Amyloid deposits in articular cartilage

E. G. L. BYWATERS and J. DORLING

From the Department of Medicine, Royal Postgraduate Medical School, London, and the M.R.C. Rheumatism Unit, Taplow, Bucks.

For many years it has been known that so-called 'primary' amyloidosis and the amyloidosis associated with multiple myeloma may present with similar joint symptoms (Parkes Weber, Cade, Stott, and Pulvertaft, 1937; Tarr and Ferris, 1939; Koletsky and Stecher, 1939), that is, stiffness, swelling, and limitation due to the deposition of amyloid in the synovial membranes of the joint or of the tendon sheaths (Fig. 1). Indeed, carpal tunnel syndrome is a well-recognized complication of such diseases (Hamilton and Bywaters, 1961). Whether this is due to passive accumulation via the bloodstream or to active cellular deposition locally has long been debated. We record briefly here two cases of primary amyloidosis in which deposition of amyloid occurred in the surface layers of articular cartilage, as originally recorded briefly by Gamarski and Baretto Netto (1959). The deposition of these fibrillar deposits may throw light on the mechanism of amyloid formation, whether it may be secreted in the cartilage by chondrocytes, may diffuse in as a soluble precursor, or may be pressed in as preformed fibrils along hidden channels.

Case reports

Case 1, a man aged 40 years at his death in 1960

In May, 1959, he experienced abdominal pain and diarrhoea. The next month he developed septic bursitis after an injury, leading to oliguria. The blood urea was 210 mg. per cent., necessitating four haemodialyses (Halton). Erythrocyte sedimentation rate 130 mm./hr (Westergren).

Biopsy of right kidney and nephrectomy due to haemorrhage.

Histology report

'Acute interstitial nephritis and tubular necrosis' with venous thrombi.

This seems to us a possible example of sulphonamide blockage, similar to the picture of acute hydronephrosis (Bywaters, 1945). Recovery followed diuresis on 53rd day.

Progress

In April, 1960, 'rheumatism' occurred in the shoulders, back, and arms.

In July, 1960 (Dr. Jennings, Royal Berkshire Hospital), he had sore mouth and throat for 2 weeks leaving painless enlargement of the tongue, dysphagia, hoarseness, difficulty in elevating the arms, bilateral carpal tunnel syndrome, and swollen left knee.

On September 24, 1960, he was admitted to Hammer- smith Hospital with woody swelling of the tongue, infiltrated nodules of the mucous membranes, swollen knee, tachycardia, exertional dyspnoea, increased jugular venous pressure, enlarged liver, and firm limited wrists. There were also pseudohypertrophy and weakness of the shoulder girdle muscles and subcutaneous nodules on the trunk.

Blood urea 25 mg. per cent. Serum electrophoresis normal. Sp. gr. 1015. Proteinuria 10-2 g./24 hrs, Bence Jones protein positive, single globulin peak.

Fig. 1 Knee joint (Case 1), showing pale, rather opaque cartilage and pale, stiff synovial fringes.

Drill biopsy of the tongue showed amyloid. Congo red 48 per cent. clearance from plasma in 30 min. Erythrocyte sedimentation rate 20 mm./1st hr. Plasmacytosis of bone marrow.

**Termination**

Despite prednisone 100 mg./day (Taplow), he deteriorated and died on December 24, 1960.

**Post mortem examination** (at Reading)

This confirmed primary amyloidosis involving the heart, skin, muscles, tendon sheaths, and spleen, and also affecting the synovial membrane of the joints and spreading over the cartilage. Amyloid was not found in the liver or kidney. Myeloma was not confirmed.

**Case 2, a man aged 43 years at his death in 1967**

In 1966 he had weakness of the shoulder girdles, aching, and stiffness, with weight loss of 10 kg. This was followed by median nerve paraesthesiae, and stiffness and pain in the wrists and elbows.

In February, 1967, a lump was noticed in the neck; this was biopsied elsewhere and 'fibrosis' was reported.

The erythrocyte sedimentation rate was 2 mm./1st hr. There was proteinuria, but no Bence Jones protein. Serum electrophoresis was normal. Serum calcium 5·9 later 7·4 mEq./l. Alkaline phosphatase 20 K.A. units.

Blood urea rising from 91 mg. per cent.

In March, 1967, he underwent removal of 'parathyroid adenoma'.

**Progress**

In April, 1967, he entered Hammersmith Hospital under the care of Dr. Graham Joplin, with husky voice and dysphagia; he was bedridden with pain and weakness. There was corneal calcification, firm nodules on the trunk and neck, and limitation and contractures of fingers, wrists, elbows, shoulders, hips, and knees.

Serum acid phosphatase 6·8 K.A. units per cent. Urea 64 mg. per cent. Uric acid 8·5 mg. per cent. Serum immunoglobulins low. Bence Jones protein (type K) in urine and plasma.

There were radiological lytic lesions in the pelvis, femora, spine, and ribs, and myeloma cells on bone marrow exploration.

**Termination**

He was treated with melphalan, but died in uraemia after 3 weeks.

**Post mortem examination** (Dr. Evans)

This confirmed amyloid deposition in the joints, muscles, and bones near the joints, but not in the liver, spleen or glomeruli. Myeloma deposits were widespread in the marrow.

**Results**

The knee joint of Case 1 is shown in Fig. 1. The cartilage was in places abnormally white and opaque and the synovial membrane appeared similarly pale and stiff, almost as if starched. Staining with iodine showed a strong uptake (Fig. 2) in the synovial membrane and in the superficial layers of the articular cartilage and the knee meniscus (as also but to a lesser extent in Case 2).
Electron microscopical study of formalin-fixed synovium showed that these deposits were primarily pericellular (Figs 4a and b), i.e. that they could be due to secretion of amyloid by the cells or to deposition from the synovial fluid, or from the capillary ultrafiltrate which forms synovial fluid, perhaps by coprecipitation with hyaluronate-protein.

In crystal violet, Congo red, and thioflavine-stained sections of both decalcified and undecalcified formalin-fixed blocks of cartilage from Case 1, and
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FIG. 4(b) High-power view of part of Fig. 4(a), showing details of synovial cell process containing ribosomal material, mitochondria, and pinocytosis. × 65,000.

Indeed also in haematoxylin and eosin stained sections, surface deposits and more deeply globular deposits of amyloid were seen (Fig. 5a, overleaf). These deposits also showed Congo red, apple green birefringence in the polarizing microscope (Fig. 5b, overleaf) and were present in articular cartilage from the humerus, femur, tibia, and patella. Alcian blue staining was lost between 0·050 and 0·1 M magnesium dichloride (Scott and Dorling, 1965). These deposits appeared along the surface and more deeply, both
FIG. 5(a) Cartilage from patella (Case 1), showing surface staining and globular deposits except in the neighbourhood of chondrocytes. Note knife mark (arrow). Congo red. × 112.

FIG. 5(b) Same section as 5(a) under polarized light, showing apple-green birefringence: the dark lines are thought to be artefacts due to wrinkles in this section. × 112.
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parallel to the surface in some superficial areas and more deeply as strings of beads along parallel straight lines extending deeply from the surface sometimes in differing sets of directions, suggesting the existence of channels (Fig. 6) perhaps the *säf-bahnen* discussed by Schaffer (1930) and thought by Linn and Sokoloff (1965) to be artefactual. There was no correspondence with knife grooves (Fig. 5a). Chondrocytes stood out clearly because they were surrounded by an amyloid-free zone. No amyloid staining was seen within chondrocytes. In several areas, fibrillation of cartilage had occurred as a precursor of osteoarthritic changes (Fig. 7). Here, amyloid deposition both superficially and more deeply in globular form followed the lines of the cartilage-fluid interface (Fig. 8), suggesting permeation from synovial fluid. In Case 2, amyloid deposition, equally great in the synovial lining cells, was considerably less in the superficial layers of cartilage and failed to penetrate deeply (Fig. 9). A method used to show fibrin (phosphotungstic acid haematoxylin, Mallory) in Case 1 showed staining at the base of the superficial layer and, in one particular area, in the centre of the unstained globular deposits (Fig. 10). The findings in Case 1 have been confirmed by electron microscopy (Figs 10, 11, and 12) which shows amyloid fibrils (but without visible channels) in small aggregations without definite polarity: the fibres measure about 75 to 100Å wide and have repeating units along their length—2 to 3 per 100Å of length conforming to the pattern described by Shirahama and Cohen (1967). No such deposition has been seen in secondary amyloidosis. In Case 2 a few marginal

**Fig. 6** Surface deposition and deeper trails of deposits, sparing chondrocyte territory in patella (Case 1). Crystal violet. × 300.

**Fig. 7** Fibrillation clefts in cartilage (Case 1) showing staining along surface of clefts as well as previous types of staining. Crystal violet. × 200.
FIG. 8 Fibrillation clefts, showing depositions on surface (Case 1). Note faint lines as a continuation of the amyloid strings of beads. These are thought to be real, not artefactual and perhaps to correspond to major collagen bundles. × 200.

FIG. 9 Metacarpal head (Case 2) showing surface deposit of amyloid. Congo red. × 650.

FIG. 10 Patella cartilage (Case 1), showing fibrin deposits below surface deposit of amyloid and centrally within the amyloid globules. Phosphotungsten and haematoxylin. × 300.
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FIG. 11 Patella cartilage (Case 1). × 4,900.
FIG. 12 Patella cartilage (Case 1). × 32,500.
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FIG. 13 Intervertebral plate and body (Case 2), showing amyloid infiltration of frayed end of ruptured fibrocartilage fragment: other amyloid is seen near traumatized sites in fibrotic tissue and blood vessels. New reparative fibrocartilage formed in situ is not stained (left). Disc cartilage at top. Congo red. × 30.

areas of ruptured (?) dead) fibrocartilage in the vertebrae contained amyloid, but the intervertebral disc cartilage contained none.

Discussion
These findings encourage conjecture regarding both amyloid deposition and cartilage structure.

It seems possible that synovial fluid might contain a soluble precursor of amyloid, although it is unlikely to be the water-extractable component of equivalent weight 7000 described by Pras, Schubert, Zucker-Franklin, Rimon, and Franklin (1968), since it is precipitated by salt concentrations beyond 0.010 mole./l. Linn and Sokoloff (1965) have shown that fluid expressed mechanically from cartilage (bovine nasal septum) had the general composition of extracellular fluid with 91 m.Eq./l. chloride (it also contained 213 mg./100 ml. protein, although whether this is plasma protein is not known). Diffusion from synovial fluid into cartilage depends on molecular size and upon charge in relation to the ground substance, which may change with depth. Efforts should be made to identify any such material in these synovial fluids.

Alternatively, amyloid fibrils might be thrown off into the synovial fluid from the synovial membrane layer and penetrate as insoluble fibres along non-visible pathways or sap alleys (saft-bahnen) in cartilage. This seems possible as there was no regular 'streaming' orientation of fibrils and the pores postulated in cow articular cartilage by McCutchen (1962) to account for weeping lubrication were thought to be somewhat less in diameter (62Å) than the width of these fibres. Furthermore, in the electron microscope sections no channels can be seen. This may be because they are stuffed with amyloid. In some sections (Fig. 8) lines or channels may be seen continuous with the amyloid chain of beads but containing no amyloid. However, sections stained for fibrin—another fibrillar material—shows in at least one area deposits at the core of the amyloid globules. This favours the non-specific pressure-infiltration process.

There remains the possibility that amyloid is secreted into cartilage matrix by chondrocytes. This is also unlikely because the amyloid deposits are not seen either in the light microscope or on electron microscope study in the immediate neigh-
bourhood of chondrocytes, unlike the fine fibrils (both intracellular and pericellular, and ultimately replacing the cellular locus) observed by Meachim and Roy (1967). These latter filaments lack any periodic banding and occur equally in superficial and deep chondrocytes, and in osteoarthritic and more normal cartilage.

It seems possible that the greater development of amyloid fibril deposits in Case 1 than in Case 2 depends upon the exposure (by loss and attrition of the superficial layer of cartilage) of the more vertically orientated collagen bundles of the mid-zone and by the permeation of these by amyloidic materials from the synovial fluid.

Superficial deposition of lipid may also occur in a similar distribution to that of urates in gout, although Ghadially, Meachim, and Collins (1965) preferred to think that it was secreted by the superficial row of chondrocytes. The globules occur as a superficial layer or in the immediate neighbourhood of chondrocytes, a distribution different thereto from that described here.

Summary

Articular cartilage in two cases of primary amyloidosis with Bence-Jones proteinuria showed infiltration of amyloid, demonstrated by methyl violet, thioflavine T, and Congo red staining, using both birefringence and ultra-violet fluorescence. Amyloid fibrils about 75–100Å long were demonstrable ultramicroscopically. These deposits, most dense superficially, were seen in one case to occur from the surface downwards as globular beads along straight tracks and also deeply along the surface of fibrillation clefts. This suggested diffusion from the synovial fluid of a soluble amyloid precursor or permeation of fine fibrils along pre-formed but hitherto unknown pathways. The deposits spared the immediate surroundings of chondrocytes. In the synovial membrane, deposits of amyloid were pericellular.

We thank Dr. Joplin and Dr. Evans of the Royal Postgraduate Medical School of London for permission to use clinical and pathological data on Case 2.

Discussion

Dr. W. H. de Haas (Amsterdam) Are there any clinical symptoms with this disease?

Prof. Bywaters The clinical symptoms relating to the joints sometimes, as I have said, bring these patients to the rheumatology clinic with a diagnosis of rheumatoid arthritis, with stiffness, swelling of the limb joints, and sometimes with pseudohypertrophy of muscle or with weakness imitating myopathy, sometimes with the carpal tunnel syndrome, sometimes with cramps or intermittent claudication or angina, and sometimes with skin thicken-

ing mimicking scleroderma. I think we are beginning to pick out more of these cases with greater knowledge of the condition, and also with greater use of immunoglobulin studies of the serum and, particularly, of the urine. These patients both had Bence Jones proteinuria, although neither of them had serum monoclonal abnormalities.

Dr. J. Ball (Manchester) This is an important study because cartilage presents an opportunity of deciding whether amyloid can be produced by connective tissue cells in an avascular tissue. In this sense the absence of amyloid around the cartilage cells is somewhat disappointing. Amyloid has a characteristic biochemistry and I should like to ask if biochemical studies were done. Normal cartilage has a low permeability, excluding even small molecules of like charge. Did you examine non-articular areas of the joint cartilage and non-articular cartilage for amyloid?

Prof. Bywaters In answer to the first question, no biochemical studies of synovial membrane have been done in these cases but I think that anyone would be satisfied with the staining and electron microscope procedures I have shown. Secondly, non-articular cartilage was not stained. We looked carefully at the intervertebral discs and there was no staining, except in small portions of cartilage which may have been regenerated cartilage herniated into the bodies of the vertebrae. There was a little marginal staining by Congo red with green birefringence in these areas.

Dr. V. Wright (Leeds) I was especially intrigued by the question of the permeability of articular cartilage. None of us will disagree with the fact that articular cartilage is permeable, but your demonstration of these juice-ways is particularly significant. In normal conditions we know that only substances of very low molecular weight enter the articular cartilage; Sokoloff’s work shows this, and it is very important in connection with the mechanisms of lubrication. Do you think these juice-ways occur only in degenerative joint disease, or do you think they are present in normal articular cartilage as well?

Prof. Bywaters In areas of articular cartilage without fissuring, amyloid appeared as a flat layer in the very superficial areas. The beaded linear deposits were seen more in the general neighbourhood of fissured cartilage, and this does indeed lead one to think that the beaded lines might correspond to the general direction of the collagen fibres in the intermediate zone of normal cartilage (which run vertically or diagonally to the surface), perhaps being exposed by wear-and-tear loss of the normal superficial zone of cartilage (characterized by transverse collagen fibres). However, as I demonstrated in the electron microscope slides, there was no cavity or gross aggregation of collagen bundles in the amyloid zones and no fibrillation of cartilage to account for this peculiar appearance.

Dr. S. Orloff (Brussels) Does the synovial fluid in these patients have any characteristic that is different from classical rheumatoid arthritis?
PROF. BYWATERS I do not think anyone knows. We did not examine the synovial fluid and I do not think there are any reports in the literature.

DR. A. ST. J. DIXON (Bath) I was wondering whether there might not be an alternative explanation for the fact that the juice-ways, or sap-alleys, or whatever you like to call them, are outlined in contact areas whereas the superficial amyloid staining as you showed very clearly, occurs all over the cartilage surface. This need not mean degeneration or wear. The contact areas are subject to enormous pressures between the two bone ends and, as the people who are studying articular lubrication have shown us, these microscopic pools of synovial fluid are trapped and compressed and these are the areas where fluid could be physically forced in.

PROF. BYWATERS That may be so.

DR. R. BLUESTONE (London) I have twice heard you mention these two patients with Bence Jones proteinuria and this peculiar amyloid formation. I think it is pertinent to note that Bence Jones proteinuria may simply reflect increased immunoglobulin synthesis. It does not necessarily imply myelomatous change. Furthermore, Waldman and his group at the National Institutes of Health, Bethesda (Waldman, 1968), have shown that some patients with primary amyloid, even excluding those who years later turn out to have myeloma, have increased rates of light chain production and decreased synthesis of normal immunoglobulin.

PROF. BYWATERS I think this may be so. The protein was identified as a K type in only one of them. The other was not examined. In neither was there evidence of myeloma post mortem.

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RÉSUMÉ

Les dépôts amyloïdes dans le cartilage articulaire

Le cartilage articulaire dans deux cas d’amylodose primaire avec de la protéinurie Bence Jones a montré une infiltration amyloïde démontrée par le méthyle violet, la thioflavine et la coloration au Congo rouge et en se servant de la biréfringence et aussi de la fluorescence ultra-violette. Les fibrilles amyloïdes longues de 75 à 100 Å pouvaient être démontrées ultra-microscopiquement. Ces dépôts, plus denses superficiellement, avaient été vus dans un cas comme commençant à la surface et allant vers l’intérieur comme des grains globulaires en lignes droites et aussi profondément sur les surfaces des fissures de fibrillation. Cela a suggéré une diffusion d’un précurseur amyloïde soluble venant du liquide synovial ou à une pénétration de fibrilles fines le long des passages déjà formés mais inconnus jusqu’ici. Ces dépôts avaient épargné les environs immédiats des chondrocytes. Les dépôts amyloïdes étaient péricellulaires dans la membrane synoviale.

SUMARIO

Depósitos amiloïdes en el cartílago articular

El cartílago articular en dos casos de amiloidosis primaria con proteinuria Bence-Jones reveló infiltración amiloidea, demostrada por violeta de metilo, tioflavina y colorante rojo del Congo, usando tanto birrefringencia como fluorescencia ultravioleta. Fibrillas amiloïdes de aproximadamente 75-100 Å eran demostrables ultramicroscópicamente. En un caso se observó que estos depósitos, en su mayoría densos superficialmente, ocurrieron de la superficie para abajo, en forma de cuentas globulares a lo largo de sendas rectas, y también intensamente sobre la superficie de hendiduras fibriformes. Esto sugirió la difusión, del fluido sinovial, de un amiloide precursor soluble, o la penetración de fibrillas finas a lo largo de sendas preexistentes, aunque hasta ahora desconocidas. Las zonas inmediatas a estos depósitos carecían de condrocytes. En la membrana sinovial, los depósitos de amiloide eran pericelulares.