Structural and metabolic disease

New knowledge of connective tissue ageing

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The biological mechanisms of ageing are poorly understood. One of the few areas in which reasonable progress is being made is in the age-related changes in connective tissues. This is understandable since connective tissues are involved in many of the gross manifestations of ageing such as wrinkling of the skin, physical disabilities of joints, and vascular disease. Even here, however, we are only beginning to learn of the nature of the age changes in precise biochemical and biophysical terms. Only recently has our knowledge of the connective tissue components collagen, proteoglycans, and elastin progressed to the point where we can frame sound questions about their changes with age.

It is important to attempt to define ageing since many studies, supposedly describing age changes, deal with changes that are more reasonably regarded as being due to growth and maturation. There is no consensus on which definition should be adopted but Strehler (1962) has suggested criteria which any change must meet if it is to be regarded as a true ageing event. True ageing changes should be (1) universal—that is, they must occur in all members of the population; (2) intrinsic to the organism—that is, a change must not be a function of diet, disease, environment, or other external influences; (3) progressive; and (4) deleterious to the organism.

While the applicability of such criteria can be questioned they nevertheless provide a framework for assessing whether changes are true age changes. In certain instances, particularly at a molecular level, it is difficult to determine whether changes might be deleterious. This is because their implications in terms of the function of a tissue or an organ are not always clear.

Another problem in studies of ageing is to differentiate between chronological and physiological age. This is particularly difficult in randomly bred (human) populations where individuals vary widely in their physiological capabilities at any particular chronological age. This may be less of a

problem in highly inbred animal strains where changes tend to be displayed more uniformly. The difficulties of deciding true physiological age may be insuperable. Chronological age may then be the only measurement that can be made.

In general, it may be assumed that ageing starts after growth and maturation have ceased—at, say, 20-25 years of age for humans—and that it will go on progressively. Although geriatricians tend to be more concerned with physical and mental deterioration during the last 10 to 20 years of the human lifespan, this period may not be so important from the point of view of true ageing processes. Of more interest may be the changes from maturity until 60 to 65 years of age. During this period changes due to ageing proper occur, making a person more susceptible to disease and to other injurious factors.

Connective tissues such as tendon, skin, bone, and cartilage perform a number of mechanical functions. Other connective tissues may fulfil different roles. The properties of each depend on the nature of its components—whether collagen, proteoglycan, elastin, or glycoprotein (Jackson, 1978; Bailey, 1978; Ireland, 1978; Muir, 1978)—and on the ways in which they interact. It is generally accepted that the tensile strength of connective tissue depends on the collagen component and that resistance to *compression* is conferred by the proteoglycans (see Kempson, 1975). Proteoglycans are also important in determining the transport properties of the tissue towards compounds such as salts and water, hormones, waste products, and gases (Preston and Snowden, 1972; Maroudas, 1973; Glatz and Massaro, 1976).

Changes in the functional properties of connective tissues play a part in certain disease processes. These changes and the underlying biochemical alterations are therefore of considerable interest. For example, mechanical factors play a part in the osteoarthrotic breakdown of human articular cartilage. Since the incidence of osteoarthritis increases with age, knowledge of changes with age

in the mechanical and biochemical properties of cartilage facilitates the definition of changes of importance in the pathogenesis of osteoarthritis. Although alterations in the connective tissues, particularly cross-linking in collagen and elastin, were once thought to be causative factors in ageing this concept is now largely abandoned. But this does not diminish the potential importance of age-related alterations in connective tissues in the pathogenesis of disease.

The connective tissues are a heterogeneous group in terms of their function, appearance, and biochemical constitution. It would be impracticable to discuss here age changes in every individual component of each tissue. We shall therefore review selected reports of biochemical and other changes in connective tissue components that may indicate general trends. As a specific example of how biochemical and mechanical properties are related and of how they may be related to disease we shall discuss the hypothesis that fatigue failure in the collagen network of articular cartilage may be a primary event in some forms of osteoarthritis.

Biochemical changes in constituents of connective tissues with age

COLLAGEN

Older studies dealt with the possibility that cross-linking between individual collagen molecules increases with age (Sinex, 1968; Kohn, 1971; Hall, 1976). However, almost all the early evidence is indirect. Many findings may be interpreted as evidence of change in the type of cross-link, with stabilisation of pre-existing cross-links, rather than as an increase in their number (Bailey and Robins, 1973; Bailey *et al.*, 1974; Bailey and Robins, 1976; see A. J. Bailey (Bailey, 1978) at page 49, and D. S. Jackson (Jackson, 1978) at page 44). That excessive cross-linking could be deleterious is illustrated by the mechanical properties of tanned leather. Tanned leather contains extra, artificial cross-links which make the material more rigid than normal; but tanned leather has a lower tensile strength than untanned leather. The tanned collagen fibres cannot adapt to local stresses because of restrictions imposed by the presence of the extra cross-links (Harkness, 1971).

Although much is known of the chemical nature of the initial stages in the cross-linking of collagen in a wide variety of tissues (Bailey, 1978) the cross-links in mature tissues have not yet been characterised. As tissues mature the reducible cross-links, whose structures are now known, gradually disappear. There is no fall in the extent of cross-linking so therefore the reducible cross-links must be converted to more stable, non-reducible forms. As yet there

is no consensus on the mechanism of stabilisation of the reducible cross-links. Reduction of the aldime type cross-links may occur *in vivo* (Mechanic *et al.*, 1971; Deshmukh and Nimni, 1972) although Bailey and his colleagues have been unable to confirm this (Robins *et al.*, 1973).

The disappearance of reducible cross-links is more or less complete in the mature animal (Robins *et al.*, 1973). However, there are changes in the properties of collagen between maturity and old age that suggest that its cross-linking characteristics may change during the ageing phase of the lifespan as well as during growth and maturation. For example, Steven (1966a, b) demonstrated changes between the ages of 25 and 65 years in the nature of the cross-links in human achilles tendon collagen: the changes were determined by a combination of chemical and enzymic techniques. Further, Hamlin and Kohn (1971; 1972) have shown increasing resistance of human diaphragm tendon collagen to attack by bacterial collagenase between maturity and old age.

It is clearly important to establish the chemical nature of the cross-links formed during maturation of collagen and to determine whether changes take place during ageing, after maturity. It is also important to learn whether the collagens of different tissues are affected similarly during ageing. To understand the relationship between possible age changes in collagen cross-linking and the mechanical properties of collagen fibres is an important goal. Hall (1976) and Piez (1969) have suggested the opposite age-related change of increased cross-linking—that is, cleavage of peptide bonds or cross-links, or both. Although such a mechanism could have important mechanical consequences—for example, in influencing fatigue failure of articular cartilage—there is little supporting biochemical evidence for this view.

The racemisation of amino-acids of collagen from the L form to the D form (Helfman *et al.*, 1977) may occur with age. All amino-acids with the exception of glycine possess an asymmetric and hence an optically active carbon atom. Thus there are both D- and L-diastereoisomers of amino-acids. But only L-amino-acids are incorporated into protein in mammalian systems. Once incorporated into proteins racemisation at the alpha-carbon can occur. The rate of racemisation is temperature dependent and it has been shown that for aspartic acid in human dentine and enamel proteins the rate of racemisation is such as to cause an enrichment in the D-aspartic acid content of about 0.1% per year (Helfman *et al.*, 1977). By the age of 60 to 70 years about 6-7% of the aspartic acid, in a metabolically stable protein, could be in the D form. Racemisation also occurs in other amino-acids but at a lower rate.

The presence of D-amino-acids would tend to disrupt the hydrogen bonding that stabilises the secondary and tertiary structure of polypeptide chains in proteins, leading to alterations in the conformation of molecules. Presumably such alterations in conformation would result in alterations in functional properties of the protein although there is no information on the nature of this change. Although racemisation might occur in any protein significant effects would be observed only in proteins turned over very slowly or not at all. Collagen and elastin could be particularly affected during ageing. Tissues other than enamel and dentine must be examined before the significance of this mechanism can be assessed.

Other changes in collagen with age such as an increase in the amount of hexose bound to the ϵ -NH₂ groups of lysine and hydroxylysine residues (Robbins and Bailey, 1972), the apparent occurrence of intramolecular pseudopeptide bonds between ϵ -NH₂ groups of lysine or hydroxylysine and the side chain COOH groups of aspartic and glutamic acid (Steven *et al.*, 1972), or the existence of other types of cross-link (Hall, 1976) have been reported. Their functional significance is not clear. The binding of hexose to the ϵ -NH₂ groups of lysine and hydroxylysine does not appear to play any part in linking other connective tissue components (glycoproteins or proteoglycans) to collagen (Bailey *et al.*, 1974). Steven *et al.* (1972) suggested that intramolecular pseudopeptide bonds could reduce the hydrophilic nature of the collagen polymerised into fibrils. The functional significance of this reduction is not clear.

High-angle x-ray diffraction experiments have provided evidence that the amianthoid change (fibrillation) during ageing in costal cartilage corresponds to an increased orientation of collagen fibrils in the affected areas (Hukins *et al.*, 1976). The collagen in such areas appears to have a normal periodicity, as judged by low-angle x-ray diffraction patterns and electron microscopy, but the fibrils have unusually large diameters. These large diameter fibrils are probably a result of fusion of smaller fibrils with their axial banding patterns in register. Studies of tissues such as skin and tendon have also demonstrated an increase in the mean diameter of collagen fibrils with age and a greater spread of values about the mean (Hall, 1976).

PROTEOGLYCANS

Considerable scope exists for age-related changes in these complex connective tissue components. Much of the present evidence on age-changes in proteoglycans has been derived from cartilage, particularly articular cartilage, because of the increased incidence

of osteoarthritis with age.

There are reports of alterations in the relative proportions of different glycosaminoglycans as a function of age in costal cartilage (Mathews and Glagov, 1966), knee joint cartilage (Greiling and Baumann, 1973), and human intervertebral disc (Buddecke and Sziegoleit, 1964; Gower and Pedrini, 1969; Szirmai, 1970). Adams and Muir (1976) noted progressive changes in chondroitin sulphate to keratan sulphate ratios in the nucleus and annulus of lumbar intervertebral discs but the changes with respect to site did not follow the same pattern at different ages. In the spines from young persons (aged 8 and 16 years) the keratan sulphate to chondroitin sulphate ratio tended to decrease in the annuli of discs taken from progressively lower regions of the spine and this ratio tended to increase in the nuclei of discs from progressively lower regions. In a spine from a person aged 44, however, the ratio decreased progressively down the spine in both annulus and nucleus. The fact that in general the ratio of keratan sulphate to total chondroitin sulphates increases with age may be explained by the possibility that proteoglycan subunits from older specimens are altered. Their molecular size may be reduced owing to a decrease in the number of chondroitin sulphate chains attached to the protein core whereas the number of keratan sulphate chains is unaffected (Inerot *et al.*, 1978). Buddecke *et al.* (1973) showed that such variations in the relative proportions of different glycosaminoglycans are not features of all connective tissues. They found no change in the relative proportion of different glycosaminoglycans in bovine thoracic aorta up to 13 years of age.

It is therefore certain that changes in the proteoglycans occur with age in certain tissues. The ability of the proteoglycans to form the large aggregates, normally present in young tissues, and their capacity to interact with collagen may also alter with age. Šimůnek and Muir (1972) showed that the amount of proteoglycan extracted from pig articular cartilage by iso-osmotic sodium acetate decreased considerably over the first 3 years of life but then remained more or less unchanged to 5 years of age. The amount of proteoglycan remaining bound to the collagen after two further extractions with 2M CaCl₂ increased up to 3 years of age and then remained constant to 5 years of age. This indicated that the proteoglycans became more tightly bound to collagen up to 3 years of age. Similarly, Quintarelli *et al.* (1975) showed that less rabbit costal cartilage proteoglycan could be extracted using 4M guanidinium chloride at 4 years of age than at 1 month of age. Adams and Muir (1976) have described similar changes in the extractability of proteoglycans

from human lumbar discs between the ages of 8, 16, and 44 years, indicating that the proteoglycans interact more strongly with collagen with advancing age in both the nucleus and the annulus.

These observations are important but difficult to interpret. The oldest of the animals tested by Šimůnek and Muir (1972) and by Quintarelli *et al.* (1975) cannot be regarded as being truly old for the species. The maximum lifespan of the rabbit is about 12 years and of the pig about 20 years (Comfort, 1964). The oldest of the human samples studied by Adams and Muir (1976) was only 44 years, and the 16-year-old sample cannot be regarded as being from a mature adult. Thus the changes described might be attributable to growth and maturation rather than to ageing. Until more detailed experiments are performed it remains difficult to make an accurate assessment. Such problems of interpretation arise in many studies of ageing. It is often impossible to examine enough points in a lifespan and there may be inadequate baseline data for the mature adult.

Proteoglycan subunits interact with each other as well as with collagen, and this second type of interaction is affected by age. Adams and Muir (1976), for example, showed that when proteoglycans extracted from human discs of the spine of a 44-year-old were chromatographed on agarose columns much smaller amounts of proteoglycans were excluded from the gel compared with those from discs from a 16-year-old and an 8-year-old. Also, of the proteoglycans that were retarded by the gel those from older discs were smaller than those from younger discs. Thus, while increasing age is associated with an increased interaction between proteoglycans and collagen it is also associated with a diminished capacity to form aggregates among proteoglycans that do not interact with the collagen.

Perricone *et al.* (1977) examined the aggregation behaviour of proteoglycans extracted from cartilages from aged persons. The cartilage appeared morphologically and histochemically normal. They found that whereas proteoglycans from other sources exist to a considerable extent as large aggregates the proteoglycans from aged hip cartilage (pooled tissue from three individuals, aged 69, 76, and 81 years) were not aggregated to any significant extent as judged by chromatography on agarose columns. Furthermore, the size of the proteoglycans was not affected by digestion with hyaluronic acid β 1-3 hydrolase, indicating that the proteoglycans were not complexed to hyaluronic acid. When isolated proteoglycan subunits from hip cartilage were incubated with hyaluronic acid little change in the elution patterns from the agarose columns was effected, in contrast to the pronounced aggregation

seen when hyaluronic acid was mixed with proteoglycan subunits from normal bovine knee joint cartilage. Thus, the proteoglycans from aged human hip joint and older lumbar discs appear unable to aggregate with hyaluronic acid, probably because of a defect in the hyaluronic acid binding region of the protein core of the proteoglycans (Adams and Muir, 1976; Perricone *et al.*, 1977).

Proteoglycans extracted from human articular cartilage of different ages were studied by Bayliss and Ali (1978). Using density gradient centrifugation techniques, they showed that whereas the bulk of the proteoglycans from young (16-year-old) femoral-head cartilage were of high density and had a low protein to uronic acid ratio those from a 78-year-old specimen were of lower density and had a higher protein to uronic acid ratio. Similar differences were noted between talus cartilage from a 5-year-old and from a 64-year-old. Significant differences were also apparent between the 5-year-old and a 12-year-old but in this case the density gradient pattern for the 12-year-old was intermediate between the two extremes. In contrast to Adams and Muir (1976) and to Perricone *et al.* (1977), Bayliss and Ali (1978) found evidence for aggregation of proteoglycans in old cartilage, although the high density aggregates observed in young cartilage were absent. The differences in density between proteoglycans from young and old specimens appeared to be a function of the differing protein-to-glycosaminoglycan ratios. However, few specimens were examined in this further study; moreover, no fully mature young adult specimens were included, making it difficult to assess whether the changes described are associated with maturation or with ageing proper.

Changes throughout a lifespan in the properties of proteoglycans from canine articular cartilage were studied by Inerot *et al.* (1978). Samples were examined from animals aged 4.5 months, 6 months, 8 months, 12 months, 17 months, 60 months, 96 months, and 126 months. The size of the proteoglycan subunits decreases with age. The size of the chondroitin sulphate chains did not alter appreciably, indicating that the proteoglycan subunits were smaller in older animals because of a reduction in the number of chondroitin sulphate chains attached to the protein core of each proteoglycan subunit. This reduction in the number of chondroitin sulphate chains was reflected in an increased content of keratan sulphate in the extracted proteoglycans. In keeping with other studies, the amount of proteoglycan extracted from the tissue decreased progressively with age, indicating a tighter binding to the collagen. In keeping with the findings of Bayliss and Ali (1978), however, Inerot *et al.* (1978) concluded that there was no

appreciable change with age in the ability of the extracted proteoglycans to bind to hyaluronic acid as measured by chromatography on agarose columns. This suggests that proteoglycans subunits at all ages possess an intact hyaluronic acid binding region. Also in keeping with the study of Bayliss and Ali (1978) the buoyant density of the extracted proteoglycans decreased with age and the protein content increased.

Changes such as those described above in the relative proportions of different glycosaminoglycans and in the aggregation behaviour of proteoglycans would be expected to affect the mechanical properties of tissues. Thus the decrease with age in the number of chondroitin sulphate chains attached to proteoglycan subunits may account for the increase in the compliance of cartilage with age found by Armstrong *et al.* (1977) and Armstrong (1977). The chondroitin sulphate-rich region is probably essential for this property because of its high content of negatively charged groups. Changes in the relative proportions of the individual glycosaminoglycans are also likely to alter the transport of substances through the extracellular matrix.

ELASTIC FIBRES

The elastic fibres of tissues such as the major elastic arteries and lung consist of elastin filaments and a microfibrillar glycoprotein surrounding these filaments (see A. J. Bailey (Bailey, 1978) at page 49). The characterisation of the basic structure of elastin has been greatly hampered by its extreme insolubility even under very harsh conditions, and subsequent studies of changes with age and disease have also been rendered difficult. Elastic fibres are often said to take on a more frayed, split, and fragmented appearance with increasing age and the elastin becomes more brittle. Such changes have led to the concept that wear and tear takes place in elastic fibres and that there must be profound underlying chemical changes. The nature of these changes and their functional implications remain unresolved. The biochemical studies that have been carried out into age changes in elastin have been limited to gross compositional analyses. Another difficulty, which particularly affects studies on human vascular tissue, is that of knowing whether observed changes are actually due to ageing or to changes wrought by the increasing incidence of atheromatous lesions with advancing years (see C. I. Levene (Levene, 1978) at page 165). Much of the literature on age changes in elastin has been reviewed by Hall (1976) and Sandberg (1976). It seems to be generally accepted that the elasticity of elastic tissues decreases with ageing, although changes in the mechanical properties of whole tissues such as elastic arteries

are complex, affecting collagen, elastin, and microfibrillar glycoprotein.

The elastin content of tissues can change, as in hypertension, when there is an increased elastin content (as well as an increased collagen content) in the hypertensive state as compared to normotensive controls (Sandberg, 1976). This increase in elastin content in large vessels is associated with an increase in lamellar units. The elastin content of different regions within the same organ may not show identical changes, however. For example, John and Thomas (1972) observed an increase with age in the elastin content of the visceral pleura of human lung expressed as a percentage of the total dry weight of the tissue but the content of elastin in parenchyma remained constant.

Studies on changes with age in the composition of elastin have yielded variable results (Sandberg, 1976). The discrepancies between results are, in all likelihood, explained, firstly, because different methods for elastin purification allow the removal of other proteins to a greater or lesser extent and, secondly, because the efficacy of the extraction procedures varies according to the age of the subject. John and Thomas (1972) studied the chemical composition of elastins from human pulmonary tissues and aorta throughout the lifespan. They observed that in elastin from visceral pleuras the aspartic acid, glutamic acid, lysine, and arginine contents increased from the end of the second decade onwards while the content of serine and threonine increased after the fifth decade. The glycine, alanine, and valine contents also tended to decrease slightly in the very old specimens. The total carbohydrate of these preparations was shown to increase from the third decade onwards. Similar compositional changes were noted in elastins from lung parenchyma and from aorta.

Keeley and Partridge (1974) and Spina and Garbin (1976) observed compositional changes similar to those described by John and Thomas (1972) for human aorta as a function of age. However, such compositional changes were shown to be due to contaminating collagen and glycoproteins. Treatment of the tissue (Keeley and Partridge, 1974) or treatment of elastins purified by standard procedures (Spina and Garbin, 1976) with EDTA before applying standard purification procedures resulted in elastin preparations which showed no significant compositional changes with age. Spina and Garbin (1976) determined the number of N-terminal amino-acid residues in elastin preparations before and after EDTA treatment. Treatment did not decrease the number of N-terminal residues in young adult elastin (17-27 years old) but almost halved the number of N-terminal

residues in old elastin (54-74 years old). This indicated that EDTA removed polypeptides with exposed N-terminal residues; further characterisation of the extracted material showed that it consisted of elastin degradation products and polar glycoproteins. It was estimated that the contamination of the young (17-27 years old) elastin preparations amounted to about 8% of the total weight of material. At 32-48 years the contamination was 14%, and at 54-74 years 17%. Removing the non-elastin contaminants from elastin apparently becomes more difficult as age increases.

Spina and Garbin (1976) found elastin degradation products (EDP) in their elastin preparations. That might be evidence in support of the concept that cleavage of peptide bonds occurs in connective tissue proteins with increasing age (Piez, 1969; Hall, 1976). However, in view of the drastic treatments (autoclaving in water at 120°C for 24 hours or treatment with 0.1 N NaOH at 100°C for extended periods) this point requires further investigation. Peptide bond cleavage is almost certain to occur under these conditions. Even if such a mechanism of deterioration can be demonstrated the extent of degradation and its functional significance will have to be determined.

John and Thomas (1972) also suggested that the cross-linking of elastin may change as a function of age. Thus the content of the cross-linking amino-acids desmosine plus isodesmosine (Bailey, 1978) in lung visceral pleuras and parenchyma was constant up to about 40 years but declined progressively after that. The content of another cross-linking amino-acid, lysinonorleucine, was constant up to this age but it increased after the third decade and was again constant thereafter. Changes were also observed in the aorta, but in this case desmosine plus isodesmosine did not decrease until after the sixth decade although the lysinonorleucine content increased after the third decade and then remained constant. Although some of the decrease in desmosine plus isodesmosine can be attributed to a dilution caused by the increase in bound glycoprotein, this contamination was insufficient (John and Thomas, 1972) to account for all the decrease. Keeley and Partridge (1974) and Spina and Garbin (1976) concluded that the content of desmosine plus isodesmosine remained constant with age and that any decrease could be entirely accounted for by dilution of the elastin by contaminating glycoproteins.

Thus the amino-acid composition and cross-link patterns of elastin do not change with age, but with increasing age the non-elastin and elastin components of elastic tissues become more difficult to separate. This tighter binding of elastin with other proteins

may be responsible for the stiffening and loss of long-range elasticity of elastin with age. Critical evaluation of such a mechanism is still awaited.

Clearly there is no increased cross-linking of elastin with advancing age by mechanisms involving the known lysine-derived cross-links. It has been recognised for some time, however, that elastin preparations contain certain fluorescent compounds and that fluorescence of elastin preparations increases with age (Hall, 1976; John and Thomas, 1972). The fluorescence of elastin may be due to the presence of cross-linking compounds derived from tyrosine, such as di-tyrosine and quinones, although there have been some suggestions that di-tyrosine may be present in contaminating glycoproteins rather than elastin itself (Hall, 1976). The possibility of such cross-links does not appear to have attracted very much attention, however, and more detailed characterisation of these putative cross-links, such as the isolation and characterisation of di-tyrosine or quinone-containing peptides which can be shown to be derived from elastin, will be required before their role in the ageing of elastin can be assessed.

A further progressive change with age that affects the physical properties of large arteries, particularly the aorta, is an increase in the degree of calcification occurring preferentially in the elastic lamellae (Hall, 1976). The calcium is deposited as calcium phosphate in the form of apatite crystallites. Studies of purified elastins from aorta have shown that there is an increase in the calcium content of elastin with age (see Kohn, 1971; Hall, 1976; Keeley and Partridge, 1974; Spina and Garbin, 1976).

Spina and Garbin (1976) thought that there might be two forms of calcium in elastin preparations. They were unable to remove all the calcium from their elastin preparations even after prolonged extraction with EDTA. The proteins extracted by EDTA contained calcium. The question arises whether the calcium in elastins prepared by the usual methods (which do not include EDTA extraction) is actually bound to the elastin or whether the preparative procedures are simply inadequate to solubilise apatite crystals even if the calcium is not bound to elastin. Keeley and Partridge (1974) argue that elastin preparation methods such as digestion in hot alkali to remove non-elastin contaminants may not in any event be expected to solubilise apatite crystals. Evidently the increase in the calcium content is concomitant with changes in amino-acid composition due to non-elastin proteins. Keeley and Partridge (1974) conclude that the increase in contamination of elastin preparations may occur because of the presence of apatite crystals which bind the contaminating proteins. They do not favour the view that the presence of contami-

nating protein bound to elastin may serve as nucleation centres for calcium phosphate deposition.

Another approach used in studying the relationship between calcium and elastin has been that of determining the ability of elastin preparations to undergo mineralisation *in vitro*. A number of studies have led to the suggestion that the calcium-binding capacity of elastin increases progressively with age. However, such studies are again difficult to interpret owing to the problems of determining whether the elastin preparations are pure or whether they still contain non-elastin proteins. Seligman *et al.* (1975), for example, carried out experiments to determine the kinetics of mineralisation of human aortic elastin of different ages. The rate of mineralisation of elastin increased with age. In elastin from young people there was a lag period, when the rate of mineralisation was low, followed by a period of sharp increase in the rate of mineralisation. This lag period decreased with age and was virtually abolished in elastins from older persons. However, the elastin preparations from the older subjects had amino-acid compositions which suggested contamination by non-elastin proteins. It is therefore difficult to assess the role of elastin itself in the changes observed in the kinetics of mineralisation.

Important questions concerning age changes in elastin remain unresolved. Answers to these problems would be easier to obtain if more satisfactory procedures for the purification of this protein in its native state could be devised.

GLYCOPROTEINS

The connective tissue glycoproteins are poorly characterised, although significant progress has been made in recent years (Anderson, 1976). It is not surprising that there have been few studies of age-related changes in the connective tissue glycoproteins.

The stabilisation of aggregates between proteoglycan subunits and hyaluronic acid in cartilage requires the participation of glycoprotein 'links' (Muir, 1978). Perricone *et al.* (1977) studied the proteoglycans extracted from aged human hip joint cartilage. They concluded that the glycoprotein 'links' generally present in the proteoglycans extracted from normal young tissues are absent from the proteoglycans extracted from aged hip joint cartilage. Presumably the region of the protein core which binds the glycoprotein 'links' was also absent in these proteoglycans as well as that region that binds hyaluronic acid. Another possibility is that the cartilage cells in older subjects do not synthesise the glycoprotein links in the same manner as the cells of young cartilage.

A study of the glycopeptides released by papain

digestion of human lumbar intervertebral discs (Pearson *et al.*, 1972) indicated that the glycoprotein content of both the annulus and the nucleus rose sharply after the fourth decade relative to both collagen and proteoglycans. Galactose, mannose, fucose, and glucose were identified as the major neutral sugars in the disc glycoproteins. The composition of the glycoproteins apparently changed with age in that the relative amounts of these sugars altered. The finding of glucose in the papain-released glycoproteins is unusual. It is not a common constituent of glycoproteins in animal tissues. However, the glycoprotein fraction in discs was not fully characterised and the exact nature and role of this fraction in discs remains to be demonstrated. The glycoprotein fraction will probably include the glycoprotein 'link' components of proteoglycan complexes. The degree of heterogeneity of the proteins in this fraction was not investigated.

The binding between elastin and non-elastin glycoprotein components becomes stronger with ageing. Although a fraction enriched in polar glycoproteins was isolated by EDTA extraction by Spina and Garbin (1976), characterisation of this fraction was incomplete. Probably much of the glycoprotein associated with old elastin is related to the microfibrillar glycoprotein component of elastic fibres, which has been partially characterised (Ross and Bornstein, 1970).

Connective tissues relatively rich in glycoproteins include the basement membranes (see J. T. Ireland (Ireland, 1978) at page 59). Although the glycoproteins of these tissues have been analysed their exact nature and functional role are not fully understood. The glycoprotein components change during growth and maturation but little attention has been paid to change during ageing. Morphological and histochemical evidence suggest chemical alterations in basement membranes during ageing, possibly affecting non-collagen glycoproteins. Some basement membranes increase in thickness with age; the range of values about the mean also increases (Kilo *et al.*, 1972; Darmady *et al.*, 1973; Leuenberger, 1973; Regnault and Kern, 1974). The changes are complex. The thickening may be focal, the membrane may reduplicate, and vacuolation is often present in older animals. The lamina densa increases in thickness with age at the expense of the laminae rarae.

The PAS-positive components of renal basement membranes increase in prominence with age; the Alcian blue-staining components decrease (Ashworth *et al.*, 1960; Rosenquist and Bernick, 1971). The PAS reaction is greatly reduced by prior trypsin but not by collagenase treatment, indicating the non-collagen glycoprotein nature of the PAS-positive material (Rosenquist and Bernick, 1971). Colloidal

iron stains the basement membranes of young but not of older animals. These findings together with the observation that neuraminidase treatment reduces staining in young animals indicate that sialic acid-containing glycoproteins are present in young basement membranes but absent in old. Detailed biochemical investigations of age changes in basement membranes are still required.

Changes in connective tissue cells with age

The biosynthesis of connective tissue components has been investigated in relation to age. The results for human skin collagen suggest little change in the rate of synthesis after the age of 10 to 20 years although collagen synthesis continues during this period. Uitto (1971) measured the rates of collagen synthesis in human skin by examining the incorporation of ^{14}C -proline into polypeptides and the synthesis of ^{14}C -hydroxyproline. He also used prolyl hydroxylase activity as an index of the rate of collagen synthesis and showed that the rates of synthesis are high in fetal and very young tissues but fall rapidly over the first 10 to 20 years of life; they remain constant thereafter. Similar conclusions have been reached from studies of the activities of prolyl hydroxylase, lysyl hydroxylase, collagen glucosyltransferase, and collagen galactosyltransferase in human skin by Tuderman and Kivirikko (1977), Anttinen *et al.* (1973), and Anttinen *et al.* (1977).

Risteli and Kivirikko (1976) also examined the activities of these four enzymes involved in collagen synthesis in rat liver up to the age of 420 days. Although 420 days cannot be regarded as truly old the results were similar to those for human skin. They showed that all enzyme activities were constant between 75 days and 420 days, having fallen from relatively higher levels earlier in the lifespan.

An interesting aspect of the study by Tuderman and Kivirikko (1977) was the determination of the relative amounts of enzyme activity and immunoreactive enzyme protein. Active prolyl hydroxylase consists of four polypeptide subunits and the enzyme exists in connective tissue cells in the active tetrameric form and also as inactive monomers. Tuderman and Kivirikko (1977) showed that 13-19% of the enzyme protein was in an active form in fetal tissues whereas only 2-4% was in the active form in adult tissues. The ratio of active to inactive enzyme did not change between maturity and old age. There was no change with age in the amount of prolyl hydroxylase in human serum. Presumably there were no profound changes in the rates of collagen synthesis in connective tissues generally.

The occurrence of inactive prolyl hydroxylase may be related to the occurrence of inactive enzymes in biochemical systems other than connective tissues, in which it has been observed that the amount of inactive enzyme protein increases with age. This has been interpreted as an indication that abnormal protein molecules accumulate in the tissues of older animals through errors in protein synthesis (see review by Rothstein, 1975). However, Risteli *et al.* (1976) have shown that in hepatic injury induced by dimethylnitrosamine the ratio of active prolyl hydroxylase to inactive enzyme increases significantly in damaged liver without change in the content of total immunoreactive enzyme protein. This presumably means that the cell is able to control the amount of active enzyme present, possibly by subunit association or by the synthesis of specific enzyme inhibitors (Risteli *et al.*, 1976). Thus, inactive enzymes may be present in old tissues because cells can control the amount of active enzyme present according to their physiological requirements. The presence of inactive enzyme protein may have nothing to do with errors in protein synthesis.

The synthesis of glycosaminoglycans is apparently high in very young tissues but decreases as growth and maturation proceed. There is relatively little change with ageing. The rate of incorporation of ^{35}S -sulphate into glycosaminoglycans in human skin and aorta is relatively high in infancy but falls rapidly up to about 20 to 30 years of age. Thereafter, however, there is a relatively slow decline in the rates of ^{35}S -sulphate incorporation up to about 70 years (Junge-Hülsing and Wagner, 1969; Lindner and Johannes, 1973). These workers observed similar changes in rat skin and aorta. The incorporation of ^{35}S -sulphate and ^{14}C -glucose into total and individual bovine aorta glycosaminoglycans has been examined by Buddecke *et al.* (1973). Here again, incorporation was high in the very young animal but decreased over the first 3 years and was then constant up to 13 years. This was observed for the total glycosaminoglycan fraction and for the individual glycosaminoglycans, heparan sulphate, chondroitin sulphate, and dermatan sulphate. The only exception was the incorporation of ^{14}C -glucose into hyaluronic acid, which rose over the first 3 years of life and then remained constant.

Glycosaminoglycan synthesis in rat xiphoid and costal cartilage follows similar patterns (Lindner, 1973). The rates of ^{35}S -sulphate incorporation fall from high levels over the first 6 months of life and remain constant thereafter up to 24 months. Incorporation of ^{35}S -sulphate into rabbit cartilage chondroitin-4- and chondroitin-6-sulphate (femoral head, humeral head, and costal cartilage) was

observed to be high in very young animals. The rate of incorporation decreased rapidly up to 2 months of age and then was fairly constant up to 12 months (Mankin, 1975). A 12-month-old rabbit is far from old, however. The exception to this pattern was costal cartilage, where incorporation was at the same low level for both glycosaminoglycans at each age studied. Mankin (1975) also showed that the ratio of radioactivity in chondroitin-4-sulphate to that in chondroitin-6-sulphate changed with age. Thus the ratio was over 3.0 in the joint cartilage at birth, declining to below 1.0 by 12 months. In costal cartilage this ratio rose between birth and two months of age from just over 1.0 to almost 2.0 but then dropped to about 1.5 at 12 months.

New knowledge of proteoglycan structure, the finding that proteoglycan subunits from cartilages of old animals do not contain as many chondroitin sulphate chains as those from cartilages of young animals, and advancing knowledge of the mechanisms of proteoglycan synthesis now permit more detailed studies of proteoglycan synthesis to be undertaken with respect to age. It would be of interest to know whether the diminution in the number of chondroitin sulphate chains in cartilage proteoglycan subunits is due to degradation of normally synthesised proteoglycans or to the synthesis of altered proteoglycans. This question could possibly be answered by studying the incorporation of radioactively labelled precursors into different glycosaminoglycan types of isolated, newly-synthesised proteoglycans or by measuring the activities of enzymes participating in glycosaminoglycan synthesis. Similarly, the reason for the failure of proteoglycans from some tissues of older animals to form aggregates might be investigated biosynthetically by determining the ability of newly-synthesised proteoglycans to interact with hyaluronic acid.

The finding that the rates of synthesis of collagen and proteoglycans are apparently relatively constant after maturity seems to correlate with studies of the morphological changes in connective tissue cells. For instance, Silberberg and Lesker (1973) observed that chondrocytes in the upper mid-zone of cartilage from the femoral heads of 3-month-old guinea-pigs contained numerous stacked sheets of endoplasmic reticulum densely covered with ribosomes and a well developed Golgi apparatus. Such cells seem to be very active in synthesising and secreting products. In contrast, the endoplasmic reticulum in chondrocytes from year-old animals is decreased in amount although the Golgi is still prominent. There then seems to be little change in these subcellular structures up to almost 6 years of age. Another feature noted in cells from older animals was the presence of

glycogen deposits and lipid inclusions, not so prominent in younger animals, and increases in the number of lysosomes and mitochondria. The application of morphometric techniques, developed to express morphological data of this kind in quantitative terms, would allow a more accurate assessment of age changes in cellular morphology and would seem to be a potentially fruitful area for further studies on ageing in chondrocytes and other connective tissue cells.

Changes in the turnover of connective tissue components as a function of age have attracted less attention. Junge-Hülsing and Wagner (1969) examined the half lives of ^{35}S -sulphate-labelled glycosaminoglycans in a variety of tissues of rats of different ages. They found that in most tissues the half lives increased with age, indicating a slower rate of breakdown in older animals. Such conclusions have also been expressed by Lindner and Johannes (1973) and Lindner (1973), but how they correlate with observed increases in the activities of various glycosaminoglycan-degrading enzymes in mid-adult life and decreases in old age (Lindner and Johannes, 1973; Silberberg and Lesker, 1973) is not clear. Ali and Bayliss (1975) studied activities of cathepsin D and cathepsin B1 in human hip joint cartilage and found that the activity of cathepsin D was constant with age while that of cathepsin B1 fell with increasing age up to about 40 years of age.

Tissue responses to applied stimuli are often delayed in older animals compared with young animals. For example, partial hepatectomy in rodents induces cell division in the remnant so that the tissue regenerates. But there is a lag period before DNA synthesis and cell division begin and this is longer in old animals compared with young (Bucher *et al.*, 1964). Induction of DNA synthesis in salivary gland cells by injection of isoproterenol (Adelman *et al.*, 1972b) and the induction of certain enzymes in the liver by a variety of means (Adelman *et al.*, 1972a) are also delayed in older animals. Attempts have also been made to see whether the reactivity of connective tissue cells is similarly affected by ageing, usually by studying the synthesis of connective tissue components after applying stimuli such as the wounding of skin or the implantation of various granuloma-inducing agents subcutaneously.

The effect of age on the kinetics of the formation of granulation tissue in response to the subcutaneous implantation of viscose cellulose sponge in rats was investigated by Heikkinen *et al.* (1971). They interpreted their results as showing a 'phase difference' between young and old in the parameters used to measure the development and metabolic activity of the granulomas. However, if one examines

the data it is difficult to detect any 'phase difference'. The patterns of development seem to be very much the same for most parameters in both young and old animals. Thus the nitrogen, hydroxyproline, DNA, and RNA contents were very similar at each time point after implantation of the sponges in both young and old animals, the values for each parameter peaking and declining at the same times at both ages. The only real difference between young and old animals is in the rates of incorporation of ^{14}C -proline into protein by the granuloma tissue and in the synthesis of hydroxyproline. Thus granulomas from young animals are much more active than those from old animals at the earliest time points studied (7 days) but those from older animals are more active at later time points. Since the curves for fibrous collagen content, as measured by hydroxyproline levels, are virtually identical in young and old animals at different time points, the results suggest differences between granulomas in young and old animals either in the conversion of newly synthesised collagen into fibrous collagen or in the rates of degradation of collagen. At the earliest time point the conversion of newly synthesised collagen into fibrous collagen appears to be relatively inefficient in the young animal compared to the old animal. Alternatively, the degradation of collagen may be higher in the young animal. The situation is reversed at later times after implantation.

Holm-Pedersen *et al.* (1974) have carried out a similar study on healing, full-thickness incisional wounds in mouse skin. There is no real evidence for any 'phase difference' between old (18 to 24-month) and young (6-week) animals in terms of DNA, RNA, and protein synthesis. There are differences between young and old, however. Cell division, measured by incorporating ^3H -thymidine into DNA, peaks four days after wounding in young animals and falls to normal after 21 days. By contrast, DNA synthesis induced by wounding in old mice increases continuously at least up to 21 days. Similar patterns are observed for RNA and protein synthesis. Counts have also been made of the number of fibroblasts per unit area in the wounds. The density of fibroblasts rises to a peak four days after wounding in both young and old animals, to about the same values. The density then falls continuously in wounds in young animals at least up to 21 days after wounding, approaching the values in unwounded skin at this time. In old mice, however, the density of fibroblasts in the wound remains more or less constant between four and 21 days after wounding. How this finding correlates with the apparent continual increase in DNA synthesis is not clear.

Studies of glycosaminoglycan synthesis in such experimental systems have been made by Junge-

Hülsing and Wagner (1969), who examined the time course of glycosaminoglycan synthesis in subcutaneous cotton pellet granulomas. The rate of glycosaminoglycan synthesis, measured by incorporating ^{35}S -sulphate, peaked at three days after cotton pellet implantation into young adult rats and then fell quickly. In old rats the rate of synthesis did not peak until seven days after implantation and remained at the same level until 10 days, after which it decreased. In these experiments, therefore, there was a definite 'phase difference' between old and young animals.

Experiments such as these are designed partly to investigate whether wound healing is impaired in older individuals. Sussman (1973) has examined the tensile strength and extensibility of wound tissue at different periods after wounding in the skin of adult (8-month-old) and old (20-month-old) rats. He observed that the tensile strength of healing wounds was identical at various time points after wounding in both young and old animals. The thickness of the wounds in old animals was less than that in young animals, however, and hence the total breaking strength of the wounds in old animals was less than that in young animals at 8 weeks and 13 weeks after wounding. This indicates that the younger animal produces a greater amount of fibrous tissue. Considerable differences were also noted between young and old animals in that the extensibility of the wounds in young animals was significantly greater at 8 weeks and at 13 weeks than in older animals, presumably because the scar tissues being laid down in animals at different ages are qualitatively different. These studies, therefore, show that there are differences between young and old animals in the amount of fibrous tissue produced in response to wounding and in the way in which collagen is laid down in the scar tissue. Presumably these differences reflect differences in the activities of fibroblasts.

Ageing of adult articular cartilage: osteoarthritis

As a specific example of how the functional properties of a connective tissue change with age, of how the changes in functional properties might be explained at a molecular level, and of how such events might be related to disease processes we discuss in this section the hypothesis that fatigue failure in the collagen network of articular cartilage may be the initiating event in the development of some forms of osteoarthritis. It is now generally accepted that mechanical factors play a role in the osteoarthrotic breakdown of human articular cartilage. Since the incidence of osteoarthritis increases with age, changes in the mechanical

properties of articular cartilage with age and the underlying biochemical events are of very considerable interest.

CHANGES IN MECHANICAL PROPERTIES WITH AGE

Tensile properties

Kempson (1978) measured the tensile stiffness and tensile strength of the collagen network by subjecting isolated specimens of cartilage to a gradually increasing stress until fracture occurred. His results for specimens from human femoral condyles (Figs. 1, 2) showed that both of these properties decreased with age after maturity.

By subjecting isolated specimens of cartilage to

Fig. 1 Relationship between tensile fracture stress and age. Specimens from superficial layer of lateral femoral condylar cartilage, aligned parallel to surface cleavage pattern. (Adapted by kind permission from Kempson (1978).)

Fig. 2 Relationship between tensile stiffness and age. Specimens from superficial layer of lateral femoral condylar cartilage, aligned parallel to surface cleavage pattern. (Adapted by kind permission from Kempson (1978).)

repeated applications of tensile stress until fracture occurs it is possible to measure the tensile fatigue strength of the collagen network. Fig. 3 shows typical fatigue curves (that is, stress versus number of cycles to fracture) for specimens from the superficial layer of human femoral heads (Weightman *et al.*, 1978). Each datum point represents one specimen; the number and letter show the age and sex of the cadaver from which the cartilage was obtained. The solid lines are the best-fit straight lines through the individual sets of data points. The interrupted lines all have the same slope (equal to the mean slope of the 20 individual fatigue curves in the complete study) and illustrate that, within the limits of accuracy of the experiment, the individual fatigue curves are parallel.

When the fatigue strength of the cartilage from each femoral head was quantified by the intercept of the interrupted lines with the stress axis (to give projected fracture stress or fatigue strength) the fatigue strength was found to decrease significantly with age (Fig. 4). The decrease in fatigue strength with age is, in fact, more dramatic than indicated by Fig. 4. If the fatigue strengths at 30 and 60 years of age are compared on the basis of projected fracture stress (that is, from Fig. 4) the fatigue strength appears to decrease by just over 20%. Physiologically, however, the decrease in fatigue strength should be assessed by the change in the number of cycles required to produce fracture at the same level of stress. Making this comparison the fatigue strength at 60 is a factor of more than 500 less than it is at 30.

Compressive properties

Armstrong (1977) has recently developed a radiographic technique for measuring the deformation of articular cartilage in loaded human hip joints (Armstrong *et al.*, 1978). Twenty-eight hip joints in the age range 26 to 83 years were injected with a radio-opaque contrast medium to indicate the cartilage-cartilage interface and loaded to five times body weight for 35 seconds. During this time radiographs were taken. Measurements of the thickness of the femoral head cartilage under load were made from the x-ray films and compared with unloaded thickness; the measurements gave cartilage deformation profiles. The average compliance of the cartilage over the whole contact area was determined by dividing the integrated deformation over the contact area by the total force applied to the joint.

This study showed that the compliance of femoral head cartilage increases with age (Armstrong *et al.*, 1977). Whereas in young specimens the cartilage was almost incompressible, reductions in thickness of 15% were common in old specimens.

Fig. 3 Examples of individual fatigue curves (Weightman *et al.*, 1978). Specimens from the superficial layer of femoral head cartilage, aligned parallel to surface cleavage pattern. S = stress. N = No. of cycles to fracture.

Fig. 4 Relationship between fatigue strength (as measured by projected fracture stress) and age (Weightman *et al.*, 1978).

FATIGUE HYPOTHESIS

In unloaded articular cartilage the swelling tendency of the proteoglycan gel is limited by, and hence induces tensile stresses in, the collagen fibre network. When a compressive load is transmitted across a synovial joint the hydrated proteoglycan gel is pressurised and the collagen network experiences increased tensile stresses. Expressed in other ways, (1) cartilage is a hydrostatic system in which the fluid element is provided by the hydration water of the proteoglycan gel and the container is provided by

the collagen fibres (Wainwright *et al.*, 1976); (2) the function of the collagen is the fixation of the elastic domains of proteoglycans when these experience deforming and displacing stresses (Serafini-Fracasini and Smith, 1974).

During activity the position of the load-bearing region within a joint and the magnitude of the transmitted load vary. Clearly, therefore, the collagen fibre network in articular cartilage experiences fluctuating tensile stresses and the proteoglycan gel experiences fluctuating fluid pressure.

In general, the strength of a material is defined as the minimum stress at which fracture occurs. The strength of a material may vary depending on whether it is subjected to tensile, compressive, or shear stresses or a combination of these. It also depends on whether the load is applied once or repeatedly. In the case of cyclically applied loads fracture can occur at a stress level which is lower than the static stress required to cause fracture. This kind of behaviour is known as fatigue, and a fatigue-prone material may be considered as being 'weaker' when subjected to a cyclically applied load than when subjected to a load that is applied only once.

In theory, mechanical failure in cartilage could occur in either the hydrated proteoglycan gel or in the collagen network. However, mechanical failure in the proteoglycan gel seems unlikely since, if the mechanism of load-carriage described above is

correct, this component acts as a fluid. While the gel might fail due to shear, the relatively small deformations produced in normal cartilage *in vivo* seem to make this unlikely. Structural failure affecting primarily the collagen network would seem, on the other hand, to be perfectly possible since the collagen fibres, or the bonds between them, might be overstressed in tension. The collagen network in particular might fracture in the face of a cyclically applied load if the fluctuating tensile stresses produced were sufficient to cause fatigue failure.

Collagen would, of course, protect itself from fatigue if its metabolic turnover rate were such that it was replaced before it could experience the number of stress cycles required to produce fracture. Present knowledge, however, indicates that the metabolic turnover rate of collagen in adult human articular cartilage is very low (Muir, 1978). It follows that the material will experience a large number of stress cycles before being replaced.

Discussion: fatigue failure of collagen

The evidence in support of the hypothesis that fatigue failure in the collagen fibre network is the initiating event in some forms of osteoarthritis has been reviewed at length elsewhere (Weightman and Chappell, 1978; Freeman and Meachim, 1978). In summary, it includes (1) the increased incidence of osteoarthritis after meniscectomy (Johnson *et al.*, 1974), a procedure which increases the contact pressure on the cartilage by a factor of three (Seedhom, 1975); (2) the breakdown of cartilage subjected to repetitive loading in the laboratory (Radin and Paul, 1971; Radin *et al.*, 1973; Weightman *et al.*, 1973); (3) the increased thickness (Meachim, 1971) and increased water content (Venn and Maroudas, 1977) of cartilage in very early osteoarthritis, both of which can be explained by changes in the collagen network which make it more distensible; (4) the abnormally wide separation of the collagen fibres in the cartilage underlying very minor surface disruptions in human knee cartilage (Meachim and Roy, 1969), which again implies increased distensibility of, and, hence mechanical changes in, the fibre network; and (5) the (variably) diminished tensile strength and tensile stiffness of histologically intact cartilage adjacent to areas of fibrillation (Kempson, 1978), which suggest that abnormality of the collagen network is a precursor to spread of fibrillation.

The hypothesis is now further supported by the deterioration in mechanical properties of cartilage with age. That is, since the changes in mechanical properties with age can all be explained by a gradual deterioration of the collagen network (which makes

it weaker and more extensible) and the incidence of osteoarthritis increases with age, a causal connection is suggested (but not proved) between the two.

The question now arises. What causes the deterioration of the collagen network with age? Collagen turnover occurs very slowly, if at all, in adult articular cartilage. Furthermore, collagenase is thought to be absent from normal cartilage, although cathepsin B, which is present, does degrade collagen slightly. For these reasons a metabolically induced abnormality in the collagen fibres themselves is unlikely to be responsible. On the other hand, the very inertness of collagen suggests the possibility of its being degraded mechanically, since over the course of a lifetime each fibre will experience a very large number of load cycles and hence may fatigue. Although progressive fatigue failure of the collagen fibres themselves may play a part in the age-related weakening of the fibre network it seems unlikely to be the main factor responsible, since the shortening of the fatigue life in aged compared with young cartilage is too great to be accounted for by the number of load cycles borne by the fibres in the intervening years (Weightman, 1976). One possible explanation concerns the 'linkage' between fibres rather than the fibres themselves.

Virtually nothing is known of the way in which individual fibres are linked together to produce a network. Possibly the fibres are simply interlocked physically, or possibly some other constituent of the matrix functions as 'glue' (see A. J. Bailey (1978) at page 49). If the latter proves to be the case changes in the bonding constituent could be responsible for the changes in mechanical properties and might have a metabolic basis. Muir (1978) suggests (1) that the proteoglycans which cannot be extracted with high-strength salt solutions might be attached at 'high affinity' sites on the collagen fibres and act as bonding agents between fibres, spanning distances that would be too great for cross-links to develop; and (2) that the gradual reduction in the number of high-affinity sites with age (and hence the number of proteoglycan bonds) might explain the changes in mechanical properties.

Unfortunately, the concept of a gradually decreasing number of bonds between collagen fibres seems to be at variance with another ageing change recently observed in human articular cartilage—namely, a gradual decrease in water content (Venn, 1978, and Fig. 5). Thus if the number of bonds between fibres decreases one would expect the water content to increase not decrease.

Similarly, if the number of bonds decreases one would expect an increase in thickness. Meachim (1971) found age to have no effect on the thickness of

Fig. 5 Relationship between water content and age. Full depth specimens from superior surface of human femoral heads.

the uncalcified matrix of the cartilage on the head of the humerus, and Venn (1978) found no change in the thickness of cartilage from the superior surface of the femoral head with age after maturity. But Armstrong (1977) and Armstrong and Gardner (1977) have reported a significant increase in thickness of femoral head cartilage in the anterosuperior zone between the ages of 18 and 45 years.

On balance, we think that the data on water content and thickness point, if anything, to an increasing number of bonds between collagen fibres. Perhaps the number of proteoglycan bonds decreases while the number of some other type of 'linkage' increases.

The question now arises: Can the changes in mechanical properties with ageing be explained on the basis of an increasing number of bonds between collagen fibres? The relation between the tensile strength and the degree of cross-linking in natural and non-crystallising rubber has been reviewed by Bueche and Berry (1959). In these materials the tensile strength first increases and then decreases as the degree of cross-linking increases. One explanation for this is that at the breaking point only those chains orientated to within a small angle of the direction of the applied load are supporting the load and that, as the cross-linking increases beyond a certain degree, the number of chains that can be so orientated decreases. It seems at least possible that a similar effect could occur in articular cartilage if the collagen fibres in cartilage behave in the same way as the molecular chains in natural rubber.

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