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THE CONNECTIVE TISSUE SYSTEM

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A popular anatomy book of the seventeenth century states:

"It is sufficient to know that all the Parts of the Body are made up of Threads, or Fibres, of which there be different Kinds; for there are some soft, flexible, and a little elastick; and these are either hollow, like small Pipes, or spongy, and full of little Cells, as the nervous and fleshy Fibres; others there are more solid and flexible, but with a strong Elasticity and Spring, as the membranous and cartilaginous Fibres; and the third Sort are hard and inflexible, as the Fibres of the Bones."

This idea of a fibrous network as the basis of human structure has gradually developed, although the actual term connective tissue—*bindegewebe*—was first used by Johann Müller in 1830 (Robb-Smith, 1954). The purely mechanical concept of the function of connective tissue, one might even say the static or passive role of the tissue, has dominated physiological and pathological thought in the past, and only during the twentieth century has its active role in nutrition and defence as well as in structure begun to be appreciated. The developments of scientific knowledge, and especially of new techniques, have led to a reawakening of interest in this very important system of the body.

The connective tissue system includes such diverse organs as the bones, the cartilage, the loose connective tissue of the skin, and the viscous fluid of the umbilical cord (Wharton's jelly), yet it is continuous throughout the body. Members of the Heberden Society must inevitably be interested in every part of the connective tissue system, but I shall confine this review to some of the problems of less highly specialized connective tissue.

Connective tissue consists of three essential elements: the cells, the fibres, and the ground sub-

stance. It is, however, freely interspersed by blood vessels, lymph vessels, and nerve fibres, and is closely associated with epithelial and other tissues.

The apparent scarcity of cellular elements in some connective tissue has led to a comparative neglect of this component. The fibrocyte or fibroblast is the essential cellular element, and four types of fibrogenic cell have been described by Fitton Jackson (1954a). In the cytoplasm of these cells cytoplasmic granules can be demonstrated by the electron microscope approximately 1μ in size (Fitton Jackson and Smith, 1955). The granules stain metachromatically and the presence of hexosamine indicates the presence of mucopolysaccharides. There is also evidence for the presence of other polysaccharides and approximately 30 per cent. of the granules are composed of protein. The close relationship of these granules, both *in vivo* and *in vitro*, with the development of collagen fibrils has inevitably led to conjecture as to their precise role. This must for the moment remain an unsolved problem. The evidence of Fitton Jackson (1954b, 1956) for the presence of fibril formation within the cell, and the lack of a well-defined cell surface, together with the absence of any definite evidence of the presence of nucleotides, suggest that the granules may provide an essential element for fibril formation or may be responsible for bringing about conditions suitable for fibril formation—even outside the cell.

The other cells seen in considerable numbers in connective tissue are the mast cells. Their function is not known, but there is evidence that they may store heparin (Jorpes, Werner, and Åberg, 1948). It has also been suggested that the mast cells secrete hyaluronic acid (Asboe-Hansen, 1950, 1951) and that they may be concerned in histamine release (Riley, 1953). Their function, in other words, appears to be related more to the ground substance than to fibril formation.

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It will be observed that no reference has been made to the presence of an elastocyte or an elastoblast; although some workers have suggested that such a cell exists, the consensus of opinion is that the fibrocyte or fibroblast is the sole cell responsible for the formation of the different fibres: reticular, collagenous, or elastic.

Perhaps in no branch of biological science has the development of knowledge been greater than in the composition, mode of formation, and structure, of fibrous proteins.

The morbid anatomist has, until recently, relied upon the use of staining and the light microscope to differentiate between the different fibres. Ehrlich (1877) first used the term *metachromasia* for the peculiar property of basophilic dyes to stain certain structures a different colour from that of the dye, and this has been used as a histological basis for the differentiation of the different fibres. Unna (1896) clearly realized that intermediate staining reactions occurred, and we were led in our early studies to conclude that "all that glitters is not gold", elastic-like or elastoid staining being obtained with altered collagen, and that it was possible as a result of enzymatic action on collagen to make a tissue stain with orcein or Weigert's stain. Gillman, Penn, Bronks, and Roux (1954, 1955) have extended these studies to pathological conditions, and have restressed some of the potential fallacies of conclusions based upon so-called normal histochemical techniques. There is still no unanimity as to the cause of metachromatic staining, whether it is due to chemical interaction between a polysaccharide or protein side-chain, or whether it is as Bank and Bungenberg de Jong (1939) suggest, a phenomenon dependent upon electric charge density. Another technique employed to differentiate between the different fibres has been the use of silver staining

methods, the introduction of which led to the differentiation of the reticular fibres.

The development of the electron microscope revealed that the fine fibres of the light microscope, often referred to as fibrils, were, under the higher magnification, merely small fibres, and that much smaller structures, fibrils, were the tissue unit. The work of Schmitt, Hall, and Jakus (1942), and of Wolpers (1943) showed that the collagen fibril was approximately 1,100 Ångstrom units in width and was characterized by cross striations regularly spaced about 640 Ångstrom units apart. This peculiar spacing is a constant feature of collagen from human tissue, although collagen with a different periodicity has been observed from other animal sources. Examination at higher magnification revealed the presence of bands within the 640 Ångstrom period (Fig. 1). Wolpers (1944) described two or three such bands, Schmitt, Hall, and Jakus (1945) described five, and eventually Hofmann, Nemetschek, and Grassmann (1952) described ten. These bands have fixed intraperiod positions and density of staining with phosphotungstic acid. They are thought to reflect the chemical composition and structure of the collagen fibril and may be said to be the finger print of collagen. Wolpers (1950) expressed the view that a normal natural collagen fibril had only two intraperiod bands and that the presence of more bands must be considered to be an indication of pathological change. This viewpoint is not generally accepted, but there is agreement that the number of intraperiod bands is not constant, and that different numbers of bands probably indicate differences of chemical structure.

The comparison of collagen fibrils from human sources shows little fundamental difference in either the gross or the minute structure at the lower powers of magnification, save that the collagen from a foetal

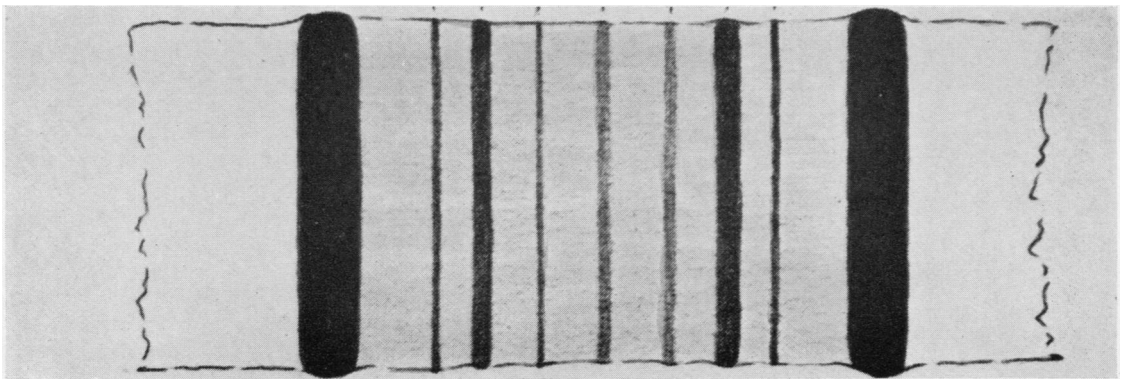


Fig. 1.—Diagrammatic representation of intraperiod bands.

Achilles tendon shows a diffuse sheet formation rather than the characteristic fibres (Fig. 2). This peculiar sheet formation in Achilles tendon is found throughout foetal life and has been seen by us up to the sixth month of infant life. However, careful analysis of fibril width has revealed differences between the fibril width in different tissues and also the presence of changes with chronological age (Grassmann, 1957; Gross, 1950; Schwarz, 1953; Pahlke, 1954). It has been shown that the fibril thickness increases with age, and this is so characteristic that the age of the fibril can be determined by such measurements. There is also a characteristic fibril width for different tissues, although for some tissues the scatter is greater than for others. Schwarz (1957), using silver impregnation methods coupled with stereoscopic electron microscopy, has shown that two types of silver staining are obtained with collagen fibrils: the silver may be uniformly spread over the surface of the fibre or it may be concentrated in the dark band of the intraperiod cross structure. Four or five granules are to be seen lying side by side in the dark bands of the fibrils. Longitudinal section has shown that this was not an artefact and that the granules were deposited in the fibrils. The internal silvering is not present in all collagenous material, in fact it is never found in the

collagen obtained from healthy sclera, but it is found in the collagen of the Achilles tendon. The development of intrafibril staining is a feature of ageing of certain tissues such as the Achilles tendon. The possible implication of these findings on the structure of collagen will be referred to later.

Another interesting electron microscope development was the report of Wyckoff (1952), confirmed by Kennedy (1955), that the collagen fibrils have a tubular structure.

Bowes and Kerten (1948) have determined the composition of collagen, using what for biologists must be considered rather vigorous methods of extraction, and have obtained a product of constant composition from calf skin. The striking feature of the amino acid analysis of collagen is the high content of glycine, over 25 per cent., the high content of hydroxyproline and proline, and, to a lesser extent, the amounts of aspartic and glutamic acids. Attempts are being made to determine the structure of collagen by dislodging proteins and polypeptides from the main collagen mass and by analysing the amino acid sequences. Perhaps the greatest recent controversy has been that over the significance of the small carbohydrate content (approximately 0.5 per cent.). Until recently, most chemists have suggested that the carbohydrate was due to the presence of

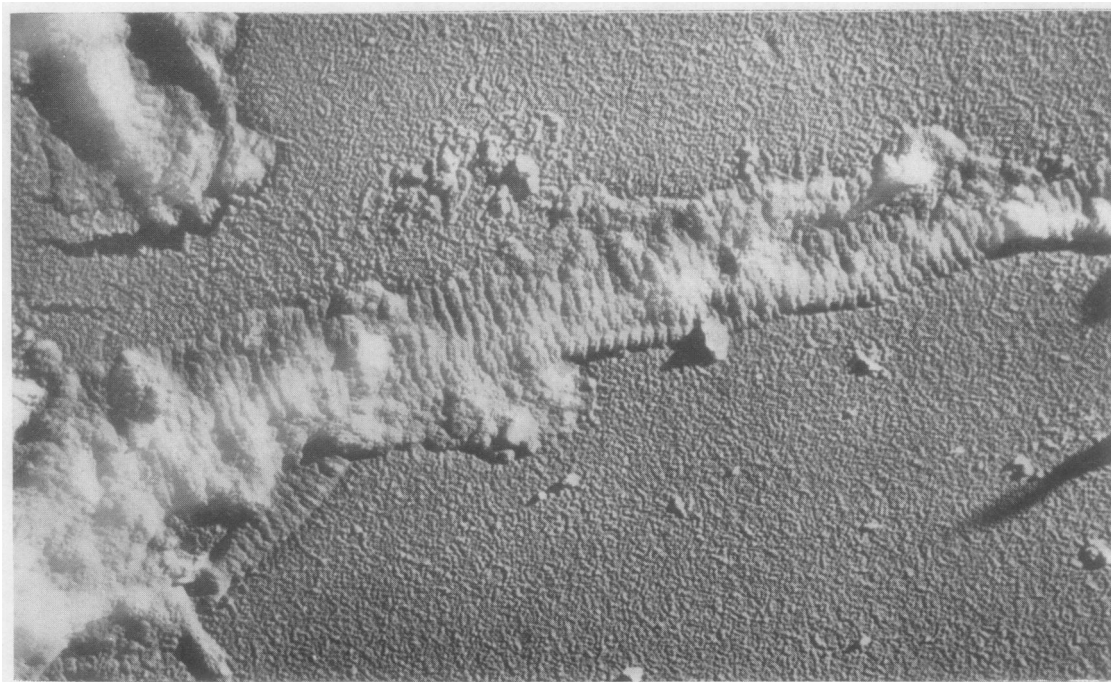


Fig 2.—Collagen from Achilles tendon of 6 months foetus. $\times 24,000$,

cement substance, or ground substance, which had been inadequately removed during preparation. Grassmann (1957) recently suggested that the carbohydrate might form an integral part of the collagen fibril. The presence of silver particles in the fibril could be interpreted as indicating their adherence to cement substance between finer fibrils or their affinity for certain amino acids such as hydroxylysine, and does not necessarily imply that the carbohydrate is an integral part of the fibril. Collagen from some animal sources contains a considerable proportion of carbohydrate, so that there is evidence from the wider biological field of a close association between polysaccharide substances and the protein molecules of collagen. D. S. Jackson (1953) has shown that conditions which are favourable for the extraction of mucopolysaccharides are associated with a marked reduction in the stability of the tissue. D. S. Jackson (1954) also suggested that chondroitin sulphate might form stabilizing linkages between collagen chains. It is interesting that Wood (1954), working in my department on the physical properties of collagen and elastic fibres, found that the treatment of native elastic tissue with formaldehyde or periodate increased the cross linkages and strengthened the material, but that when collagen-free tissue was used these reagents had very little effect.

The complexity of the collagen problem is perhaps even more fully appreciated when one examines the products of extraction. Nageotte (1927) first reported the solubility of rat tail tendon in dilute acetic acid. Orekhovitch, Tustanovsky, Orekhovitch, and Plotnikova (1948) isolated a citrate-soluble fraction which yielded fibrils on dialysis. The precipitated fibrils had the characteristic cross-striation of collagen under the electron microscope, and Bowes, Elliott, and Moss (1953) have found that the amino acid analysis of citrate soluble collagen was very similar, if not quite identical, to that of natural collagen. Considerable study of the soluble fractions of collagen in both acids and alkalis, and the factors concerned in the precipitation of fibrils (dialysis, salt concentration, and mucopolysaccharides) have been made both by the Massachusetts group, Schmitt and Gross in particular, and also by the King's College group, Fitton Jackson and Randall (1953) and Randall, Booth, Burge, Fitton Jackson, and Kelly (1955). Schmitt, Gross, and Highberger (1955) reviewed their findings and discussed three forms of collagen: standard fibrous collagen, fibrous long-spacing (F.L.S.), and segment long-spacing (S.L.S.). The fibrous long-spacing material (F.L.S.) can be produced by the addition of a variety of substances, chondroitin sulphate,

thrombin, heparin (Randall, Fraser, Fitton Jackson, Martin, and North, 1952), whereas originally Highberger, Gross, and Schmitt (1951) had supposed that only mucoprotein would induce the change after dialysis with ichthyocol acid filtrate. The fine structure of F.L.S. varies considerably, but the amino acid composition is qualitatively the same as collagen, and the high-angle x-ray diffraction picture is similar to that of collagen. The striking feature is the variability within, as it were, the overall general similarity of pattern. The S.L.S. is also similar in chemical analysis to collagen, but there are structural differences and these segments are produced most readily by the addition of A.T.P. to ichthyocol acid filtrate.

These findings serve to indicate the complexity of collagen as regards structure, chemical composition, and reactivity, and to render less disturbing the findings of our group on the action of alkalis and enzymes upon collagen (Hall, Keech, Reed, Saxl, Tunbridge, and Wood, 1955; Burton, Hall, Keech, Reed, Saxl, Tunbridge, and Wood, 1955) have shown that alkaline buffer solutions, pH range 7.0 to 10.4, and phthalate buffer solution of pH 5.0 cause profound changes in the collagen fibrils, producing products which have the tinctorial properties and the morphological appearance under the electron microscope of elastic tissue fibres. These findings might of course be artefacts, but the structural changes are accompanied by chemical changes, the modification of the collagen fibrils being accompanied by the release of proteins or polypeptides rich in hydroxyproline and arginine and also of material rich in reducing sugars (Burton and others, 1955). I do not wish to overstress the significance of these results, also confirmed by Banga, Baló, and Szabó (1954, 1956), but merely to underline the complexity of collagen and elastic tissue fibres.

Astbury and Bell (1939), largely on the basis of x-ray diffraction data, classified the fibrous proteins into two broad groups: the keratin, myosin, epidermin, fibrinogen (K.M.E.F.) group, and the collagen group. The K.M.E.F. group, in addition to having common structural features, which is the reason for their similar and characteristic diffraction patterns, all exhibit long range elasticity. The collagen group also have a characteristic diffraction pattern which is quite distinct from that of the K.M.E.F. group.

Elastic tissue fibres have presented a problem. Their structure is much less well-defined than that of collagen; Hall, Reed, and Tunbridge (1955) have described three common components (branching fibres, sheetlike structures, and debris material) of elastic tissue fibres from many human and other

animal sources (Fig. 3). The *x*-ray diffraction pattern is quite distinct from that of collagen and the chemical analysis of Partridge and Davis (1955), Partridge, Davis, and Adair (1955), and Partridge (1957) would suggest that there are very marked differences in the amino acid composition, mainly the higher amount of valine and the small amount of hydroxyproline and proline. Despite these differences and the marked contrast in physical properties, *i.e.* the much greater elasticity, the absence of any real evidence for an elastoblast, and the fact that the formation of collagen would appear to be a prerequisite for the formation of elastic tissue fibres, available evidence would indicate that elastic tissue fibres are probably members of the collagen group of fibres rather than of the K.M.E.F. group. Furthermore, elastogenesis is usually associated with the presence of large amounts of metachromatic staining material, and since many mucopolysaccharides exhibit metachromatic staining mucopolysaccharides may play

an important role in determining the formation of elastic tissue fibres and possibly an even more important role than in the case of collagen.

The discovery by Baló and Banga (1949a, b, 1950) of a new enzyme elastase in the pancreas has assisted greatly in the study of elastic tissue. Hall (1957) has shown that there are two components of the enzyme, a fraction E_1 , with maximum activity at pH 7.8, which attacks mucopolysaccharides, and a second fraction E_2 , with a maximum activity at pH 8.7, which is proteolytic. These findings have been confirmed independently by Banga, Baló, and Szabó (1954, 1956). In natural tissue, collagen and elastic fibrils are intimately related, so that separation without the risk of degradation has been well-nigh impossible. The specificity of action of the enzyme elastase has rendered possible the removal of elastic fibres as well as permitting a fuller analysis of the structure and breakdown products of elastic tissue fibres themselves.

The third fibre, the reticular fibre, was first

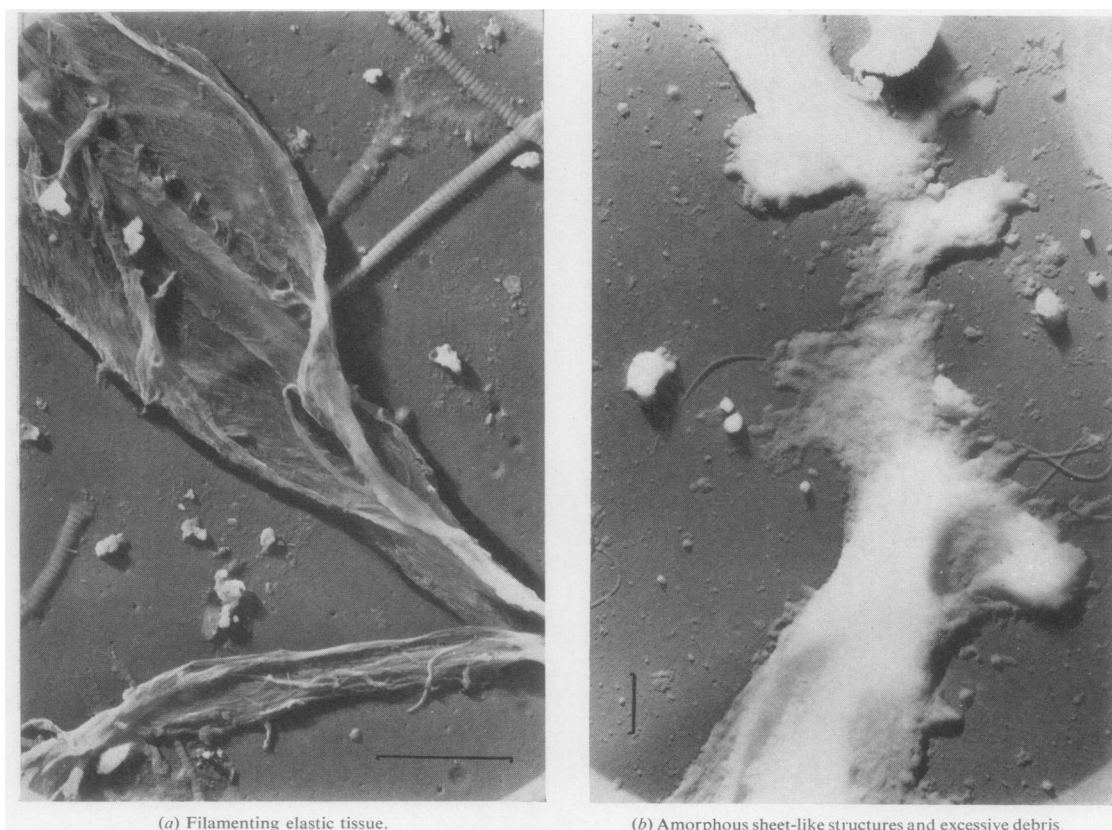


Fig. 3.—Examples of elastic tissue. (The mark indicates 1μ)

isolated by Mall (1888), from lymph nodes. The fact that on boiling the fibres failed to produce gelatin led him to conclude that they were not collagenous. The reticular fibres, often referred to as reticulin, are very fine fibres which branch and anastomose with one another to form a fine network or reticulum. Reticular fibres are most numerous in young tissue and basement membrane. They are not birefringent in polarized light, and they stain poorly with Van Gieson's stain, but with silver staining techniques they readily absorb silver particles. On the other hand, reticulin reacts to certain enzymes such as trypsin and collagenase, as does collagen. Kramer and Little (1953) have clearly shown, with the aid of the electron microscope, that the fibrils are soluble in boiling water, the reason for their resistance being the presence of a very stable amorphous component, rich in both protein and carbohydrate. Windrum, Kent, and Eastoe (1955) have shown that renal cortical reticulin has 4.2 per cent. of non-hexosamine carbohydrate and 10.9 per cent. of bound fatty acids, predominantly myristic acid. The fibrillar element of reticulin, however, has a cross-striation similar to that of collagen, but with a periodicity of approximately 210 Ångstrom units. The amino acid content also closely resembles that of collagen. There is thus very sound evidence for thinking that the fibrillary element of reticulin is a variety of collagen, but the view that reticulin is necessarily a precursor of collagen is not universally accepted.

The last component of connective tissue is the ground substance. The universality of the ground substance, filling as it does all the interfibrillary spaces, renders the study of the substance or group of substances difficult. It is not easy to be certain of the purity of the extracts or that extracts of fibrillary components have always been obtained without including some of the ground substance components. The ground substance plays a very important role in the regulation of water balance and is extremely susceptible to changes of hydrogen ion concentration and of other ions. Excellent reviews of this aspect of the function of connective tissue are available and no further reference will be made. Meyer (1954), in reviewing the ground substance of connective tissue, stressed that the products of extraction are largely dependent on the method employed, but that mucopolysaccharides and proteins are the important constituents. The following mucopolysaccharides have been isolated from ground substance: hyaluronic acid, chondroitin sulphates, A, B, C, chondroitin, and keratosulphate. The ground substance of the different tissues varies in the quantity and quality of mucopolysaccharides

present. The ubiquitous nature of so many of the components of ground substance can and does interfere with the interpretation of staining reactions and even with the chemical analysis of the fibrous components of connective tissue; this is a point of particular significance when studying and attempting to interpret pathological changes.

This review of some of the basic properties of the elements of the connective tissue system may serve to illustrate the complexity of the system and to show how this very complexity may indicate the variety of responses which may occur to a given stimulus. The influence of age upon the response of the fibrous elements to enzymes is very important, and the work of Keech (1955) upon the action of collagenase upon collagen prepared from the skin of the abdominal wall from patients of different ages is of great interest to rheumatologists. The increased susceptibility of the collagen below the age of 25 to enzyme action might afford an explanation for the vulnerability of the collagenous structures of the heart valves in rheumatic fever (Table I, overleaf).

The increasing prevalence of certain disorders, peri-arteritis nodosa, lupus erythematosus, scleroderma and dermatomyositis, all of unknown aetiology and with many features in common, the panorama of symptoms and signs, the varied mode of onset and presentation, and the diffuse pathological findings in the vascular and connective tissue systems led Klemperer (1950) to classify them as "collagen diseases". This term has been applied in a way that Klemperer never intended, and he has clearly indicated that evidence that these disorders are primary diseases of collagen is not forthcoming. Nevertheless, the novelty of the term, and possibly a slight scientific glamour attaching to it, has caught the imagination of the medical profession, and there is a tendency to include an increasing number of disorders, especially rheumatoid arthritis, under this generic title. The recent review by Sinclair and Cruickshank (1956) of the pathological findings in fatal cases of rheumatoid arthritis provides sufficient evidence, in so far as rheumatoid arthritis is concerned, to exclude the disease from the collagen disorders. Similar studies of lupus erythematosus and periarteritis nodosa (Klemperer, 1948) have revealed that the pathological changes in these two disorders are not confined to the connective tissue system, let alone to the collagenous component. Klinge (1933) demonstrated that the essential pathological lesion in rheumatoid arthritis and rheumatic fever was fibrinoid degeneration and mucoid swelling. Bien and Ziff (1951) found, by chemical analysis of fibrinoid material, that it lacked proline and hydroxyproline and so could not be considered

TABLE I

THE MOTH-EATEN FIBRES INDICATE A DEGENERATIVE CHANGE IN THE COLLAGEN FIBRES. AFTER 3 HRS' INCUBATION, MOTH-EATEN FIBRES ARE VERY NUMEROUS IN THE AGE RANGE 1-30 YRS.

Age Group	No. of Cases	Incubation Time			
		10 min.	1 hr	3 hrs	24 hrs
Erythroblastosis foetalis	7		BF+	BF+ B++	BF++ B+++
Newborn	5		NOT DONE	BF+ B+++	BF+ B+++
1-6 mths	4		BF+	BF+ B+	BF+ B+++
1-5 yrs	11		BF+ B++	BF+ B++	BF+ B+++
6-10 yrs	9		BF+ B+	BF++ B++	BF++ B+++
11-15 yrs	5		BF+ B+	BF+ B+	B++
16-20 yrs	5		BF+ B+	BF+	BF+ B++
21-30 yrs	5		BF+	BF+ B+	BF+ B++
31-40 yrs	2	NOT DONE		B++	BF+ B+
41-50 yrs	5	NOT DONE			B++

□ "Standard" Change

■ "Moth-Eaten" Fibres

▨ "Granular Degenerating" Fibres

BF Beaded Fibril

B "Beads"
(From Keech, 1955).

to be collagen. Kellgren, Ball, Astbury, Reed, and Beighton (1951) reported that the amorphous fibrinoid tissue did not give an x-ray pattern of collagen. Gueft and Laufer (1954) suggest that the protein of fibrinoid may be nuclear rather than collagenous in origin.

My personal awakening to the potentialities of the connective tissue system arose from a study of senile purpura (Tunbridge, Tattersall, Hall, Astbury, and Reed, 1952). The limited areas of skin involved (the dorsal aspect of the forearm and the face), coupled with the severe degenerative changes in the collagen of the affected sites, led us to study other disorders known to exhibit abnormalities of the skin and to attempt to produce similar degenerative changes in skin collagen both *in vitro* and *in vivo*. We reported upon the staining and morphological abnormalities with both the light and electron microscopes in pseudoxanthoma elasticum, Ehlers-Danlos syndrome, and Werner's syndrome. Table II summarizes our findings at that time. The assessment of the collagen was made on the qualitative appearances under the light and electron microscopes. We made no quantitative studies, nor did we include the reactions to enzymes and physical agents. As a result of the developments in knowledge and newer

TABLE II

COMPARISON OF NORMAL HISTOLOGY AND ELECTRON-MICROSCOPY IN CERTAIN DERMAL ABNORMALITIES.

Condition	Histology		Electron-Microscopy		
	Elastic Staining	Collagen	Elastic Fibres	Collagen Fibres	Debris
Senile Elastosis	Excessive	Normal	Trace	Fragmented	Abundant trypsin-soluble
Ehlers-Danlos Syndrome	Excessive	Scanty	Excessive	Trace	Fairly abundant Small scale
Pseudoxanthoma Elasticum	Increased or faint	Normal or aggregated	Nil	Abundant mainly normal	Normal
Werner's Syndrome	Normal	Abnormal	Nil	Grossly degraded	Abundant trypsin-soluble

From Tunbridge and others (1952). *Clinical Science*, 11, p. 322.

techniques, I feel that some of our findings as recorded in this Table will need to be modified.

Pseudoxanthoma elasticum, first described by Balzer (1884), is a developmental dysplastic disease which is rarely manifest before the second decade. The skin becomes thickened and leather-like and in the involved areas becomes lax and redundant though relatively inelastic, hence the term pseudo-elasticum. Because parts of the skin, particularly the grooves between the folds, become yellow in colour, the earlier clinicians considered the condition to be of a xanthematous origin. Two other features have been described in the disorder: angioid streaking of the fundus and arterial changes due to an increased tendency to calcification and a liability to haemorrhage. The histological changes occur in the deeper layers of the skin and consist of an increase of elastic staining material and the aggregation of some of the collagenous fibres into whorled masses. Under the electron microscope there was no evidence of gross deformation of the collagen fibres, or of the presence of abnormal amounts of amorphous material, nor any increase in the true elastic fibres. The findings vary considerably in different cases, and in some instances there was a suggestion of a slight abnormality in the collagen fibrils. An occasional finding is the presence of fatty material with evidence of calcification, and there are fatty-looking nodules to be seen in the optic fundus. These findings suggested to us that there might be a minor disorder of the lipoids in addition to changes in other connective tissue elements, and they emphasized the scanty attention which has been given to the possible role of lipoids and lipoid-like material in studying the connective tissue system.

The other interesting abnormality of the connective tissue of the skin that we have studied has been the Ehlers-Danlos syndrome or rubber skin (Ehlers, 1901; Danlos, 1908). McKusick (1956) states that the first recorded case of this syndrome was described in 1882 by Job van Meekerven of Amsterdam. The disorder is exceedingly rare. It is essentially a condition of infancy and childhood and is characterized by excessive hyperextensibility of the skin. The latter is soft, almost velvety in appearance, and often pale in colour, in fact it may have an almost pearly-white appearance. The condition may be generalized, but it is more frequently localized particularly to the skin around the elbows and knees and may affect only one knee or one elbow. The laxness of the skin is often associated with some *hypermobility of the joint* and there is a pronounced tendency to haemorrhage. The skin is easily damaged and the combination of haemorrhage and trauma tends to scarring as well as to excessive scar formation often

resembling keloid or leading to tumour formation. Another interesting clinical feature is that the condition often disappears as the child grows or at least becomes much less pronounced.

Examination of the affected areas by normal histological methods reveals the presence of an excess of elastic staining material. Under the electron microscope we have shown this to be due to a true increase in elastic tissue fibres, which may form as much as 75 per cent. of the material examined, in contrast with the very small percentage (less than 5 per cent.) of elastic tissue found in normal skin. The remaining fibrils appear to be those of normal collagen. The connective tissue in adjoining normal skin is normal in appearance under both the light and electron microscopes. Jansen (1955) has suggested that the primary defect is a difference in structure of the collagen, more particularly in the alignment of the collagen fibrils, but he offers no explanation for the quantitative increase in the elastic fibrils. The elastic tissue fibres are readily destroyed by elastase, a further indication of their true nature.

Baló and Banga (1949a, b), after discovering elastase in pancreatic tissue, were able to prove the presence of inhibitors in serum which prevented the action of the enzyme *in vivo*. Saxl and Graham (1956), working in my department, have confirmed the presence of an inhibitor. Moreover, they have found an increased amount of inhibitor in the serum of patients suffering from the Ehlers-Danlos syndrome. This observation might offer a ready explanation if there were always a generalized increase in the formation of elastic tissue, but it is difficult to explain the localized increase of formation other than by assuming an alteration in the tissue of the affected area. The affected area, however, does not seem to be entirely abnormal, because as I have already mentioned, trauma and haemorrhage are often followed by excessive scar formation, in which normal-looking collagen predominates, although clinically the appearance may resemble keloid formation.

The possibility of strictly localized inhibition or the converse of the action of the enzyme naturally brought to mind the patchy distribution of degenerative changes in arteries, particularly the larger arteries where the elastic laminae predominate. Analysis of the aorta has shown that the elastic layer contains a considerable amount of collagen and mucopolysaccharide beside elastic fibres. Mucopolysaccharide is found in greater quantity in the subintimal than in the subadventitial zone. Saxl (1957), working in my department, decided to try the action of Hall's two fractions of elastase (E_1 the

mucolytic enzyme and E_2 the proteolytic enzyme) on consecutive sections of aorta. Fig. 4 shows that the proteolytic enzyme, E_2 , acted maximally upon the middle zone of the elastic coat and to a lesser extent on the other portions, whereas E_1 , the mucolytic enzyme, acted maximally in the subintimal zone. Furthermore, detailed study of the latter areas revealed evidence of the release of fatty material. It would be premature to draw too many conclusions from these results, particularly as to their bearing upon the all important problem of arterial degeneration. They do, however, serve to re-emphasize that alterations in the local conditions of a tissue may predispose to pathological changes, and that the variations in response to a common stimulus may be determined by such local variations.

In this review I have tried to show that the connective tissue system, although so interconnected throughout the human body, and formed of similar elements, cells, collagen, elastic and reticular fibres, and ground substances, exhibits important variations, both qualitatively and quantitatively, in the component parts, yet nevertheless maintains an overall identity. Many of the manifestations of disease may be determined by the condition of the connective tissue elements, even though changes in their structure may not be apparent with the means of analysis available to-day.

In reviewing this subject, I should like to pay tribute to my colleagues, not only for their important contributions, but for the very great stimulus, help and loyal co-operation they have given to me during the past 10 years.

Mr. President, I trust that these remarks may encourage clinicians to continue the accurate observation of bizarre and often anomalous phenomena, because so often such apparent trivialities have provided the stimulus to fundamental scientific observations. Likewise, I hope that the fascinating problems of the connective tissue system may stimulate the younger clinicians to develop and maintain an active interest in at least one small section of medical science and thus enable themselves to utilize more readily the advances of knowledge which must ultimately be so important for the better treatment of their patients.

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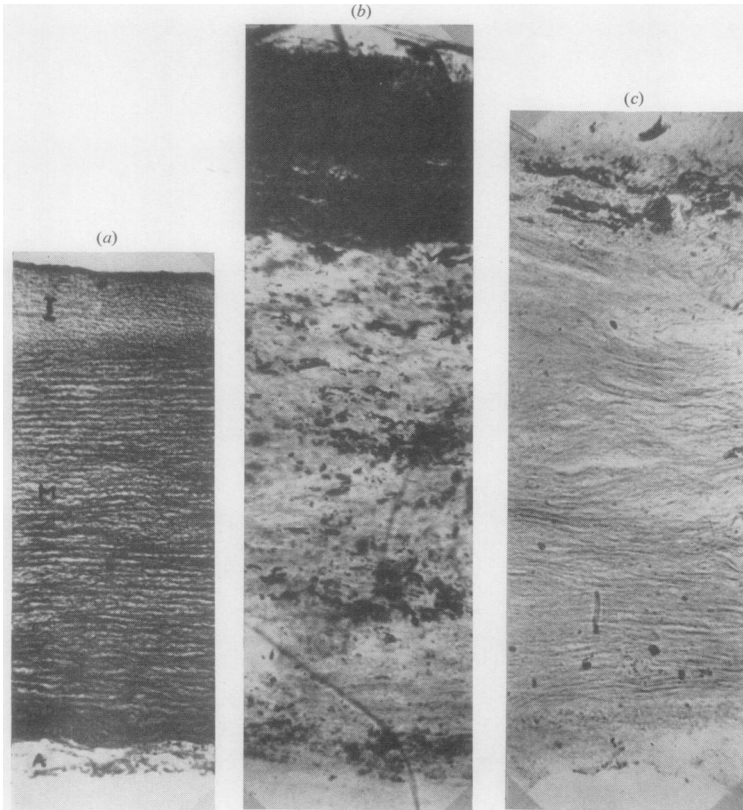


Fig. 4.

(a) Section of untreated aortic media stained by Wiegert's stain.

(b) Similar section of aortic media, stained after treatment by the proteolytic component E_2 of the enzyme elastase. It will be noted that there are widespread degenerative changes in all parts of the media.

(c) Similar section of aortic media stained after action of the mucolytic component E_1 of the enzyme elastase. The major degenerative change in this section is to be seen in the subintimal layer.

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