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THE CONNECTIVE TISSUE*

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Klemperer and his colleagues (1942) were the first to draw attention to the possibility that certain conditions with common morphological aspects could be due to widespread disorders of the connective tissue, and thus was born the appellation "collagen diseases." The clinical similarities in conditions with these like pathological findings were quickly recognized, and rheumatic fever, rheumatoid arthritis, systemic lupus erythematosus, polyarteritis nodosa, progressive systemic sclerosis (scleroderma), and dermatomyositis have, by more or less general consent, been grouped as diffuse disorders of the connective tissue for some years. Some others have been added to them with the passage of time, examples being thrombotic thrombocytopenic purpura and relapsing febrile nodular non-suppurative panniculitis (Weber-Christian disease).

It was, however, realized from the first that common pathological changes, and even similar clinical features, were no evidence that these states had a common aetiology, and an additional objection was raised to this grouping of diseases on the score that more than collagen, or even connective tissue as a whole, was involved. The substitution of the term "connective tissue diseases" for "collagen diseases" thus did not satisfy the critics. In spite of the various distortions of his original conception, Klemperer (1956) once more supported his contention that this grouping was useful in that it introduced a concept of a diffuse intercellular pathology, whereas previously attention had been devoted exclusively to the cellular aspects of these conditions. Neuberger (1960) has given his support to Klemperer's conception, and so has Robb-Smith (1960) in a carefully reasoned argument.

Most clinicians also would wish to preserve this grouping, at any rate for the present, as it provides a starting-point for the consideration of certain complex clinical problems.

Before turning to the discussion of the little which is known of the aetiology and pathogenesis of the connective-tissue disorders, it is necessary to review some of the knowledge which is reasonably established concerning the fundamental aspects of connective tissue.

The connective-tissue system forms a continuous matrix throughout the body, and includes the bones, cartilages, the loose connective tissue of the skin, the tendons, ligaments, fascia, joint capsules, and the capsules of organs. The content of collagen in structures varies with the degree of strength which is required from them. Collagen in tendons, for instance, amounts

to 25 to 35% of their total wet weight, whereas in the vitreous humour it constitutes only 0.05% (Slack, 1959).

The connective tissue consists of three elements—cellular, fibrillary, and the ground substance. These are all closely associated with epithelial and other tissues, such as blood vessels, lymph vessels, and nerve fibres, and this is evidenced by the clinical studies of the so-called connective-tissue diseases, in many of which vascular disorders dominate the clinical picture. Some of the morphological and biochemical characteristics of the cellular and fibrillary elements, and of the ground substance, have already been reviewed (Richardson, 1959).

Cellular Elements

The cells of the connective tissue are of two types—the fibroblasts and the mast cells. Fibroblasts are the more numerous cells, and their main function is the formation and liberation of collagen into the extracellular spaces. They contain in their cytoplasm granules composed of mucopolysaccharides and protein, and it is possible that these granules are responsible for the development of collagen fibrils, but this is still unproved.

The other cells, the mast cells, are mesenchymal in origin. They contain metachromatic granules in their cytoplasm which stain with basic dyes such as toluidine blue. These granules are composed of mucopolysaccharides which enter the ground substance from the mast cells. The mast cells are associated with sulphate metabolism and may be responsible for the storage of heparin and the secretion of hyaluronic acid, and are probably concerned with histamine release. The function of the mast cells therefore appears to be related to the ground substance rather than to fibril formation.

Fibrillary Elements

These are believed to be produced entirely by the fibroblast, which is thought to be solely responsible for the formation of different fibres, which may be collagenous, reticular, or elastic.

Collagen

The electron microscope has greatly altered and enlarged our conception of the appearances of connective tissue. Its use has demonstrated that the collagen fibril is approximately 1,010 Ångstrom units in width, and is characterized by regularly occurring cross-striations spaced at intervals of 640 Ångstrom units (an Ångstrom unit is 0.1 m μ). This particular spacing is constant for collagen for human tissue from whatever site it is taken. There are also inter-period bands between the 640-Ångstrom-unit period; but here the

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number is not fixed, and the different numbers of bands probably indicate differences of chemical structure.

The width of collagen fibrils varies with different structures but tends to increase with age. Age also affects the destructive action of the enzyme collagenase on collagen, as it is greater in young tissue, and the increased susceptibility of collagen to this enzyme below the age of 25 years might afford an explanation for the vulnerability of collagenous structures in young heart valves in such conditions as rheumatic fever. The effect of age on connective tissue depends to some extent on the gradual reduction of the very high water content which is present in the embryo, and this is thought to lead to the side-chains of the polypeptides becoming drawn together and losing some of their physical and chemical activity. On the basis of this view ageing of the connective tissue is a drying-out process.

Chemically, collagen is protein and contains over 25% of the amino-acid glycine, and has a high hydroxyproline and proline content.

The polypeptic chain of collagen is stabilized by cross-linkages with other adjacent chains. The effective linkage is the hydrogen bond between the NH group of one chain and the oxygen of a carbonyl group of another. When these hydrogen bonds are disrupted by heat the collagen fibre shrinks and takes up its natural shape, that of a random coil. The estimation of the shrinkage temperature (T_s) at the level where shrinkage begins (T_{s1}) and where it ends (T_{s2}) is a means of determining the effect of various substances on collagen. It has been shown that a reduction in shrinkage temperature can be achieved by hydrogen-bond breakers, and Glynn (1959) has found that sodium salicylate and related compounds are the most active.

When a collagen fibre has shrunk—that is, has had its hydrogen bonds broken—it is, *in vitro*, readily digested by proteolytic enzymes such as trypsin. It is possible that, *in vivo*, dissolution of collagen is preceded by the action of some hydrogen-bond breaker (Glynn, 1959).

The fibrous protein of collagen is highly resistant to proteolytic enzymes and to absorption *in vivo*. The structure and stability of collagen have been reviewed by Glynn (1959), who studied the resistance to absorption of collagen by means of implants. He found that collagen—that is, facial strips, taken from a freshly killed rabbit and implanted subcutaneously—persisted virtually unchanged for 13 weeks, with an almost identical appearance after over a year, whereas subcutaneous implants of liver and heart muscle disappeared in four weeks, and of kidney in six weeks. When the implants were made in the erector spinae muscle, only fascia remained after four weeks.

Neuberger and Slack (1953) had previously demonstrated the stability of collagen in another way by showing how extremely slow was the turnover of limb collagen when glycine labelled with the radioactive isotope C-14 was fed to adult rats.

Reticulin and Elastic Fibres

The second fibre in the connective tissue is the reticular fibre, sometimes called reticulin as it forms a fine network or reticulum. The reticular fibre contains carbohydrate, and this can be demonstrated by various oxidative methods, and has a cross-striation under the electron microscope similar to that of collagen. There is evidence in support of the suggestion that the fibrillary element of reticulin is a variety of collagen, having a

similar protein constitution, but there is an abundant amorphous matrix in reticulin, rich in carbohydrate, in which the arrangement of the reticular fibrils is random and unlike the orderly parallel bundles of collagen.

The third element, elastic tissue, has a different appearance from collagen under the electron microscope, and also a different chemical composition, containing more of the amino-acid valine, and less hydroxyproline and proline.

The Ground Substance

Alteration in the chemical composition and physical properties of the ground substance is probably the basis of many of the connective-tissue disorders.

The mast cell is thought to be responsible for the production of ground substance, but it may not be solely concerned, as there is some evidence that fibroblasts may play a part. The ground substance, lying between the meshes formed by the fibrils of the various fibrillar structures which constitute the protein framework of the connective tissue, contains protein derived from the extracellular fluid, together with a high content of mucopolysaccharides, chiefly hyaluronic acid, chondroitin sulphate, A, B, and C chondroitin, and keratosulphate. It also contains mucopolysaccharides and protein complexes known as glycoproteins. These glycoproteins tend to be the least soluble at the basement membrane, where they are in close relationship with collagen and reticulin fibres.

The ground substance is normally the consistency of a gel, and stains metachromatically, both hyaluronate and sulphate mucopolysaccharides staining in this way, but in certain disorders it loses this staining characteristic. Scurvy is an instance of this, the ground substance ceasing to stain metachromatically. Further evidence of alteration in the ground substance in this disorder is an increase of glycoproteins in the blood.

Hyaluronic acid is responsible for certain physico-chemical properties of ground substance—namely, its water-binding capacity, its gelatinous character, and its viscosity. It appears to act as a lubricant and, as Karl Meyer (1954) says, a shock-absorber.

Changes in the permeability of the ground substance can be effected by means of the enzyme hyaluronidase, which increases permeability by removal of hyaluronic acid from the meshes of the protein fabric. This process can be reversed by diffusion with large macromolecules, which suggests that flow takes place through spaces of such a size that they can be blocked more readily by large macromolecules than by smaller ones.

The endocrine glands have their influences on the chemical state and function of the connective tissue, and various well-recognized clinical features depend upon this.

In severe cases of hypopituitarism and in pretibial myxoedema the collagen bundles and elastic fibres may be swollen and degenerate, with an increase in the hyaluronic acid and chondroitin acid content of the ground substance and in the number of mast cells in it. Examination of the ground substance shows a reduction in mucopolysaccharides and mast cells, as well as degeneration of the collagen and elastic fibres, which stain poorly. Hormonal deficiency or overactivity, therefore, can express itself in marked changes in the connective tissue, and especially in the ground substance.

The wide purple striae found on the abdomen and around the hips and shoulder girdles in adrenal hypercorticism are due to a reduction in the tensile

elasticity and strength of the skin. Histologically the skin is atrophic, and the ground substance contains little mucopolysaccharide. Mast cells are usually scanty and the connective tissue as a whole acellular. Both collagen fibrils and elastic fibres show degenerative signs and stain faintly. The connective tissue, therefore, and the ground substance in particular, is severely affected by deficient or overactive hormonal influences.

Neuberger (1960) has expressed the view that, interesting as are the studies in changes in collagen synthesis or its breakdown, the biochemistry of the components of the ground substance, such as the mucopolysaccharides, is likely to be of greater importance in elucidating the essential factors in the connective-tissue disorders. Further, he suggests that the same might well be true of the study of immunological reactions and of systemic antibodies.

Antigenicity of Connective Tissue

Immuno-histochemical techniques have been used to investigate the antigenicity of connective tissue (Scott, 1959). This technique depends on labelling a specially prepared antibody globulin with a substance which will fluoresce under the fluorescent microscope. If the fluorescing antibody globulin is then exposed to tissue containing its specific antigen, it will be fixed by the antigen and the site of the antigens identified by fluorescent microscopy. Using two different labels on different preparations of antibody globulin, Scott was able to recognize three connective-tissue antigens or groups of antigens. These he called basement membrane antigen, reticulin antigen, and fibrous-tissue antigen.

The basement membrane antigen reacted only with antiglomerulous conjugates. The other two reacted not only to antiglomerulous conjugates, but also to anti-synovium, the difference between them being that fibrous-tissue antigen reacted preferentially with anti-synovium whereas reticulin did not.

Scott makes it clear that there is not as yet enough information to enable any correlation between results obtained by immuno-histological methods with ones from chemical and physico-chemical analyses. This method of investigating the properties of connective tissue is, however, likely to yield much valuable information in the future.

Evidence of hypersensitivity has been put forward as aetiologically significant in each of the conditions commonly included as connective-tissue disorders. There is in fibrinoid degeneration a pathological change common to all these disorders and to allergic reactions. This change is not, however, confined to these conditions, and is found in such diverse states as malignant hypertension and thromboangiitis obliterans, where it affects the vessel walls, in inflammations of serous, mucous, synovial, and endothelial surfaces, in the base of peptic ulcers, and also in hygromas. It has been described in addition in the placenta, although the fibrinoid material in this situation is regarded as being of a different character to that found elsewhere. It can be produced by trauma to tissue such as squeezing, or by prolonged passive hyperaemia. It is seen in various acute bacterial infections and has followed the use of large doses of deoxycortone acetate.

It is clear, therefore, that fibrinoid change is not specific for any particular group of disorders, although it occurs regularly in allergic conditions.

There is evidence that fibrinoid material contains inspissated fibrin as one of its constituents. Other suggestions are that fibrinoid may be due to changes in ground substance or to necrosis of collagen, or to a combination of these processes together with the products of degenerated smooth muscle.

Study of rheumatoid nodules by x-ray defraction technique suggests that when the fibrinoid change is far advanced there is complete destruction of collagen, whereas in the intermediate stages the x-ray pattern shows the presence of amorphous material and disintegrating collagen fibres.

Chemically, fibrinoid material lacks both proline and hydroxyproline, and in this it does not resemble collagen. It also has no close resemblance to collagen under the electron microscope. Under the light microscope it appears as a homogeneous eosinophilic and relatively acellular refractile substance. It has some of the tinctorial properties of fibrin, and it was these appearances and staining properties that led to its introduction as a descriptive term.

There is some evidence of hypersensitivity in the therapeutic response of most of the so-called connective-tissue diseases, as well as frankly allergic states, to corticosteroids or corticotrophin. Drugs such as testosterone, sulphonamides, the antibiotics, and the thiouracils have been indicated as causative agents in the development of polyarteritis nodosa, and the rise in the incidence of systemic lupus erythematosus has been attributed to the increased use of the sulphonamides and of antibiotics, notably penicillin. Again, a condition closely similar to that of systemic lupus erythematosus has been shown to follow the use of the hypotensive drug hydralazine.

These associations could have a basis in hypersensitivity, as it has been shown that a hapten, a non-antigenic substance foreign to the body—for example, a drug—can combine with a body protein, and by so doing render it antigenic.

The presence of the rheumatoid arthritis factor and of the two lupus erythematosus cell factors in systemic lupus erythematosus (Bencze *et al.*, 1959) suggests that there are circulating antibodies in these two conditions.

Whether or not the rheumatoid arthritis factor and the lupus erythematosus factor are more than interesting by-products of these conditions remains to be decided, and it must be admitted that the level in the blood of the rheumatoid arthritis factor does not parallel the clinical course of these diseases, and its addition does not increase the severity of the patient's condition. Furthermore, the transmission of the factor to volunteers has not produced the disease, and people can carry the rheumatoid arthritis factor without having any clinical manifestations of the disease—so-called false-positive individuals. These are sometimes the siblings of patients with rheumatoid-arthritis-factor-positive rheumatoid arthritis.

Auto-immunity

The possibility that an auto-immune reaction may be the underlying process has been recently suggested.

An auto-immune reaction was first demonstrated in Hashimoto's thyroiditis. Antibodies were shown to have been produced against, not foreign protein, but one of the body's own components, in this instance thyroglobulin. There thus must be some mechanism normally present which allows the body's immunological processes to distinguish between its own products and foreign ones,

as otherwise there would be a constant production of auto-antibodies. Until recently the idea that this could ever happen was contrary to all accepted immunological theory, and speculation now centres about the nature of the normally efficient mechanism for distinguishing between "self" and "not self."

It has been shown that the power of antibody-forming tissue to react is largely dependent on the presence of antigens at the time the tissues reach maturity, and that tolerance can be induced by presenting the tissues with antigen some time before the production of antibodies begins. This is somewhere round about the time of birth. It is possible that if the antigen cannot gain access to antibody-producing tissues at this time no tolerance is acquired, and later in life the antigen from the person's own tissues may lead to antibody production.

Macfarlane Burnet (1959), in his clonal selection theory of immunity, has suggested that body cells possess specific immunological properties or competence which are genetically determined. The antigen, according to this theory, does not, as in the classical theory of immunity, imprint its pattern by imparting information which results in the production of an antibody complementary to itself, a template, but it simply stimulates certain mesenchymal cell clones which are genetically determined to respond by antibody to it. A clone can be defined as a group of individuals of like genetic constitution obtained by asexual reproduction from a single original individual. When the clones multiply either their destruction may result, as normally happens, or proliferation may take place and a disease process follow. The clones of cells are called "forbidden clones" if the production of their union with antigen does not lead, as it normally does, to their destruction and elimination from the body, but rather to their proliferation and the further production of antigen, with the establishment of a vicious circle.

The antigen therefore, according to this theory, stimulates cells to produce the specific antibody which they have been genetically determined to produce, and does not itself determine the specificity of the antibody.

An alternative hypothesis is that antibody-producing cells may undergo somatic mutation themselves, with the result that they produce auto-antibodies and thus auto-immune disease.

Auto-immunity is an attractive theory by which to explain the association of neoplasms with dermatomyositis, and the presence of widespread vascular lesions with thromboses in patients with malignant growths. It could be postulated that the neoplasm produces breakdown products to which cells either lose their immunological tolerance or have genetically determined specific immunological properties.

That auto-immunity can occur is beyond doubt, but the nature of the breakdown of the normal mechanism existing to prevent the continual production of auto-antibodies is far from certain. It is also uncertain whether the auto-immunity is primary and a causative factor, or secondary and the result rather than the cause.

The position concerning the presence and significance of various and increasingly numerous auto-immune reactions in systemic lupus erythematosus has been reviewed most profitably in the *British Medical Journal* (1960). There can be some overlap between thyroid antibodies and antinuclear antibodies in Hashimoto's disease, and between the antinuclear factors and the

rheumatoid arthritis factor in both systemic lupus erythematosus and rheumatoid arthritis. It seems that the evidence at present suggests that in systemic lupus erythematosus, at any rate, auto-immune antibodies are the result rather than the cause of the disease.

The evidence that the connective-tissue disorders are in part due to auto-immunity, or even to hypersensitivity, is thus very far from complete, and it is sobering to reflect that if the role of hypersensitivity is disproved there is little at present to put in its place.

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ATHEROSCLEROSIS: RESULTS OF A PILOT SURVEY IN A NORTH LONDON AREA

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The object of this survey, which was exploratory and limited in extent, was to examine the pattern of aortic atherosclerosis as it presented in a North London area, so as to study its natural history and to see if it differed in any way from that found in other parts of the world.

Currently, several surveys of atherosclerosis are being carried out on a geographical basis so as to ascertain the importance of the various factors of race, climate, terrain, social behaviour, and diet as aetiological agents in this disease (W.H.O., 1958), if, indeed, atherosclerosis is a disease as distinct from the physiological process of ageing.

As it is impossible to examine the progress of the disease in its various stages in the same human subject the only recourse is to examine trends in several hundreds of patients at necropsy. As is recounted below, there have been difficulties in assessing the extent and severity of the various lesions, both objectively and precisely, so as to be reproducible in the form of mathematical data.

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