by their staining reactions and their appearance under the optical microscope, both types of fibre are in fact made up of the same macromolecular collagen fibrils (Schmitt, Hall, and Jakus, 1942), the reticulin fibres simply representing smaller aggregations of these molecular fibrils. Thus the fibrous elements of the connective tissues are almost entirely composed of collagen.

At the chemical or molecular level collagen is a relatively insoluble fibrous protein which is converted to gelatin by boiling. It contains a high proportion of certain amino-acids, such as glycine, proline, and hydroxyproline (Astbury, 1940; Bowes and Kenten, 1948). Indeed, glycine accounts for about one-third of all the amino-acid residues of collagen. It is the only body protein to contain much hydroxyproline, and the quantity of collagen in any given tissue may be estimated in terms of its hydroxyproline content (Neuman and Logan, 1950). However, the most characteristic feature of collagen is its macromolecular configuration, which has been so brilliantly defined by Astbury (1940, 1947) in this country, and by Schmitt, Hall, and Jakus (1942), Bear (1942), and others in America. The x-ray diffraction photographs show a highly characteristic pattern, the main features of which are equatorial spots at about 11 Å and a meridional arc at 2.86 Å, there being a basic repeat along the fibre axis at a spacing of 2.86 Å. A much longer spacing of about 640 Å can also be demonstrated. The electron microscope reveals fibrils which are typically cross-banded, the bands corresponding to the 640 Å spacing revealed by the x-ray photographs (Figs. 1 and 2). These physical techniques provide at present the most certain method of recognizing normal collagen, and their application to the study of connective-tissue abnormalities is proving of great value.

Mucoïd

Histologically the mucoïd forms the ground substance which lies between the connective-tissue fibres and fibrils and the cement which binds the macromolecular fibrils into

the much larger histological fibrils, and forms the basement membrane which separates connective tissue from epithelium and endothelium. Mucoïd is not readily seen in ordinary histological preparations, though it can be demonstrated by the use of special staining techniques. Thus it may show metachromasia with toluidine blue (Lison, 1935; Sylvén, 1941), and it may stain deeply with the Hale (1946) and McManus (1946) techniques.

Chemically the extractable mucoïd is largely made up of substances which Meyer (1947, 1950) has broadly named mucopolysaccharides. These substances are highly polymerized long-chain forms of a basic disaccharide composed of equimolecular parts of glucuronic acid and an amino-sugar. In the connective tissues they are mainly of two kinds: hyaluronic acid, in which the amino-sugar is N-acetylglucosamine, and chondroitin sulphate, in which the sugar is galactosamine, and which also contains a sulphate radical on this moiety. The chondroitin sulphates may be subdivided according to the configuration of the contained galactose; thus in cartilage it is racemic, while in skin and tendon it is largely dextrorotatory. The D-galactosamine-containing chondroitins are often conjugated with protein in the tissues,

FIG. 1.—X-ray diffraction diagrams by Professor W. T. Astbury and E. Beighton of (a) normal oriented collagen fibres; (b) part of a rheumatoid nodule showing histologically normal collagen (partly disoriented); and (c) part of rheumatoid nodule showing advanced fibrinoid change.

FIG. 2.—Electron micrographs by Dr. R. Reed of (a) part of rheumatoid nodule showing histologically normal collagen; (b) part of rheumatoid nodule showing advanced fibrinoid change; and (c) part of nodule showing intermediate degree of fibrinoid change.

forming relatively insoluble glycoproteins; these proteins are unlike collagen in that they contain tyrosine and tryptophan, and it seems likely that they represent the water-insoluble glycoproteins of the basement membrane and ground substance described by Gersh and Catchpole (1949).

Most polysaccharides are soluble in water, and when highly polymerized they form viscous solutions; thus measurements of viscosity are often used to estimate their degree of polymerization (Chain and Duthie, 1940). Polysaccharides in solution are readily depolymerized by certain testicular and bacterial extracts which have been called hyaluronidases, though this name is misleading, since many of these extracts are also active against some forms of chondroitin sulphate (Meyer, 1950). In the tissues the polysaccharides are normally highly polymerized, and the increased spread of fluid in the tissues which follows the introduction of hyaluronidase-containing extracts has been attributed to depolymerization of the tissue polysaccharide, though direct evidence for this is still lacking, and there may be other factors which influence spreading (Duran-Reynals, 1942).

The polysaccharide content of tissue is usually estimated chemically in terms of its amino-sugar content, and in the case of chondroitin sulphate the sulphur content of extracts has also been used as a quantitative measure. Although the staining techniques already mentioned provide a tinctorial indication of the polysaccharide content of the tissues, for various reasons they cannot be used for accurate quantitative studies. Furthermore, the specificity of these staining techniques is not yet firmly established, although the periodic acid-Schiff technique of McManus appears to be the most reliable. Thus statements about tissue polysaccharide based exclusively on staining properties should be treated with some reserve.

Of the two polysaccharides hyaluronic acid is found chiefly in synovial fluid and in situations where lubrication is required, whereas chondroitin sulphate is found more in cartilage and generally in situations where stability and tensile strength are required.

Although both collagen fibrils and solutions of polysaccharides can be extracted from connective tissue, Partridge (1948) has shown that these two substances may be conjugated, and that it is probably better to think of connective tissue as a collagen-polysaccharide complex. Thus he found that only about one-third of the chondroitin sulphate could be extracted from ground-up cartilage by distilled water at room temperature. This may be regarded as the free polysaccharide. An additional one-third of chondroitin was released after heat-shrinkage of the collagen fibres in the cartilage. This chondroitin still gave a viscous solution, and may be thought of as made up from the long-chain polysaccharide cross-links of the connective-tissue lattice which had been released by the contraction of the collagen fibres. The remaining one-third of chondroitin could be extracted by drastic treatment with heat, alkali, and calcium chloride, but the resulting solution was no longer viscous, so that the chondroitin extracted by this method was depolymerized, and, furthermore, substantial quantities of gelatin were also obtained. This suggests that the last portion of chondroitin was intimately bound to the collagen and could be released only by breaking down both collagenous and polysaccharide macro-molecules.

Although the connective tissues contain a certain amount of elastin and a small proportion of protein residue, which still remains to be properly characterized, the normal adult connective tissues may be thought of as a highly polymerized collagen-polysaccharide complex. The difference between individual tissues such as cartilage, ligament, or synovia are largely determined by the relative proportion and spatial orientation of the collagenous and polysaccharide parts of the complex. The connective-tissue cells, which presumably play an important part in the laying down and maintenance of this intercellular complex, contribute very

little to the physical properties of the tissues which enable them to perform their supporting and lubricating functions.

Connective-tissue Metabolism

The oxygen consumption of the adult connective tissues is negligible (Bywaters, 1937), so that their metabolic activity under normal circumstances is presumably low.

Although collagen accounts for more than one-third of the body protein, the only available studies of its metabolic turnover have been carried out by Dr. Slack, of our research centre, working with members of the staff of the National Institute for Medical Research (Neuberger, Perrone, and Slack, 1951; Perrone and Slack, 1951). They gave rats single doses of radioactive glycine labelled with C^{14} , and then estimated the specific radioactivity of the glycine recovered from the collagen of tendon, skin, bone, and liver of these animals at varying periods after the administration of the radioactive glycine. Both old and young adult and actively growing young rats were used, and the specific radioactivity of whole muscle and that of liver were estimated simultaneously for comparison.

In the old rats only small amounts of radioactive glycine entered the tissue collagen at any stage, but in the young growing rats considerable radioactivity was found in the collagen glycine after the first week; but subsequently the specific activity of the collagen remained fairly constant for as long as eight weeks provided growth dilution caused by the laying down of new collagen was allowed for. These findings in collagen contrasted strikingly with the findings in liver and muscle protein, in which the specific radioactivity rose rapidly to a high level and then fell again within a few weeks—thus confirming other observations on the rapid metabolic turnover of cellular and muscle protein.

These experiments suggest that collagen behaves differently from many other body proteins. Once laid down during growth, it appears to escape from the general metabolic pool and in the normal animal may remain in a state of relative metabolic inertia for long periods. From this it follows that rapidly developing disorders of collagen in adults are unlikely to be the result of faulty collagen synthesis. But this statement may require modification, since recent work suggests that there are soluble collagen precursors (Bresler, Finogenov, and Ya Frenkel, 1950) the metabolism of which has not yet been investigated.

Whether the polysaccharides of connective tissue behave in a similar manner is still unknown, and studies of their normal metabolic turnover are urgently required.

In the intact animal the formation of connective tissue, like other tissues, is stimulated by growth hormone. A local reawakening of connective-tissue formation may also follow local injury and inflammation, and both this local activity and growth appear to be quantitatively suppressed by cortisone in high dosage. Ascorbic acid is also essential for the proper formation of both mucoid and fibrous components of the intercellular material (Wolbach, 1933; Gersh and Catchpole, 1949; Penney and Balfour, 1949).

That local mechanical factors play a dominant part in both the development and maintenance of the connective tissues is well known; indeed, the practice of orthopaedic surgery is largely based upon this. The effect of mechanical factors on bone needs no elaboration. But we are apt to forget that a synovial cavity can develop anywhere following the application of repeated shearing strains, and that immobilization leads to loss of gliding planes throughout the tissues. Immobilization may also produce pronounced changes in articular cartilage and other joint structures (Scaglietti and Casuccio, 1936; Abramson and Kamberg, 1949). In these changes both cellular and intercellular components of the connective tissues may be involved, but we know too little about the connective-tissue mechanisms responsible and what is going on at the molecular level.

Tissue-culture studies (Porter and Vanamee, 1949) have shown that the collagenous components of connective tissue can be formed outside the body, and that the collagen protofibrils appear to be formed at some distance from the fibroblasts. Furthermore, macroscopic collagen fibres can be precipitated from clear solutions (Nageotte, 1927; Buzagh, 1942), so that collagen fibres can apparently be formed without the direct participation of the fibroblast or any other cell.

In bone, however, the dominant part played by the osteoblasts and osteoclasts seems firmly established, but cells with such definite remodelling functions have not been demonstrated in other connective tissues, and the part played by the fibroblasts in the laying down and maintenance of the connective tissues still remains undefined.

Connective-tissue Lesions

Striking changes in the connective tissues can be produced by trauma or by bacterial infection. Severe lesions may also be associated with certain metabolic aberrations such as those found in gout and alkaptonuria, but in all these instances the connective-tissue changes are clearly of a secondary nature. There are, however, two connective-tissue lesions which are often considered to be characteristic of rheumatic diseases: I refer to the fibrinoid change which is the hallmark of what Klemperer (1950) has called the collagen diseases, and the chondromalacia and disk degeneration which appear to be the initial lesion in degenerative joint disease. A fuller understanding of the nature and pathogenesis of these connective-tissue lesions would be of great value.

Fibrinoid

The fibrinoid change was first described by Neumann (1880), but it was not until Klinge (1933, 1934) demonstrated its widespread occurrence in the connective tissues of patients suffering from rheumatic fever, rheumatoid arthritis, and other related syndromes that fibrinoid, together with the accompanying inflammatory reaction, was accepted as the hallmark of this group of rheumatic diseases. Material resembling fibrinoid in histological preparations may occur in other circumstances, as, for instance, in the base of peptic ulcers (Askanazy, 1921). It can also be produced experimentally by such diverse procedures as the injection of heterologous serum (Klinge, 1933; Rich and Gregory, 1943) and local mechanical trauma (Wu, 1937). Histologically defined fibrinoid is therefore clearly not a specific lesion. Nevertheless widespread fibrinoid change in the connective tissues is characteristically found in diseases such as rheumatic fever, rheumatoid arthritis, polyarteritis, lupus erythematosus, scleroderma, dermatomyositis, and serum sickness.

In rheumatoid arthritis, which is the disease we have studied most intensively, the fibrinoid change occurs in minute spots scattered sparsely throughout the affected tissues, but on the elbows and elsewhere sizable aggregates may form, which with their surrounding zones of fibrous and inflammatory tissue constitute the classical subcutaneous nodules.

Fibrinoid areas can often be seen with the naked eye as yellowish opaque spots in the semi-translucent connective tissue; but they can be recognized with certainty only by histological examination, when they are distinguished by the following characteristics. In a fibrinoid area connective-tissue cells are either few or absent altogether, and the regular fibre pattern of normal connective tissue is replaced by a disorderly mesh of swollen indistinct bundles or clumps of amorphous material which stains more intensely with eosin than the surrounding connective tissue. In frozen sections stained by Lillie's reticulin method and Van Gieson's stain a fibrinoid area may show three different effects—fine silver-staining reticulin fibres, occasional clumps of material staining reddish brown like normal collagen, and an indistinct refractile material which takes the yellow

colour of the Van Gieson stain. In sections treated by the McManus technique the fibrinoid areas stain strongly with the pink or magenta colour which is considered by many histologists to indicate the presence of polysaccharides. Histological evidence therefore suggests that a fibrinoid focus is not necessarily homogeneous and that it corresponds to alterations in both the collagen and polysaccharides of connective tissue.

It would be a substantial advance if these histological appearances could be correlated directly with observations at or near the molecular level.

Dr. Ball, of our unit, has recently been collaborating with Professor Astbury and his colleagues at Leeds in such a study, using the techniques of x-ray diffraction and electron microscopy. Owing to the random distribution and minute size of most fibrinoid lesions the first requisite was an accurate sampling technique. Details of this technique have recently been published (Kellgren *et al.*, 1951), and it suffices to say that excellent correlation between histological appearances and biophysical findings was eventually obtained. The results of these studies show that the parts of a rheumatoid nodule which show the histological appearances of normal collagen give an x-ray diagram of more or less disorientated collagen, and show plenty of normal collagen fibrils under the electron microscope. In the parts that on histological examination show advanced fibrinoid change the x-ray diagram typical of collagen may be entirely absent and is replaced by a diffuse ring with a spacing of about 4.8 Å. Material from such a spot, when examined under the electron microscope, may show only amorphous material and no collagen fibrils at all. Intermediate areas showing varying mixtures of normal collagen and abnormal material were found by all these techniques, and in such intermediate zones the electron microscope revealed broken and disintegrating collagen fibrils as well as increasing amounts of amorphous material. These results are illustrated in Figs. 1 and 2 (page 1094).

The histological staining reactions suggest that fibrinoid contains polysaccharide, and chemical analysis appears to support this view. Thus Ziff and his colleagues (1951, personal communication) have extracted most of the non-collagenous material from rheumatoid nodules with a solution of sodium hydroxide. The extracted material, which they assumed to be largely fibrinoid, was found to contain over 7% of polysaccharide—a value greater than the 6% which they found in umbilical cord, a tissue known to have an exceptionally high content. Furthermore, hydroxyproline, the amino-acid characteristically found in collagen, was not found in significant amounts in this extract, but they did find considerable quantities of tyrosine, which is not present to any extent in normal collagen.

Somewhat similar findings have been reported by Consden, Glynn, and Stanier (1952). They took nodules from cases of rheumatic fever, removed the collagen by autoclaving, extracted the fat with suitable solvents, and then analysed the residue, which they assumed to be largely fibrinoid. They found that the residue from the nodules (25–40%) was greater than the residue obtained from normal connective tissue (10–15%) treated in a similar manner. Both residues were hydrolysed and their amino-acid and sugar content estimated. The normal residue gave an amino-acid pattern similar to that of elastin, but the fibrinoid residue was more complex. In conformity with Ziff, they found a low content of hydroxyproline and considerable quantities of tyrosine, and also a high content of a reducing sugar. They suggest that fibrinoid in rheumatic fever is a mixture of substances, including some fibrin, but in our studies of fibrinoid from patients with rheumatoid arthritis no signs of fibrin appeared on any of the x-ray diagrams. Leaving aside these minor differences, it is clear that fibrinoid differs markedly from normal collagen and may be either a mixture of substances or a single glycoprotein which may well be related to the insoluble glycoproteins of the basement membrane and ground substance postulated by Gersh and Catchpole (1949).

At present we can only speculate on the mode of formation of fibrinoid, but the slow metabolic turnover in normal collagen which was revealed by the tracer studies makes it unlikely that fibrinoid results from a failure of collagen synthesis, and it seems more probable that the collagen-polysaccharide complex of normal connective tissue is somehow destroyed, the collagen being removed but the polysaccharides remaining in an unusually insoluble form.

Chondromalacia

The initial lesion of degenerative arthritis is found in the superficial layers of the articular cartilage, where it becomes softened and fibrillated. With constant joint movement this fibrillated cartilage becomes worn away, exposing the underlying bone, which in its turn becomes worn away. This process is accompanied by a compensatory condensation of the subchondral bone and marginal overgrowth of both cartilage and bone—the whole process eventually leading to severe disorganization of the joint.

This degenerative process occurs to some extent with advancing age in most individuals, but it may come on prematurely in many joints in certain subjects (Heine, 1926). It also comes on prematurely in individual joints that have been damaged by trauma, infection, or aseptic necrosis of adjacent bone, and also in joints submitted to unusual wear and tear through faulty mechanics or unusual occupational stress, or through loss of the normal protection afforded by intact pain and proprioceptive sensibility. These local factors are well known, and examples of their action are seen daily in clinical practice; they have also been studied experimentally (Mau, 1929; Bauer and Bennett, 1936). Thus we know a great deal about the causes of premature degeneration of individual joints, but we still do not understand the connective-tissue mechanism through which they work, and we know little of the constitutional disturbance which presumably underlies the widespread degeneration of old age and the premature onset of degeneration in many joints.

Let us therefore consider the initial connective-tissue lesion. This has been studied extensively by the Stockholm school, and an excellent description of the malacic process, as seen in the articular cartilage of the patella, has been published by Hirsch (1944). He was able to show that the earliest change consisted in a loss of elasticity in the cartilage, and that where this occurred the metachromatic staining which gives a tinctorial picture of the chondroitin content of the cartilage was grossly deficient. Furthermore, chemical analysis showed that the sulphur content of such cartilage was in fact reduced. This led to the conclusion that the primary lesion was a loss of the chondroitin sulphate moiety of the chondroitin-collagen complex of normal cartilage. Hirsch's methods, however, provided no information about the behaviour of the collagen moiety, but the fibrillary nature of the abnormal cartilage suggests that the collagenous fibres are to a large extent preserved, at least in the early stages.

A typical malacic lesion in cartilage is shown in Fig. 3a, and it is instructive to compare this with the area of fibrinoid connective tissue shown in Fig. 3b. Both preparations have been stained by the McManus technique to show up their polysaccharide content. It will be seen that the cartilage, which normally stains strongly, has become quite pale in the malacic area and that its normal hyaline texture is becoming fibrillary. By contrast the fibrinoid area stains strongly and is relatively amorphous compared with the fibrillary nature of the surrounding connective tissue.

Similar studies have been carried out on the nucleus pulposus of the intervertebral disks by Sylvén and his co-workers (1951). They found that normal disks from young subjects contained much hydrophilic mucoid which stained metachromatically and which from electrophoretic and other data they believe to be chondroitin sulphate. This chondroitin appears to be firmly bound to a mass of fibrils, which it largely obscures in ordinary preparations; but by various technical devices they were able to unmask these

fibrils, which then showed the typical cross-banding of collagen under the electron microscope. In a degenerate disk from an elderly subject the mucoid was much reduced and metachromatic staining was defective, while the electron microscope revealed large numbers of well-formed fibrils showing the cross-banding of normal collagen. Thus according to Sylvén the ageing process in the disk consists in a progressive loss of chondroitin sulphate, leading to the unmasking of the collagen fibrils normally embedded within it.

From these various studies it would appear that the initial lesion of degenerative arthritis consists in a loss of the

FIG. 3.—Micrographs of abnormal connective tissue by Dr. J. Ball, stained by the McManus technique to show polysaccharide content. (a) Malacic articular cartilage from human knee showing degenerative changes ($\times 65$). (b) Rheumatoid subcutaneous nodule ($\times 55$).

polysaccharide moiety of the collagen-polysaccharide complex of the connective tissues. Whether this results from a failure of polysaccharide synthesis or from the action of enzymes of the hyaluronidase type, or from some other mechanism, remains to be discovered.

These studies of the fibrinoid and malacic change have enabled us to observe more closely some of the ways in which the connective tissues may go wrong and to characterize at least two essential connective-tissue lesions at the molecular level—the degenerative change, in which there is a primary loss of polysaccharide, leaving the collagen fibres unsupported so that they may eventually disintegrate from wear and tear; and the fibrinoid change, in which the

collagen fibrils are destroyed at an early stage, leaving behind a material rich in polysaccharide which may be in the nature of a glycoprotein.

These two lesions should be thought of as typical tissue responses resulting from some interference with the normal mechanisms of connective-tissue maintenance, but these mechanisms may, of course, be interfered with in many ways, so that many different causative factors are likely to be involved in the production of these lesions.

(Figs. 1a, 1b, and 2a appeared in *Nature*, 1951, 168, 493, and are reproduced by permission.)

[The second Lecture, with a list of references, will be published in our next issue.]

MEGALOBlastic ANAEMIA IN YOUNG ADULTS

AETIOLOGICAL IMPORTANCE OF OCCULT IDIOPATHIC STEATORRHOEA

BY

HUGH CONWAY, M.B., B.Sc., M.R.C.P.

(From the Gardiner Institute of Medicine, University of Glasgow, and the Western Infirmary, Glasgow)

The addition of folic acid and vitamin B₁₂ to the list of preparations available for the treatment of megaloblastic anaemia has placed a new importance on the accurate differentiation of the megaloblastic anaemia of idiopathic steatorrhoea from Addisonian pernicious anaemia. Fortunately, this is not likely to be difficult when the features are classical, and, in particular, the presence of free HCl in the gastric juice will be decisive in excluding Addisonian pernicious anaemia. It is now well recognized, however, that anaemia and other deficiencies may develop as a result of steatorrhoea in the absence of a history of intestinal symptoms. For this reason the diagnosis may easily be missed unless specifically thought of and looked for (Tuck and Whittaker, 1950).

Addisonian pernicious anaemia is rare under the age of 30; only 2.8% of the 1,600 patients collected by Wilkinson (1949) were in this age group. The occurrence of megaloblastic anaemia, unassociated with pregnancy, before the age of 30 is therefore an indication for very careful assessment before the diagnosis of Addisonian pernicious anaemia is made.

The present investigation serves to stress the difficulty that may exist in distinguishing the megaloblastic anaemia of idiopathic steatorrhoea from Addisonian pernicious anaemia. The initial observation was that a patient in whom Addisonian pernicious anaemia had been diagnosed ten years before, at the age of 26, had a macrocytic anaemia refractory to parenteral liver. The early age of onset of the anaemia and its ultimate complete refractoriness suggested an error in diagnosis, and investigation revealed that the patient was suffering from idiopathic steatorrhoea, although bowel symptoms had never been troublesome. It was decided, therefore, to re-examine, if possible, all cases of Addisonian pernicious anaemia diagnosed before the age of 30 in one medical unit during the 13-year period 1937-49. There were eight such cases out of a total of 131 with that diagnosis. Four were regular attenders at a haematological clinic; one had died in another hospital with severe refractory macrocytic anaemia two years after discharge; the remaining three were easily traced. The

seven surviving patients were thoroughly reinvestigated during 1950 irrespective of their condition at that time.

Investigation

The patients investigated fall into two distinct groups: those proved to be suffering from idiopathic steatorrhoea and those in whom the diagnosis of Addisonian pernicious anaemia was confirmed; for ease of description, the former will henceforward be considered as Group A and the latter as Group B.

The investigation of each patient included a complete clinical and haematological examination, fractional gastric analysis, oral glucose-tolerance test using 50 g. of glucose, estimation of the prothrombin index and the serum calcium, phosphorus, and alkaline phosphatase, and radiological examination of the small intestine.

In addition, with a diet containing 70 g. of fat a day, the stools were collected over four-day periods, which were marked off from one another; each period was ended by a bowel wash-out. Three collection periods were used in two patients and two in the remaining five. The stools obtained during each period were dried in bulk and the fat content was estimated by the Soxhlet extraction method.

On completion of the investigation all anaemic patients were treated intensively for a few weeks with a potent intra-muscular liver extract; blood examinations were then repeated.

Idiopathic Steatorrhoea Misdiagnosed as Addisonian Pernicious Anaemia.—As a result of the investigation the diagnosis of Addisonian anaemia was confirmed in three patients, but four were proved to be suffering from idiopathic steatorrhoea. The latter patients are described below and the points of difference from Addisonian pernicious anaemia are discussed. It will be shown that the distinction is not merely of academic interest, for in three cases the change of diagnosis was followed by a more effective therapeutic regime.

Case 1

A woman now aged 36 was diagnosed in June, 1940, as suffering from Addisonian pernicious anaemia on the evidence of a megaloblastic anaemia combined with histamine-fast achlorhydria. There were no bowel symptoms. Parenteral liver therapy produced a very poor reticulocyte response and a slow rise in red blood cells. No abnormality of the stools was noted. Between 1940 and 1947 the red-cell count was found to be above 4 million per c.mm. on only one occasion despite intensive liver therapy. She never really felt well, but did not suffer from diarrhoea.

During the next two years her health was good, but towards the end of 1949 she again relapsed and was readmitted to hospital in January, 1950. There was still no complaint of diarrhoea or any other alimentary upset. Her face and neck and the dorsal surfaces of her hands were diffusely pigmented and presented an exaggerated effect of sunburn. The fingers and the toes were clubbed. Haematological examination revealed a moderately severe megaloblastic

TABLE I.—Fat Content of the Faeces on a Diet Containing 70 g. of Fat a Day

Group	Case No. in Text	Faecal Fat Output during 4-day Periods (g.)		
		1	2	3
A	1	58.8	70.0	—
	2	34.0	22.5	33.0
	3	35.0	31.8	—
	4	39.2	54.0	—
B	—	11.8	5.6	—
	—	11.2	14.0	19.0
	—	14.1	8.4	—
Control	—	8.1	14.4	—
	—	12.3	16.5	14.3
	—	15.3	—	—
	—	5.1	10.2	—
	—	6.3	—	—