THE EFFECT OF MECHANICAL STRESS ON THE STIFFNESS OF ARTICULAR CARTILAGE AND ITS ROLE IN THE AETIOLOGY OF OSTEOARTHROSIS

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Submitted in accordance with the requirements
for the degree of Ph.D.

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June 1988.

"What is Cartylage? It is a substance as it were of the kynde of bones, but it is softer or sowpler than the bone is."

Robert Copland, 1541.

ABSTRACT

Although a substantial amount is known about the pathogenesis of osteoarthrosis, its aetiology and in particular the role that mechanical factors play, remains unclear.

One particular hypothesis suggests that cartilage adapts mechanically so that it may transmit, without sustaining damage, the stresses to which it is predominantly subjected, and that damage to the cartilage is caused by infrequent high stresses in excess of the predominant level. As a corollary, it was suggested that highly stressed cartilage should be stiffer than lowly stressed cartilage.

A survey of the mechanical properties of normal articular cartilage from unembalmed cadaveric knee and ankle joints was undertaken to test this hypothesis. For this purpose, a specially developed indentation test apparatus was commissioned. Tests of the machine's measurement capabilities indicate that coefficients of variation of 2.14% and 1.20% for indentation and cartilage thickness measurement could be expected. The maximum percentage errors in the calculated creep modulus value which could result from these typical measurement errors, were 4.2% and 2.9% respectively.

Creep modulus values, calculated from these measurements, were used in topographical comparisons of cartilage stiffness. The stiffest areas of cartilage in the knee joint were the femoral condyles and areas of the tibia covered by the menisci. Cartilage on the patellar surfaces of the femur and in areas exposed by the menisci was significantly softer. Cartilage from the ankle joint was considerably stiffer than cartilage from the knee.

Comparisons between the cartilage stiffness and levels of stress which act in the knee and ankle joints during normal ambulatory activity, showed the stiffest areas of cartilage to be subjected to the greater stresses. Correlations of averaged data values indicated a significant ($p < 0.01$) direct relationship between cartilage stiffness and stress. This relationship and the consistency with which osteoarthrotic lesions were found in areas subjected to damaging patterns of stress supported the hypothesis under examination.

The lack of correlation found between the proteoglycan content and cartilage stiffness suggested that structural rather than compositional factors may be more important in influencing the compressive stiffness of normal articular cartilage.

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INTRODUCTION

Articular cartilage is the thin layer of specialized connective tissue which protects the underlying subchondral bone substrate in articular joints. It consists of a complex extra-cellular matrix of collagen fibers embedded in an extremely hydrophilic aggregation of muco-polysaccarides. When healthy, articular cartilage is bluish white and shiney in appearance and is approximately two orders of magnitude softer than the underlying subchondral bone. When loaded, it deforms, increasing the areas over which loads are transmitted and thereby minimizing the streses to which the subchondral bone is subjected. Of equal import to normal joint function, is the highly effective combination of articular cartilage with synovial fluid, in providing near frictionless movement of the opposing joint surfaces during load bearing.

Although articular cartilage is a remarkably resilient material, it can undergo degenerative changes which may place the whole joint at risk. Osteoarthrosis, or degenerative joint disease as it is alternatively known, affects as much as 60% of the population at one time or other. Clinically, osteoarthrosis is characterised by pain, restrictions in joint mobility and joint stiffness, whilst gross remodelling of the subchondral bone, marginal osteophyte formation and subchondral bone cysts are other changes commonly seen in the later stages of the disease. Unlike rheumatoid arthritis, evidence of osteoarthrosis dates as far back as Neanderthal man (40,000 B.C.) and even earlier in the fossilized remains of animals from the Mesozoic period (20 million B.C). Even so, our understanding of the aetiology of osteoarthrosis remains unclear.

Although studies of the mechanical properties of articular cartilage, the loads and stresses to which cartilage is physiologically subjected and of the possible mechanisms through which cartilage may fail, have all led to improvements in the understanding of the disease, there is still no established consensus of opinion regarding the role of bio-mechanical factors in the development of osteoarthrosis. Most contemporary mechanical hypotheses of osteoarthrosis fail to encompass fully the existing body of facts already known about osteoarthrosis.

One of the more recent hypothesis of osteoarthrosis, which concentrates on the magnitudes and patterns of mechanical stresses to which the cartilage is subjected (Seedhom et al,1979) embodies a substantial amount of evidence regarding the location and incidence of osteoarthrotic lesions. The hypothesis, which cites infrequent but excessive stresses as being the initiating mechanical factor in osteoarthrosis, is based on the observation that osteoarthrotic lesions in the patello-femoral compartment of the knee are located predominantly in areas corresponding to contact areas at 40° to 80° of flexion. More interestingly however, is the peculiar pattern of stressing to which these areas were subjected. Like the rest of the patella surface, the typical stresses acting in these areas are low, around 1 MN/M^2. Where these areas differ however, is that during certain less frequent ambulatory activities, eg climbing or descending stairs, the cartilage is subjected to much higher levels of stress, around 4 to 6 MN/M^2.

Seedhom et al (1979) proposed that these peculiar patterns of stressing were directly responsible for triggering osteoarthrosis. As a corollary to their hypothesis, they suggested that the stiffness

of articular cartilage underwent adaptation in response to the predominant level of stress to which it was subjected. It follows that cartilage subjected primarily to high stress should be stiffer than cartilage subjected primarily to low stress.

The primary objective of the present study was to test this hypothesis. In particular, the investigation was focused on the corollary regarding cartilage adaptation to mechanical stress. Does articular cartilage adapt in response to the level of mechanical stress to which it is predominantly subjected? For this purpose, a complete survey of the compressive stiffness of articular cartilage from the knee and ankle joints was intended. The ankle joint is a highly stressed joint; estimates for the stresses of up to 5 MN/m^2 have been made. In contrast, the stresses in the knee joint are much smaller, although distinct differences between the stress levels acting in the two compartments have been observed.

The majority of attempts to measure the compressive stiffness of articular cartilage have been made with some form of indentation test. Such a test involves loading the articular cartilage in situ on the subchondral bone with either a hemispherically or plane ended cylindrical indenter. The resulting depth of indentation, the load applied and the geometry of the probe can be then related to the compressive stiffness of the cartilage and its undeformed thickness. The nature of formulae relating these parameters, especially in connection with the testing of articular cartilage, has been the subject of considerable debate. The basis of their derivation is an inherent assumption of material elasticity, isotropy and homogeneity. Concern regarding the accuracy of techniques for correcting for the effects of finite specimen thickness has also been expressed.

For this investigation, a much improved indentation technique has been developed which alleviated many of the time consuming procedures previously adopted in performing indentation tests. The machine, with small modification, was also used to make accurate measurements of the undeformed cartilage thickness. The extent to which the compressive modulus values obtained with this apparatus can reliably be used to characterise the stiffness of articular cartilage has been evaluated.

It was also intended as part of this study, to reinvestigate the extent to which the mechanical properties of cartilage are related to its biomechanical constituents. Conflicting results regarding this relationship have been previously reported. Such an investigation was intended to elucidate possible mechanisms through which articular cartilage may adapt to mechanical stress.

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LITERATURE REVIEW

1.1 Articular cartilage

Articular cartilage is the thin protective layer of specialized connective tissue which lines the articulating surfaces of synovial joints. When healthy, articular cartilage is smooth, shiny and bluish white in colour, although with increasing age it becomes rather yellowish in appearance, probably as a result of pigmentation associated with the non-collagenous proteins found in cartilage (Korst et al, 1968). It is devoid of nerves and blood vessels.

Articular cartilage follows closely the shape of the joint surfaces although its thickness varies both from joint to joint and topographically across a joint surface Simon (1970). In the main load bearing joints of the lower limb, the thickness of articular cartilage ranges from 1 mm to 5 mm. In the smaller more peripheral joints, articular cartilage is much thinner. It is much softer than bone, resembling a thin sheet of rubber, which deforms when subjected to compressive loads with a typical visco-elastic response.

1.1.1 Structure

Articular cartilage consists of an extracellular matrix of collagen fibres embedded in an extremely hydrophilic aggregation of proteoglycans (synonymous with glycosaminoglycans or mucopoly-saccarides). Dispersed throughout the matrix are a relatively small number of cells called chondrocytes. The distribution of chondrocytes and of the matrix components varies considerably depending on the depth of the cartilage from the surface. Collins (1949) conveniently classified cartilage into four layers: (i) the superficial layer, (ii) the intermediate layer, (iii) the deep layer

and (iv) the calcified layer. In human femoral condylar cartilage the superficial layer accounts for 5% to 10% of total thickness, the intermediate layer and deep layers, about 30%-40% each and the calcified layer 5%-10% (Meachim and Stockwell, 1979). Interfaces between the uppermost three layers are gradual and imperceptible, whilst the upper boundary of the calcified layer can be distinguished by a basophilic line or 'tide mark' (Fawns and Landells, 1953).

In the superficial layers of normal articular cartilage, collagen fibrills are relatively small in diameter, approximately 30 nm. The fibrills tend to join together forming tightly packed bundles, arranged tangential to the articular surface. Depending on their topographical location, these superficial bundles of collagen fibres take up preferred orientations (Bullough and Goodfellow, 1968; Benninghoff, 1925) which appear to be related to the direction of mechanical strain to which the cartilage is subjected. Proteoglycan levels in superficial layers are relatively low, whilst the density of chondrocytes appears to be greater than anywhere else in cartilage. The chondrocytes tend to be discoidal in shape with their long axis parallel to the articular surface.

Collagen fibrills in the intermediate layers are larger (approx 80 nm in diameter) than those occurring in the superficial layer. They are more widely spread out and have no apparent preferred orientation. The proteoglycan content is greater in this layer than in cartilage from the superficial layer; and the chondrocytes, which tend to be spheroidal in shape, are more evenly but less densely distributed.

In the deep layer, the predominant orientation of the collagen is perpendicular to the cartilage bone interface. The fibres are still

larger in diameter, approximately 140 nm, and extend into the calcified areas of the cartilage, providing the cartilage with a firm attachment to the subchondral bone. Proteoglycan levels are much the same as in the intermediate layers, although it is thought that there are generally fewer chondrocytes. Polarised light microscopy (Bullough and Goodfellow, 1968) and electron microscopy (Weiss et al, 1968; McCall, 1969) have been largely responsible for confirming these ideas regarding the collagenous structure of articular cartilage.

By weight, normal articular cartilage is approximately 70%-75% water, 15%-20% collagen and 6%-10% proteoglycan. The remainder is made up by the chondrocytes and glycoproteins (Maroudas, 1979; Muir, 1980).

1.1.2 Function

Articular cartilage has two main functions. First, it plays an important role in the transmission of intra-articular loads. As cartilage is loaded it deforms. This deformation results in an improvement in joint congruity which increases the size of contact areas. The stresses acting on the underlying subchondral bone are thus limited. Secondly, the cartilage layer provides an extremely efficient bearing surface. Although the exact modes of lubrication are still not fully understood, tribologically it is known that with synovial fluid as a lubricant, cartilage on cartilage has a remarkably low coefficient of friction. Estimates of the coefficient of friction give $\mu = 0.02$ (Jones, 1936). Compared to the coefficient for ice on ice ($\mu = 0.05$) or for perspex on steel, a combination commonly used in prosthetic devices ($\mu = 0.3$), synovial joints are extremely effective in minimizing frictional forces.

1.1.3 Biochemistry of the matrix components

Collagen

The collagen in most mammalian cartilage consists of three polypeptide chains which, as a result of their repeated sequences of tripeptides, are intertwined in a triple helix structure. Collagen found in hyaline cartilage (articular cartilage) is primarily type II collagen (Miller, 1976) although small quantities of other types of collagen have been isolated (Burgeson et al,1982; Duance et al, 1982). Type II collagen is distinguished by its three identical polypeptide chains whilst other collagen types comprise combinations of different polypeptides. These helical structures form themselves by molecular cross linking into fibrils which can be considered as the basic biological unit of collagen. Further cross linking between individual fibrils results in a complex fibrillar network which affords cartilage its tensile strength.

Proteoglycan

Proteoglycan consists primarily of long carbohydrate chains attached covalently to central core proteins. The carbohydrate chains are predominantly chondroitin and keratin sulphate chains, although a small number of other oligosaccharides have been identified (Hascall and Sadjera, 1970). Chondroitin and keratin sulphate both consist of repeated disaccharide units, chondroitin sulphate of N-acely-lactosamine and glucouronic acid and keratin sulphate of N-acelyglucosamine and galactose (Hardingham and Muir, 1973).

In adult human articular cartilage, chondroitin sulphate is the predominant carbohydrate chain found in proteoglycan. Its estimated molecular weight of 20,000 is large compared to that of keratin sulphate (approx 5,500) and the other oligosaccharides (approx 2,000)

found in articular cartilage. It therefore constitutes a large proportion of the weight of proteoglycan, approximately 80%-85%. Keratin sulphate accounts for less than 10% of the weight of proteoglycan whilst the core protein between 7%-12%, (Hascall and Sadjera, 1970).

Two types of chondroitin sulphate have been identified in articular cartilage, chondroitin-6 sulphate and chondroitin-4 sulphate. These are distinguished by differences in the attachment of the sulphate groups to the carbohydrate. Although no biological significance has been attributed to the difference, it has been shown that the sulphate groups in chondroitin-6 sulphate project further from the core protein suggesting a greater interference with the collagen matrix.

Proteoglycan aggregation and its interaction with the collagen matrix

In the presence of hyaluronic acid the proteoglycans readily combine to form large aggregations with estimated molecular weights in excess of 350×10^6 (Hardingham et al, 1981). As a result, free hyaluronic acid is rarely found in cartilage. Because of the high density of sulphate and carboxylate groups associated with the chondoitin and keratin sulphate molecules, proteoglycan has a large net negative charge. In isolation, proteoglycan aggregations swell extensively, imbibing large amounts of water and take the form of a gel. In cartilage, however, the proteoglycans are immobilized in a complex matrix of collagen fibres which restricts its ability to swell. It is this restriction in the swelling of the proteoglycan which provides cartilage with its mechanical resilience. The

interaction between the two components of articular cartilage is essentially a mechanical one, the proteoglycans being entangled in the collagen matrix. It is however suspected that a minimal amount of chemical bonding occurs between proteoglycan and collagen (Bland and Cooper, 1984), although the extent to which this affects the mechanical properties of cartilage is not known.

The Chondrocytes

Although sparsely distributed within articular cartilage, the chondrocytes are responsible for the maintenance of the extra-cellular matrix of proteoglycan and collagen. Chondrocytes readily respond to mechanical, biochemical and micro-environmental stimuli by increasing their synthesis of proteoglycans, type II collagen and enzymes such as cathepsin. In normal mature articular cartilage, the synthesis of proteoglycan is rapid, reflecting its relatively short half life of as litle as eight days (Mankin and Lippieto, 1969). In comparison, collagen synthesis is slow and thus its turnover in normal mature articular cartilage is minimal (Libby et al, 1964).

1.1.4. Mechanical properties

In healthy synovial joints frictional forces are minimal because of the exceptionally low coefficient of friction of cartilage on cartilage. During ambulatory and for that matter athletic activity, articular cartilage is therefore predominantly subject to compressive loads.

In compression, the constitutive behaviour of articular cartilage is that of a visco-elastic material. That is, it exhibits both the characteristics of a fluid (visco) and a solid (elastic).

Conventionally, the response of visco-elastic materials to statically applied loads are recorded in the form of deformation versus time curves, otherwise known as creep curves. Such curves have been obtained for normal articular cartilage by a number of workers (Bär, 1926; Göcke, 1927; Hirsch, 1944; Elmore et al, 1963; Kempson et al, 1971). In each case, an instantaneous deformation of the cartilage was followed by further creep deformations. Where the recovery of the cartilage was monitored following the removal of the applied load, an initial instantaneous but partial recovery of the cartilage was observed. This was followed by a further time dependent recovery of the cartilage.

Characterizing the properties of articular cartilage is further complicated by its visco-elastic, anisotropic and non-homogeneous nature. The design of suitable techniques for making such measurements is also hindered by the curved geometry and layered structure of the cartilage. Most commonly, compressive or shear modulus values have been calculated for cartilage using formulae derived for elastic, isotropic and homogeneous materials (Kempson et al, 1971a; Hayes et al, 1972). In characterizing the mechanical properties of articular cartilage in this way, it is necessary to assume that cartilage behaves as an elastic, isotropic and homogeneous material. This single phase elastic approach to the measurement of the mechanical properties of cartilage has been used in a number of studies (Kempson et al, 1971b; Coletti et al, 1972; Hayes et al, 1972; Hori and Mockros, 1976; Roberts et al, 1986; Jurvelin et al, 1986).

More recently, efforts to describe mathematically the behaviour of articular cartilage in terms of the interstitial fluid flow within the matrix and the mechanical properties of the matrix itself (Mow et al, 1980) prompted the development of more rigorously controlled confined compression tests. Comparisons between the mathematically predicted behaviour of cartilage and that observed when cartilage is mechanically tested have indicated that the model is realistic.

Mak et al (1987) further extended the biphasic theory in order that the behaviour of cartilage under plane-ended or spherical indenters could be studied.

1.2 Osteoarthrosis

Osteoarthrosis or as it is alternatively known, degenerative joint disease, is a common and disabling disorder of the synovial joints, particularly of the main weight bearing joints. Observations of osteoarthrosis in man date back to 40.000 B.C. and Neanderthal man (Straus and Cave,1957). In animal species, fossilized remains of dinosaur skeletons from as far back as the Mesozoic period (20,000,000 B.C.) have indicated degenerative joint changes (Moodic, 1923).

Pathologically, osteoarthrosis is initially characterized by the erosion of the articular cartilage layer and then subsequently by remodelling of the subchondral bone, marginal osteophyte formation and subarticular bone cysts. Clinically, the commonest complaint of osteoarthrosis is pain, particularly during joint flexion and weight bearing, although joint stiffness and limitation in the range of joint flexion are also regularly reported.

The characteristic changes seen in articular cartilage and in its immediate surrounds during osteoarthrosis are the consequence of a complex and involved sequence of mechanical and biochemical alterations and responses. The exact aetiology of osteoarthrosis, however, remains unresolved. Investigations are complicated as it is inevitable that the disease has no unique aetiological pathway. Progress in the understanding of osteoarthrosis is further hampered by the difficulty in designing and conducting suitable experiments to answer the current questions raised about its aetiology.

1.2.1 Mechanical factors and osteoarthrosis

That mechanical factors play an important role in the aetiology of osteoarthrosis is no longer disputed. There is however much debate about the mechanisms which are involved and where they fit into the sequence of aetiological events.

One of the earliest hypotheses regarding the role of mechanical factors in osteoarthrosis was made by Freund (1939). He stated that a certain range of loading was healthy for cartilage and that above and below this range, cartilage degenerates. No attempt was made to specify the range of loading nor to account for the mechanisms which served to regulate cartilage health.

Cyclic loading was suggested by Saaf (1950) and Linn and Sokoloff (1965) as an important factor in maintaining healthy cartilage. In exerting a pumping action on the interstitial fluid, essential metabolites could be circulated to and from the chondrocytes. In the absence of such loading,. cartilage was apt to degenerate due to inadequate nutrition. Observations of disuse atrophy of cartilage in

paralysis and degenerative changes in the unused areas of the hip and elbow joints (Trueta, 1963, Goodfellow and Bullough, 1967) further fuelled belief that insufficient mechanical stimulation was aetiologically important in osteoarthrosis.

Freeman (1972) suggested that the failure of the collagen in articular cartilage was the initial mechanical change in osteoarthrosis. In particular, he regarded tensile fatigue failure as the likely mode of failure. Kempson et al (1971b) had previously suggested that excessive levels of stress could conceivably cause collagen failure in the superficial layers, especially in cartilage where proteoglycan levels were reduced and the superficial layers were subjected to larger strains.

Weightman (1976) investigated the fatigue properties of articular cartilage and found that in tension it exhibited a typical fatigue behaviour. His results, in particular the relationship found between the fatigue failure stress and age, have been subsequently criticized by Seedhom and Swann (1986) for exhibiting (as the author himself had admitted) considerable scatter. In a subsequent and more accurate study, Weightman (1978) indicated that his original results had underestimated the decrease in fatigue strength of cartilage with age. As a consequence, cartilage appeared more susceptible to fatigue failure than originally thought. Even so, Weightman was cautious in relating his laboratory results to the in vivo failure of cartilage, as he was unable to demonstrate comparability between physiological stresses acting on the collagen and those used during the laboratory fatigue testing. Seedhom and colleagues (1979) had previously observed examples of near perfect cartilage in knee

specimen in their seventh and eighth decade and often advanced osteoarthritic damage in specimens as young as their second decade. Such observations suggested that collagen fatigue failure was less significant aetiologically than originally thought by Freeman (1972) and Weightman (1973).

Radin (1973) popularized the view that the initial events in osteoarthrosis were changes in the subchondral bone rather than changes in the cartilage itself. By repeatedly impacting immobilized hind legs of Canadian white rabbits, Radin induced stiffening of the underlying subchondral bone. He suggested that as a result of a process of microfracture and remodelling, increases in subchondral bone stiffness disposed the overlying cartilage to mechanical failure. Later, in a histological study of the effects of cyclic impact loading on rabbit cartilage, Radin (1984) indicated that mechanical failure of the collagen preceded any cellular or biochemical alterations in the cartilage.

A more recent mechanical hypothesis of osteoarthrosis (Seedhom et al, 1979) suggested that a particular mode of stressing was responsible for initiating osteoarthrosis. Based on previous observations of osteoarthrosis in the patello-femoral compartment made by Emery and Meachim (1973) and Meachim and Emery (1974) a study of the location of such lesions indicated that they were not randomly located on the patella surfaces but confined primarily to those areas in contact with the patella at angles of flexion of 40° to 80°. Seedhom and colleagues (1979) showed that during walking, the patella surfaces of the femur were subject to low levels of stress, 1 MN/m^2. During less frequent activities such as climbing or descending stairs or

walking up or down inclined surfaces, the areas of the patellar surfaces of the femur in contact with the patella at angle of flexion of 40°-80° were subject to much higher stresses ranging from 4 and 6 MN/m^2.

It was suggested that cartilage becomes conditioned mechanically to transmit, without sustaining damage, the stresses to which it is most commonly subjected. As a consequence, it was proposed that highly stressed cartilage should be stiffer than lowly stressed cartilage. In the event of infrequent stresses in excess of the predominant level, it was thought that damage to the cartilage occurred. Such a pattern of stressing whereby, for the most, stresses are low but for short periods are in excess of the predominant level, was therefore regarded as an important aetiological factor in osteoarthrosis.

Subsequent experiments Seedhom and Hargreaves (1979) demonstrated that similar patterns of stress could also occur in the tibio-femoral compartment of the knee, particularly in areas of the tibia exposed by the menisci to direct articular contact with the femur. These areas are the most susceptible to osteoarthrosis on the tibial plateau.

1.2.2 Other aetiological factors

Although biochemical, enzymatic and cellular changes have been consistently observed in osteoarthritic cartilage, the exact aetiogical role of these factors is unclear. How such changes fit into a sequence of aetiological changes and responses is also unknown.

In normal articular cartilage a regulated cellular activity is of great importance if the matrix is to be successfully maintained. Stimulation of the chondrocytes either directly as a result of mechanical stresses (the effects of which may vary with alterations in the mechanical properties of the cartilage caused by a deficiency in either of the two main matrix components) or indirectly by chemical means are just two possible mechanisms through which cellular activity could be moderated.

In osteoarthrosis an imbalance occurs whereby the processes of matrix degradation and re-synthesis are disturbed, and as a consequence articular cartilage undergoes distinct biochemical changes. Observed increases in the chondrocyte synthesis of collagen, proteoglycan and degenerative enzymes such as cathepsin (Rothwell and Bentley, 1973; Herman et al, 1982) indicate that significant biochemical responses occur in osteoarthrosis, although they appear to be either inadequate or perhaps more likely inappropriate for re-establishing the original biochemical balance. This may be particularly so, if the factors responsible for initiating such responses are still present.

1.3 The mechanics of synovial joints; forces, contact areas and stresses.

Understanding the aetiology of osteoarthrosis, and in particular, the influence that mechanical factors have, requires a knowledge of the forces and stresses to which joints and cartilage are typically subjected.

Forces acting in the knee joint have been calculated for a number of typical ambulatory activities (Morrison, 1967; Paul, 1976; Seedhom et

al, 1977) as well as for other less frequent activities (Seedhom and Terayama, 1976; Ellis et al, 1979). During level walking, the typical tibio-femoral loads are between three and four times body weight, whereas the maximum loads in the patello-femoral joint are as little as 0.6 times body weight. During activities such as climbing or descending stairs, loads acting in the tibio-femoral do not differ greatly from those occurring during level walking. Loads acting in the patello-femoral joint during stair ascent and descent can however be as much as 2.7 times body weight. In more athletic activities, loads of approximately 20 times body weight have been estimated to act in both the tibio-femoral and the patello-femoral compartments (Smith, 1971).

The need to estimate the size of stress levels occurring in synovial joints inspired several attempts to measure the size of contact areas in the hip as well as in both the tibio-femoral and patello-femoral compartments of the knee. The technique of injecting dye into physiologically positioned and loaded cadaveric knee joints was developed by Dean (1970) and Greenwald (1970). The technique was limited as only one contact area could be determined in any one joint. Roentgenographic and casting techniques were subsequently developed by Kettlecamp and Jacobs (1972) and Walker and Hajek (1972). It should be noted however, that Walker and Hajek, having removed the menisci, must have significantly disturbed the mechanics of the joint, and that Kettlecamp and Jacobs used loads which were significantly smaller than those which occur physiologically. This prompted the development of an improved arthographic technique (Maquet et al, 1975). During flexion of the knee, they showed that contact areas on the tibial surfaces moved posteriorly and

progressively diminished in size from 20 cm^2 to 11 cm^2. Following meniscectomy, contact areas were much reduced, in some cases by as much as 50%. This result has since been confirmed using different techniques (Seedhom and Hargreaves, 1979; Fukubayashi and Kirosawa,1980).

Seedhom and Tsubuku (1977) made further modifications to the casting technique to account for creep deformation of the cartilage during the period taken for the casting material to cure. They estimated patello-femoral contact areas to be approximately 5 cm^2 during level walking.

Walker and Erkman (1975) developed another casting technique for determining tibio-femoral contact areas which relied on the expression of half cured methylmethacrylate from between loaded joint surfaces. With large loads, contact occurred both in areas exposed and covered by the menisci. In flexion, contact areas tended to move posteriorly over the tibial surfaces.

The combination of results from studies of contact areas and of joint forces enabled estimates of average stress levels acting within joints to be made. Seedhom et al (1979) indicated that a typical value of stress acting in the patello-femoral compartment during level walking was approximately 1 MN/m^2. Likewise, similar estimates were made for the tibio-femoral compartment (Seedhom and Hargreaves, 1979), showed that stresses acting in the lateral compartment were slightly greater than those acting in the medial compartment, but more importantly that stresses acting on areas underlying the menisci were generally greater than stresses acting on areas exposed to direct articular contact.

Attempts to measure directly the contact pressures occurring in synovial joints have been numerous. Walker and Erkman (1975) placed a mini-transducer (3.6 mm diameter and 0.75 mm thickness) directly between the opposing surfaces in the tibio-femoral compartment. Adams and Swanson (1985) and Marahi et al (1980) inserted load transducers into the cartilage from the underlying subchondral bone, whilst Brown and Muratori (1979) recessed numerous small piezoresistive transducers into the cartilage surface. Ahmed and Burke (1983) placed a thin micro-indentation transducer (thickness 0.285 mm) between the joint surfaces.

The main criticism of these direct contact pressure measurement techniques is the disruption to the congruity of the opposing joint surfaces. It is likely that the presence of such transducers causes stress concentrations, resulting in unrealistic measured stress values.

In attempting to determine the proportion of load carried by the menisci, Inaba and Arai (1987) measured contact pressures by inserting mini-pressure tubes into the cartilage layer from the underlying subchondral bone. Using saline as both a lubricant and a pressure medium they obtained stresses comparable to previously estimated average stress levels (Seedhom and Hargreaves,1979).

Pressure sensitive paper has also been used to estimate contact pressures within the knee (Fukubayashi and Kurosawi, 1980; Huberti et al, 1983) and within the hip joint (Afoke et al, 1987). Values of 3 MN/m^2 and 10 MN/m^2 . were obtained for the maximum contact presures acting in the knee and the hip during ambulation. These

values were noticeably larger than corresponding values estimated indirectly. Including two protective layers of clingfilm, the total thickness of the pressure sensitive paper, when inserted into the joint space, was 0.25 mm. This could have significantly effected the congruity of the joint. The insertion of the paper also required the incision of the the ligamentous structures surrounding the joint. This could easily leave the joint in an unstable, non-physiological and perhaps less congruent position. This fact may well explain the excessive estimations of contact pressures obtained using this technique.

1.4 Mechanical testing of cartilage

1.4.1 The indentation test

The earliest attempts to indent articular cartilage (Bär, 1926; Göcke, 1927) were made using 'elastometers', similar to that described by Schäde (1912). They comprised a cylindrical indenter, whose movement was mechanically magnified and recorded by way of a pen attached to the end of a long counter-balanced lever arm. Both machines were plagued by problems of dry friction. In 1944 Hirsch constructed an indentation machine of his own. Although in principle it was essentially the same as those of Bär and Göcke, Hirsch paid particular attention to eliminating any frictional problems. Although it is difficult to assess exactly how successful Hirsch was in overcoming such problems, his attempts to relate his data regarding the mechanical properties of cartilage with the appearance of histological sections must be regarded as pioneering.

The apparatus of Elmore et al (1963) (which was later used by Sokoloff, 1966 and Simon, 1970, 1971) marked the end of dry friction

as a problem. Their 'compressometer' was the first to include an electrical displacement transducer coupled via amplification circuitry to a mechanical chart recorder. This avoided any need for a lever arm recording device. Once the compressor foot had been aligned perpendicular to the articular surface by way of a gimbal, the no-load position of the compressor foot, ie its position when it was just in contact with the un-deformed cartilage surface, was determined. Such a measurement was necessary in order that any movement of the indenter prior to it coming into contact with the cartilage could be excluded from the overall indentation measurement. Measurement of the no-load position was achieved by applying a small tare load of approximately 0.05 g to the cartilage via a counter balanced beam and a fixed excess load positioned some way along the beams excursion scale. This was thought sufficient to maintain contact without producing any significant indentation in the cartilage surface, although it seems likely that it was not. As such a load would be insufficient to overcome the surface tension of a layer of water on the articular surface, it suggests either that much larger loads were applied or that errors in the measurement of the no-load position were incurred.

The apparatus used by Kempson (1971a) differed little in principle from that of Elmore et al (1963). Specimen alignment was again made using a gimbal, the perpendicularity of which was assessed by viewing the area of contact between the cartilage surface and a substitute transparent perspex indenter whilst it rested gently on the articular surface, under the restraint of a tare load of 2 g. Any eccentricity about the axis of the indenter of the contact area was rectified by further realignment of the specimen by way of the gimbal. At the

same time, Kempson was also able to measure the position of the un-deformed cartilage surface. The fact that he continued to repeat this measurement until three consecutive readings were obtained suggested that the procedure was not totally ideal. Problems of poor indenter-cartilage contact or the deformation of the cartilage surface seem to have reduced significantly the reliability of the procedure. The fully loaded indenter was released from an arbitrary position just above the cartilage surface, using a manual, cam-operated release mechanism. This mechanism allowed sudden and reproducible loads to be applied to the articular cartilage, although no investigation of the actual loads applied to the cartilage was reported. The position of the indenter was continuously monitored using an L.V.D.T. type displacement transducer, and recorded using an X-Y pen recorder.

Hori and Mockros (1976) carried out indentation tests on excised cartilage-bone plugs using a modified version of apparatus originally designed by Hayes and Mockros (1971) for testing cartilage in confined compression. The mechanism for applying loads was identical to those used previously by Kempson (1971a) and Elmore (1963). Tare loads of 0.18 N were used in determining the position of the un-deformed cartilage surface whilst the perpendicularity of alignment to the indenter was reliant on the geometry of the specimen being that of a right cylinder.

Scrutiny of the apparatus and procedures used to indent articular cartilage, revealed certain deficiencies that affected measurement reliability. The two main areas of contention were the detection of the no-load position of the indenter and the application of load to the cartilage.

(a) Detecting the no-load position of the indenter.

The procedure in which small tare loads were applied to the cartilage surface in order that the no-load position of the articular surface to be measured was not only a tedious affair, increasing greatly the time required to perform each test (an important criterion in survey work, where a large number of test are envisaged) but was also a potential source of error. This fact was first acknowledged by Hori and Mockros (1976) who corrected their measurements by adding an estimated tare indentation to each depth of indentation. Based on the tare loads and radii of indenters used by Elmore et al (1963), Kempson et al (1971) and Hori and Mockros (1976), tare indentations of as much as 0.02 mm could occur. An alternative system for detecting the un-deformed cartilage surface which did not resort to pre-loading the cartilage would significantly improve the accuracy of measurements and the speed at which each test could be performed.

(b) Load application

Although it was often reported that loads were applied to the cartilage in a reproducible manner, no attempt has ever been made to measure the exact applied loads. Transient loads of twice the static value were measured by the author when performing indentations using an undamped indenter released from a height of approximately 0.1 mm above the cartilage surface. Excessive loads of this nature increased the size of the resulting indentations and introduced the possibility of large errors in the overall measurements.

1.4.2 Cartilage thickness measurement

The calculation of modulus values from the depths of indentations made in thin layered materials, requires a knowledge of the un-deformed thickness of the material.

Direct measurements of cartilage thickness were made by Kempson et al (1971) who used a trepanning instrument to prise plugs of cartilage from the underlying subchondral bone. Measurements of the cartilage thickness were made using vernier callipers. Simon (1971), Parsons and Black (1977) and Jurvelin et al (1986) all used microscopes to measure the cartilage thickness from slices of their specimen taken perpendicular to the articular surface. Although apparently straightforward, direct measurement of cartilage thickness is fraught with problems. Cutting cartilage immediately disrupts the cartilage structure allowing undesirable changes in shape and size, either as a result of unnatural and rapid dehydration or from excessive swelling should the specimen be soaked in saline prior to measurement.

Rushfeldt et al (1981) reported a successful technique for measuring the thickness of cartilage on the acetabular surfaces of the hip, using ultrasound. The thickness of cartilage was calculated from the difference in time required for ultrasonic reflections to return from the surface and bone interface of the cartilage. The experience of the present author however, in using ultrasound to measure the thickness of cartilage resulted in certain reservations regarding this technique. Even with immaculate cartilage, signal attenuation was pronounced, making measurements extremely difficult to obtain.

Another more successful approach to measuring cartilage thickness was made by Hori and Mockros (1976), who obtained results by pushing a blunt needle attached to a standard depth micrometer through the cartilage layer. The technique was later modified by Hoch et al (1983), who ramped a sharp needle attached to a load transducer through cartilage at 20 μm/s. The time taken from detecting surface

contact to detecting bone contact was used to calculate the distance the needle had moved and hence the cartilage thickness.

CHAPTER 2

THE INDENTATION TEST AS A METHOD FOR COMPARING CARTILAGE STIFFNESS

THE INDENTATION TEST AS A METHOD FOR COMPARING CARTILAGE STIFFNESS

2.1 Introduction

Although the indentation test has been regularly used (Hirsch, 1944; Sokoloff, 1966; Kempson et al, 1971; Simon, 1971; Black et al, 1979; Lane et al, 1979; Altman et al, 1984; Jurvelin et al, 1987) as a method for measuring the stiffness of articular cartilage, there has been much debate on whether or not modulus values calculated from indentations are representative of the mechanical properties of cartilage. Criticism has been focused mainly on the validity of assumptions made in the derivation of formulae relating the depth of indentation and the cartilage thickness to the compressive stiffness.

The calculation of modulus values from indentation tests was initially based on the theoretical solution to the problem of pressure distribution between two semi-infinite hemispherical bodies in contact. The solution, originally solved by Hertz (1881), assumed material elasticity, isotropy and homogeneity and that during contact the strains produced in the material did not exceed the materials elastic limit.

Hirsch (1944) was first to apply the Hertzian theory to the indentation of cartilage and in doing so, calculated modulus values for both normal and degenerate patellar cartilage using equation 2.1.

$$E = \frac{0.681\ P}{R^{1/2}\ d^{3/2}} \qquad \text{---------- (2.1)}$$

Where E is the elastic modulus of the material being tested, P is the load applied, R the indenter radius and d the resulting depth of

Indentation. He unfortunately neglected to account for the finite thickness of cartilage, arguing that, for cartilage thicker than 2 mm, the effects of distortions in the distribution of stress within the cartilage layer, due to the proximity of the stiffer underlying subchondral bone, were negligible. As this is not true, the modulus values he obtained were most likely overestimates of the actual cartilage stiffness.

Waters (1965) accounted for finite thickness by multiplying modulus values, calculated directly from the Hertzian theory, with an empirically derived dimensionless correction function, obtained from indentation tests carried out on thin rubber sheets of various thickness and compressive stiffness. In this way, the effects of changes in the stress distribution around the indenter, due to the finite thickness, could be accounted for, equation 2.2:

$$E = \frac{9P}{16r^{1/2}} \left[\frac{1 - e^{-0.42t/a}}{d} \right] 3/2 \quad \text{----- (2.2)}$$

Where E, the elastic modulus, is related to the depth of indentation, d; the undeformed cartilage thickness, t; the applied load; P; the radius of the hemisperical indenter, r and a, the radius of the area of contact between the indenter and the cartilage.

Kempson et al (1971a) made use of Waters' formula in carrying out a survey of the stiffness of both normal and degenerate cartilage from the femoral head. They observed distinct topographical variations in the stiffness of articular cartilage, the stiffest areas extending in a band from the superior surfaces around both the anterior and posterior aspects of the articular surface (Kempson et al (1971b).

These areas largely corresponded to the areas in contact with the acetabular cup during walking. Their results yielded creep modulus values for normal articular cartilage which ranged from approximately 5.0 to 14.0 MN/m^2.

The problem of finite thickness was also addressed by Hayes et al (1972), who derived theoretically a functionally similar formula to that of Kempson et al (1971a), relating the shear modulus, G of a thin layer of material to the depth of indentation, d, equation 2.3.

$$G = \frac{P(1-v)}{4akd} \qquad \text{----------} \qquad (2.3)$$

Where P is the applied load, v the poisson ratio, a, the radius of the indenter and k is a function of t, the thickness, a and v.

The analysis was again based on the assumptions of material elasticity, isotropy and homogeneity and small material strains. Hayes et al (1971) further restricted the use of their theory to indentations made either instantaneously (t=0) or at equilibrium ($t=\infty$) when cartilage had ceased to exhibit creep deformation. They argued, that in both the cases, the fluid flow within the matrix was negligible and thus the cartilage behaved most like a single phase elastic material.

2.2 A critical assessment of the indentation test

Doubt has been cast on the validity of the indentation test as a method for measuring the stiffness of articular cartilage by a number of workers. Simon (1971) concluded that the dimensionless correction function derived by Waters (1965) failed to account completely for the effects that finite material thickness has on the calculated

values for the creep moduli. He found from his indentation tests performed on cartilage using a number of indenters of different radii, that variations in the modulus values were greater when calculated using the formula of Waters, which corrected for finite thickness, than when they were calculated directly from Hertzian theory, in which thickness effects were ignored. As a consequence, Simon used his indentation measurements to compare cartilage stiffness, claiming that it was more appropriate to do this than to use calculated modulus values.

Hori and Mockros (1976), using the formula of Hayes et al (1972) to calculate shear moduli, demonstrated that modulus values predicted from indentation tests on thin sheets of polyurethane rubber were comparable with those obtained from conventional direct mechanical tests. For articular cartilage however, they noted much larger variations in the values obtained for the shear moduli. They attributed these increased variations partly to experimental errors, particularly in determining the no-load position of the articular surface, and partly to the non-homogeneity and anisotropy of the cartilage.

Mow et al (1984) legitimately criticized all attempts using single phase elastic or visco-elastic models to predict modulus values for articular cartilage. They deemed them to be inadequate, as in their view, such models bore little resemblance to either the mechanical structure of cartilage or the material behaviour they sought to describe. As a consequence, Mow and his colleagues embarked on the development of a biphasic model for articular cartilage, which described cartilage behaviour under load, in terms of the flow of

interstitial fluid within the confines of a soft deformable solid matrix.

2.3 Modulus values for comparative studies of cartilage stiffness

Although it has been argued for a variety of reasons that modulus values obtained from indentation tests do not reflect the true mechanical properties of articular cartilage, they can still be of considerable use as a measure of cartilage stiffness. This is especially so in a comparative study where the objective is to distinguish between stiff and soft cartilage rather than to obtain precise values for the stiffness of cartilage.

To justify the use of calculated modulus values one must demonstrate that variations in the modulus values, due to departures from the theoretical assumptions made during the derivation of the formula employed, are small in comparison with the differences in stiffness of the cartilage itself.

In this study, modulus values were calculated from indentations measured two second after load application, using equation 2.2. Such values are a measure of the slope of the cord to the stress strain curve, drawn from the origin to a point on the curve corresponding to two seconds. Variations in these values were assesed by performing a series of indentation tests on a variety of materials. For this purpose, the experimental apparatus and measurement techniques described in chapter 3 were used. Modulus values calculated directly from Hertzian theory (equation 2.1) provided a basis for comparison.

2.4 Experimental factors affecting modulus calculation

A series of indentation . tests were performed with the intention of determining how one particular parameter affected the calculated

modulus value. The effects of (a) the radius of the indenter, (b) the applied load and (c) the thickness of the specimen were each examined in turn. In examining the effects of finite thickness, two series of indentation tests were performed on materials whose stiffnesses were comparable to the softest and stiffest cartilage found in either the ankle or the knee joint.

2.4.1 Indenter radius

A single sheet of vulcanized rubber, 3.0 mm in thickness, was bonded using cyanoacrylate cement to a flat steel plate and subjected to a series of indentation tests made with three indenters of different radii, 0.79 mm, 1.58 mm and 2.37 mm. Nine separate tests were performed with each indenter.

Creep modulus values, calculated from the mean depths of indentation made with each indenter two seconds after load application, (Table 2.1) varied more when corrected for the finite thickness of the rubber sheet than when the specimens were all assumed to be semi-infinite in thickness. The magnitude of the calculated modulus value was thus dependent on the radius of the indenter used to perform the test. This finding largely confirmed the results of a similar study by Simon (1971).

2.4.2 Specimen thickness

The effect that specimen thickness has on the calculated modulus was examined by performing indentation tests on two specially cast materials, (a) Silicon rubber (RVT Silastic) and (b) Polyurethane rubber sheets. These sheets varied from 1.0 mm to 10.0 mm in thickness. The compressive modulus of these two materials roughly

Table 2.1 Mean depths of indentation and corresponding creep modulus values for vulcanized rubber sheet of thickness t = 3.0 mm, corrected and uncorrected for the effects of finite thickness. Applied load P = 10.93 Newtons

Radius of Indenter	Mean depth of Indentation	Creep modulus	
		Uncorrected	Corrected
mm	mm	MN/m^2	MN/m^2
0.7939	0.6140	14.35	10.34
1.5875	0.5133	13.27	7.11
2.3780	0.4676	12.47	5.64

corresponded to the highest and lowest modulus values obtained for cartilage from the knee joint.

(a) Silicon rubber

Nine sheets cast from silicon rubber, were bonded using cyanoacrylate cement to a flat steel plate and subjected to 10 indentation tests, each being performed at a different test site. A load of 0.317 kg (3.108 Newtons) was applied through a hemispherical indenter of radius = 1.5875mm. The resulting depths of indentation, measured two seconds after load application, ranged from 0.2 mm to 0.5 mm. These were comparable in depth with indentations subsequently made in the articular cartilage using an indenter of similar radius but with a larger load, 1.115 kg.

Two second creep modulus values were calculated using the formula derived by Waters (1965) (corrected for finite sheet thickness) and directly from Hertzian theory (not corrected for finite sheet thickness) using the mean depths of indentation made in each sheet, Table 2.2. These values, which are plotted against sheet thickness in Figure 2.1, demonstrate the marked effect of correction for thickness. The uncorrected modulus values decreased sharply with increased thickness, from a maximum of 9.5 MN/m^2 (t = 1.0 mm) to an asymptotic value of approximately 3.45 MN/m^2 ($t \geq 10.0$ mm). The effective modulus values for the thinner sheets were exaggerated because of the proximity of the much stiffer underlying steel plate.

The corrected modulus values, calculated using the formula of Waters (1965), increased gradually with increased specimen thickness, from 2.11 MN/m^2 (t = 1.0 mm) to the same asymptotic value of

Table 2.2 Mean depths of indentation and corresponding creep modulus values, corrected and uncorrected for finite thickness of the silicon rubber sheets. Radius of indenter = 1.5875 mm, applied load P = 3.108 Newtons.

Sheet Thickness	Mean depth of Indentation	Creep Modulus	
		Uncorrected	Corrected
mm	mm	MN/m^2	MN/m^2
1.0	0.2808	9.325	2.119
1.5	0.3397	7.008	2.285
2.0	0.3899	5.699	2.346
2.5	0.4298	4.924	2.346
3.0	0.4538	4.538	2.535
4.0	0.4846	4.113	2.781
5.0	0.5119	3.788	2.888
7.5	0.5257	3.640	3.268
10.0	0.5423	3.474	3.320

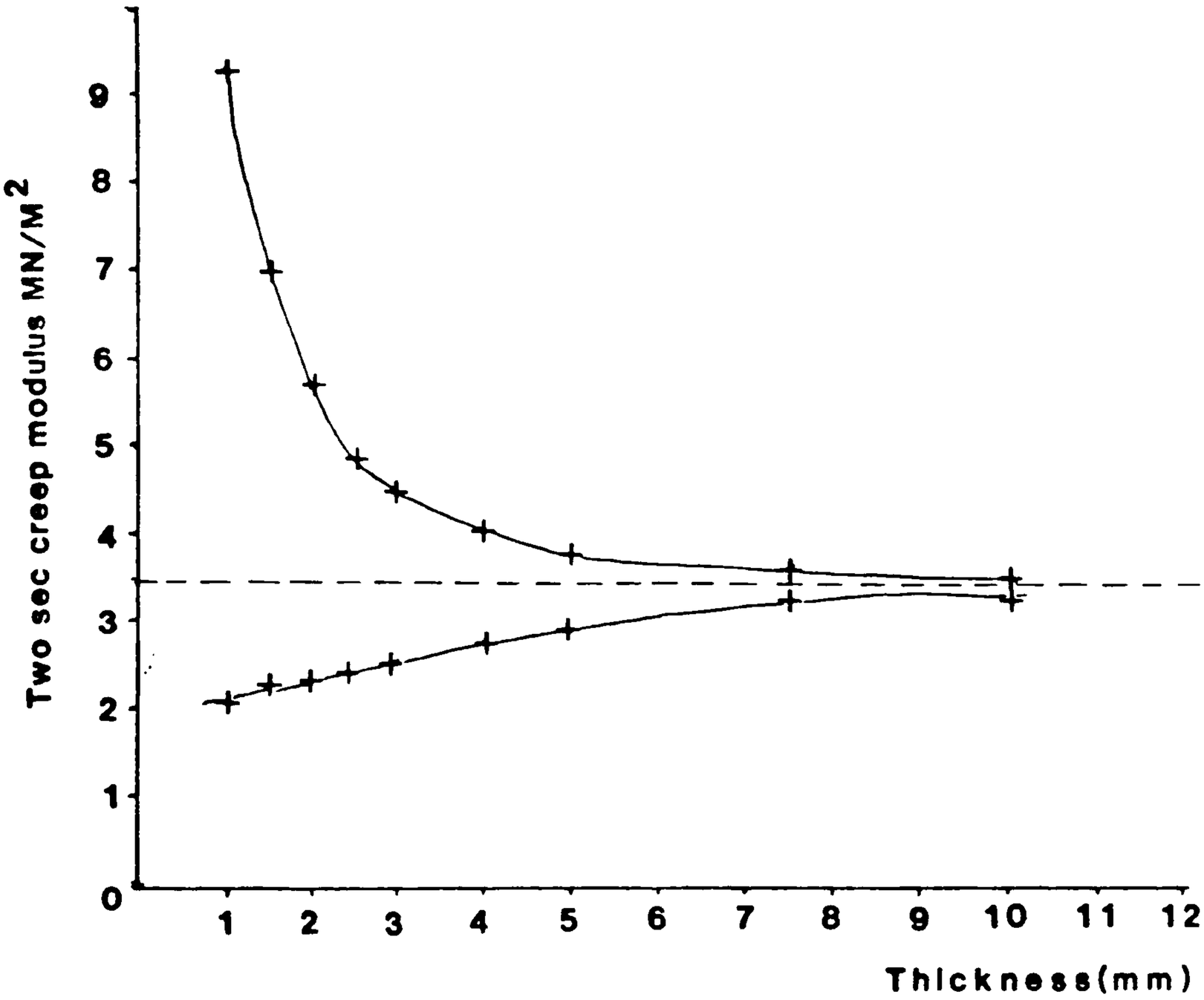


Figure 2.1 Creep modulus values, calculated with and without correction for finite thickness from indentations made in sheets of silicon rubber, plotted as a function of sheet thickness.

3.45 MN/m^2 ($t \geq 10.0$ mm). The modulus values, calculated from the mean depth of indentations made in sheets of 1 mm and 3 mm in thickness, which is typical of the thickness of cartilage in the knee and ankle joint, had a mean value of 2.326 MN/m^2 (s.d. = ±0.133). Expressed in terms of a coeffient of variation, this was equivalent to approximately 5.7%.

As can be seen from Figure 2.1, correction for finite thickness resulted in larger underestimates of the compressive stiffness for the thinner sheets than for the thicker sheets. These variations associated with specimen thickness were however much smaller than corresponding variations in modulus values calculated directly from Hertzian theory and hence not subject to finite thickness correction. A mean value of 6.29 (s.d = ±1.73) was obtained for these uncorrected modulus values.

It was also interesting to note that a value of 3.07 MN/m^2 was obtained from a large cylindrical sample of the silicon rubber, tested in direct compression. The 12% difference between this value and the asymptotic value of 3.45 MN/m^2 obtained from the indentation tests could be attributed to the differences in the nature of the two tests. Nevertheless, these results indicate that calculated modulus values, whether corrected or uncorrected for finite thickness do resemble the actual properties of the material, especially when the effects of finite thickness are less significant with specimen of larger thickness.

(b) Polyurethane rubber

Indentation tests were performed on six sheets of polyurethane rubber, cast to thicknesses varying from 1.0 mm to 5.0 mm. Each

sheet was bonded to a flat steel plate using cyanoacrylic cement and subjected to four separate indentation tests, using an indenter of radius 1.5875 mm and a load of 1.115 kg (10.92 Newtons). Depths of indentations ranged from 0.3 mm to 0.5 mm. The mean depths of indentation were again used to calculate modulus values using Hertzian theory and the formula derived by Waters (1965), Table 2.3. These modulus values are also plotted against layer thickness in Figure 2.2.

Variations in both sets of modulus values obtained from the polyurethane, were similar to those obtained from the silicon rubber. Modulus values calculated without correction for finite thickness decreased sharply with increased thickness to an asymptotic value of approximately 15.0 MN/m^2. Modulus values, calculated with correction for finite thickness, increased gradually with increased thickness to the same asymptotic value. The mean of modulus values calculated from the mean depths of indentation made in sheets of 1 mm and 3 mm was 13.75 MN/m^2 (s.d. = ±2.24). When expressed in terms of a coefficient of variation, this was equivalent to 16.3%. These large variations were attributed to the non-uniform compressive stiffness of the material, which was caused by the inclusion of air bubbles in the polyurethane during casting. The presence of air bubbles was confirmed by slicing the test samples after each indentation test. Although several attempts to minimize the quantity of air included in the cast material were made, it was not possible to exclude air totally. This problem was compounded by the extremely short curing time of the material.

Table 2.3 Mean depths of indentation and corresponding creep modulus values, corrected and uncorrected for finite thickness of polyurethane sheets. Radius of indenter r = 1.5875 mm, applied load P = 10.92 Newtons.

Sheet Thickness	Mean depth of Indentation	Creep Modulus	
		Uncorrected	Corrected
mm	mm	MN/m^2	MN/m^2
1.0	0.3094	47.07	10.71
1.5	0.3654	34.19	12.64
2.0	0.4038	29.17	13.97
2.5	0.4232	25.15	13.72
3.0	0.4709	21.32	17.53
5.0	0.5088	15.58	15.08

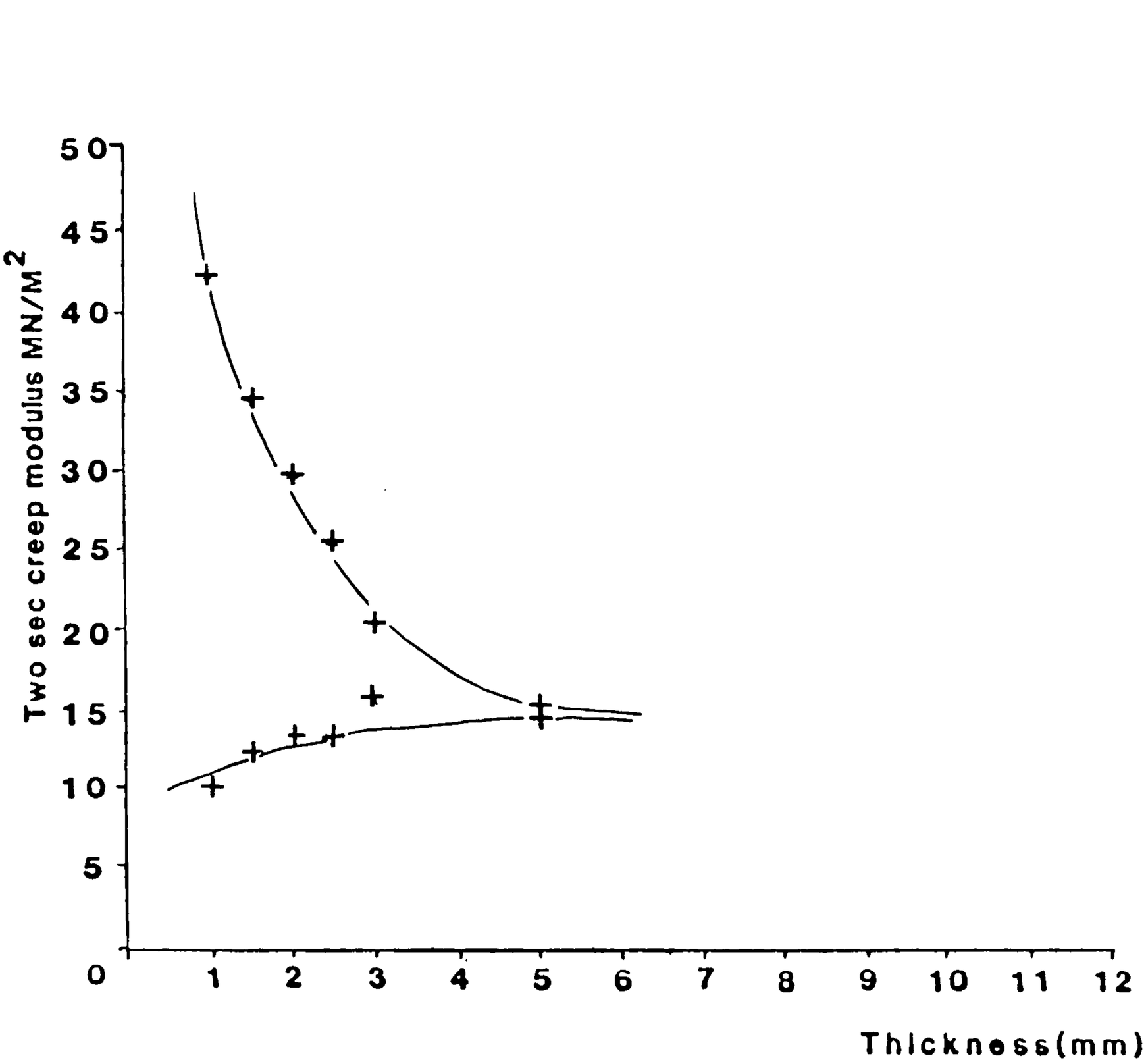


Figure 2.2 Creep modulus values, calculated with and without correction for finite thickness from indentations made in sheets of polyurethane rubber, plotted as a function of sheet thickness.

2.4.3 Load

Two separate series of indentation tests were carried out on six sheets of polyurethane rubber, using a hemispherical ended indenter of radius 1.5875 mm, but with different loads, 1.114 kg (10.92 Newtons) and 0.659 kg (6.732 Newtons). Creep modulus values calculated, with and without correction for finite thickness, from indentations made with the heavier of the two loads are given in Table 2.3. Similarly, modulus values calculated from indentations made with the smaller load are given in Table 2.4. All these data are plotted against sheet thickness in Figure 2.3. Modulus values, calculated without correction for finite thickness and which were obtained from indentation tests using the smaller of the two loads, were significantly larger ($p < 0.015$) than corresponding modulus values calculated from indentations made using the heavier load. The mean difference between corresponding modulus values was 2.54 MN/m^2. There was however no significant difference between the two sets of corrected modulus values.

2.5 Improving the correction for thickness

Even when the finite thickness is taken into account, estimated modulus values are still dependent on the thickness of the specimen. They are however significantly less dependent than when finite thickness effects are ignored. Two attempts were made to improve correction for finite thickness so that calculated modulus values were less dependent on specimen thickness. First, the constant governing the exponential form of the correction factor which appears in the formula derived by Waters (1965) was re-calculated on the basis of the data obtained from the indentation tests carried out on the silicon rubber samples. Secondly, a further empirical correction

Table 2.4 Mean depths of indentation and corresponding creep modulus values, corrected and uncorrected for finite thickness of polyurethane sheets. Radius of indenter r = 1.5875 mm, applied load P = 6.732 Newtons.

Sheet Thickness	Mean depth of Indentation	Creep Modulus	
		Uncorrected	Corrected
mm	mm	MN/m^2	MN/m^2
1.0	0.1979	35.11	9.93
1.5	0.2132	31.40	13.10
2.0	0.2330	27.49	14.36
2.5	0.2467	25.23	15.42
3.0	0.2958	19.21	16.53
5.0	0.3586	14.39	14.11

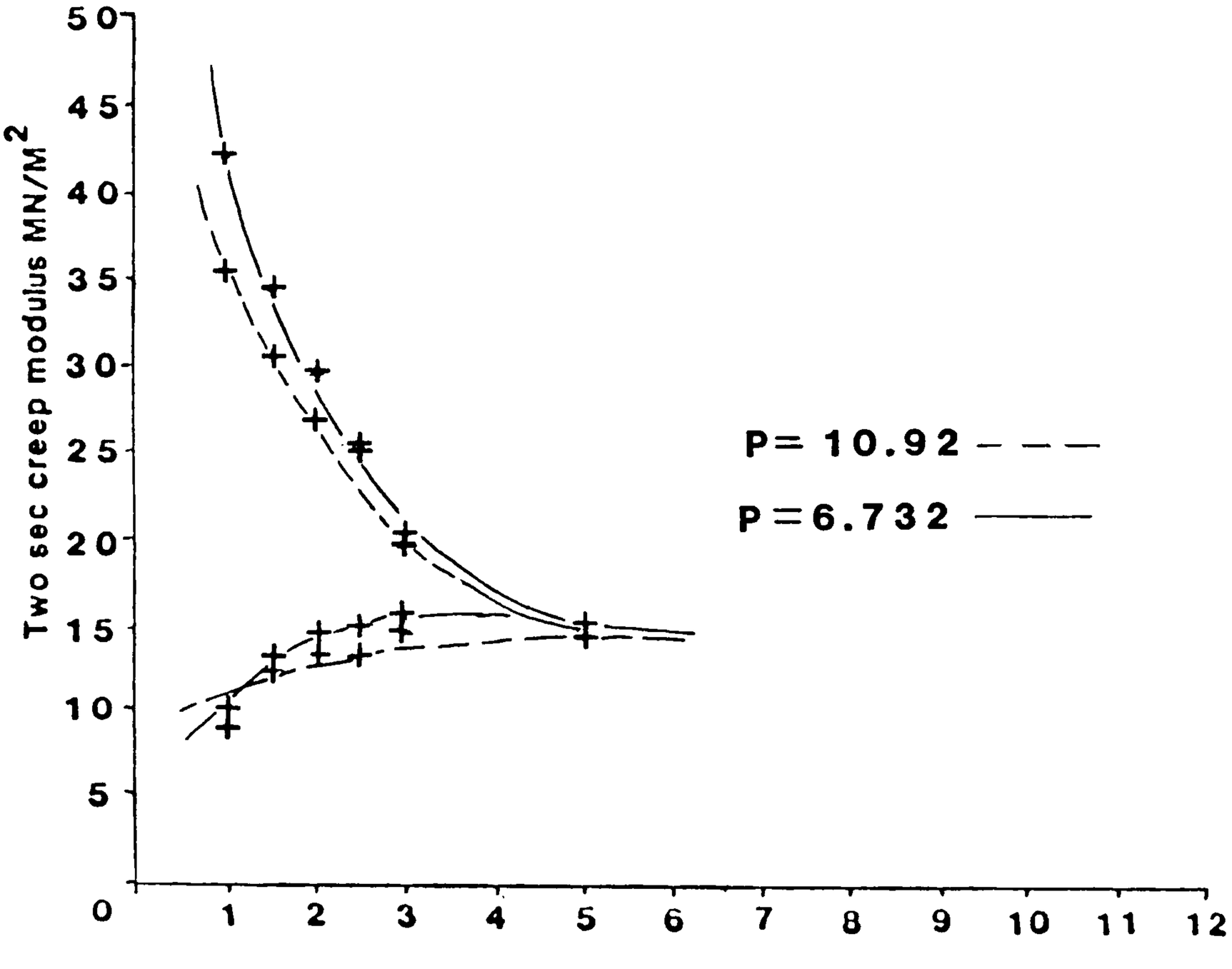


Figure 2.3 Creep modulus values, calculated with and without correction for finite thickness from indentations made in sheets of Polyurethane rubber using an indenter of radius 1.5875 and loads of 10.92 and 6.32 Newtons, plotted as a function of sheet thickness.

factor, in addition to that already incorporated in Waters's formula was derived.

2.5.1 Recalculation of the correction factor

The exponential form of the function used in Waters's formula for correcting modulus values for finite thickness is governed by the constant A in equation 2.4.

$$E = \frac{9P}{16\, r^{1/2}} \left[\frac{1 - e^{-A(t/a)}}{d} \right] 3/2 \text{ --- } (2.4)$$

Waters obtained a value for A by plotting his data in the form of $\log_e (1 - f(t/a))$ against t/a, where the two dimensionless groups, f(t/a) and t/a, are given by:-

$$f(t/a) = \frac{d}{K_1} \sqrt[3]{\frac{E^2\, r}{P^2}} \text{ ---------- } (2.5)$$

and

$$t/a = \frac{t}{r\, d} \text{ ---------- } (2.6)$$

Figure 2.4 is a reproduction of Waters's original graph of f(t/a) versus t/a, with the data obtained from the silicon rubber superimposed. The new data, which all lie to the left of the curve fitted by Waters to his original data, suggested that the value of 0.42, estimated by Waters for A, was insufficient for full thickness correction. A new value for A was obtained by plotting the data obtained from the silicon rubber in the form $\log_e (1 - f(t/a))$ versus t/a, Figure 2.5, and estimating the gradient of the most suitable straight line fitted to the data. A value of 0.449 was obtained.

Modulus values for the silicon rubber sheets were re-calculated using A = 0.449 and have been plotted against t, the sheet thickness in

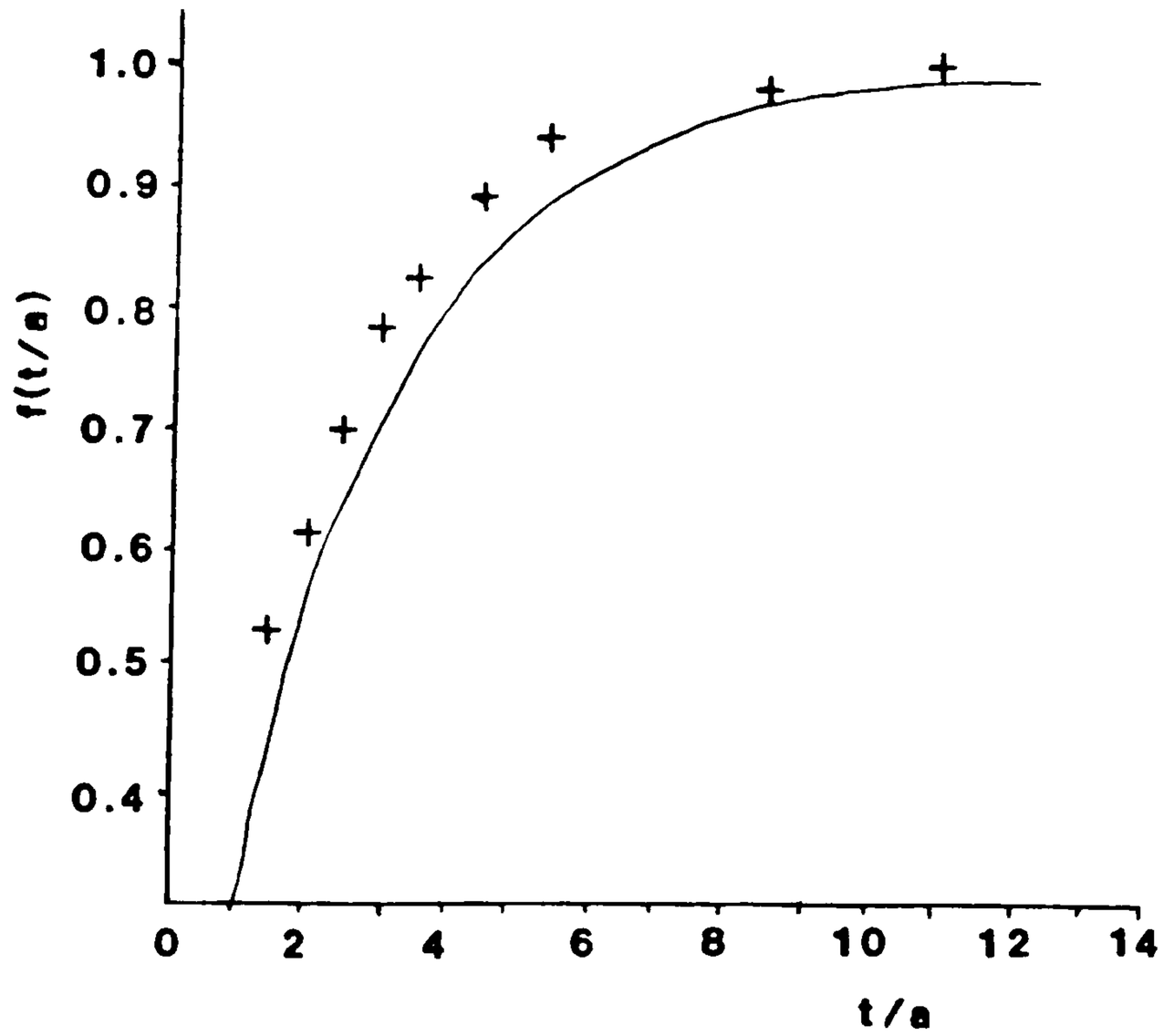


Figure 2.4 The function f(t/a). The curve represents that fitted by Waters to his original data. The data points, which all lie to the left of Waters's curve, are those obtained from the series of indentation tests carried out on the silicon rubber sheets.

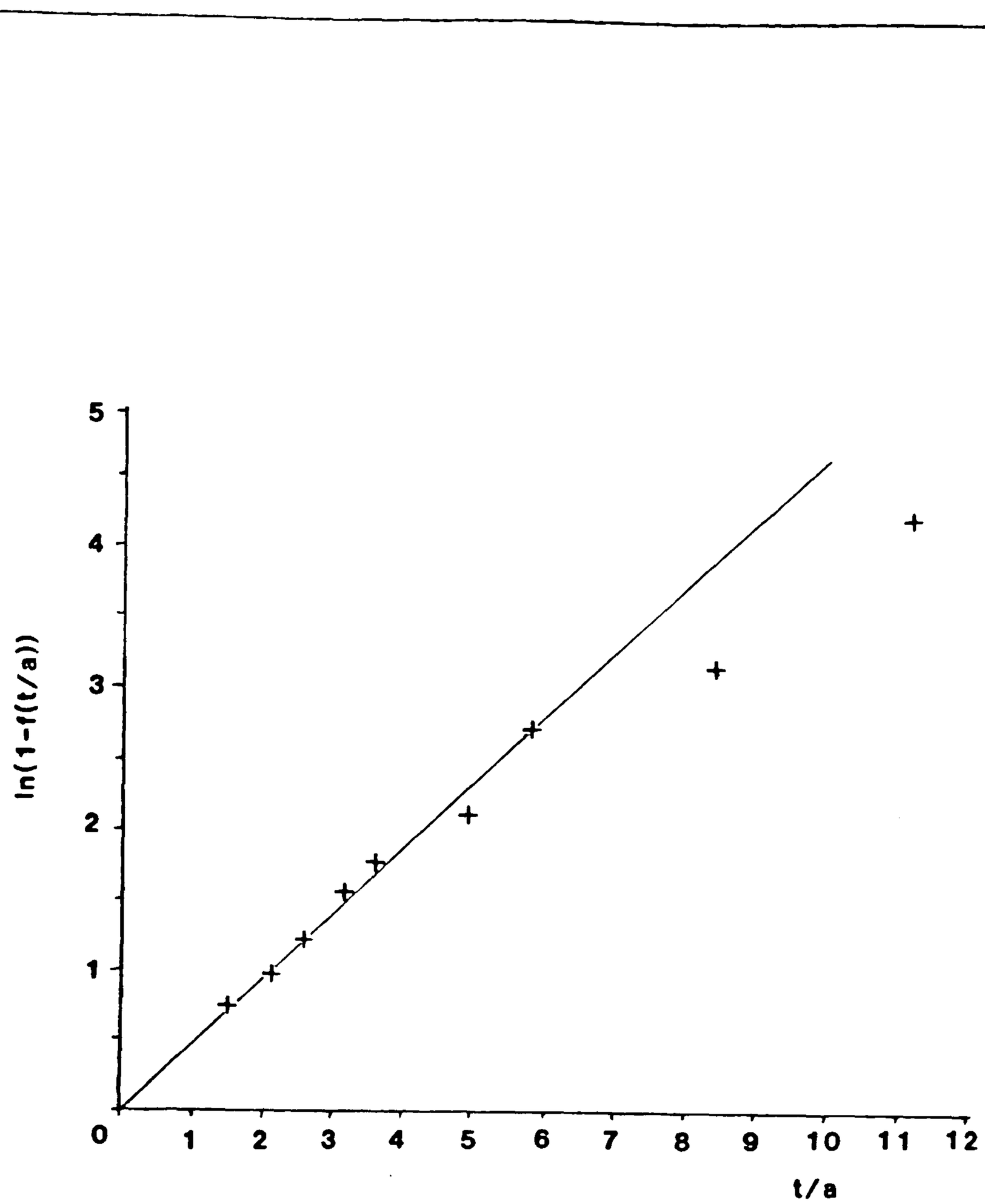


Figure 2.5 The graph of ln(1-f(t/a)) verses t/a. A value of 0.449 was estimated for the constant A in equation 2.3 from the gradient of the line which was the best fit to the data.

Figure 2.6. Comparisons of the two sets of data revealed no significant improvement in the variation of the calculated creep moduls values with thickness. The mean value of modulii obtained from sheets of thickness 1 mm to 3 mm was 2.49 MN/m^2 (s.d = ±0.14), thw corresponding coefficient of variation was 5.6%. Failure to obtain any significantly improvement in the correction of the modulus values, highlighted one limitation of this empirical approach for correcting modulus valves for thickness correction.

2.5.2 An additional empirical correction for finite thickness

Modulus values calculated from the indentations made in the silicon rubber sheets were used as the basis for an attempt to correct further creep modulus values calculated from depths of indentation for finite thickness. Between the thicknesses of 1 mm and 3 mm, the graph of the corrected creep modulus against thickness was essentially a straight line, Figure 2.7. The difference between E_a, the asymptotic value of 3.45 MN/m^2 and E_2, the calculated two second creep modulus value, was used to obtained a further correction function f'(t) given by :-

$$E'(t) = E_a(t) - E_2(t) \quad \text{---------- (2.7)}$$

Using the data obtained from the silicon rubber, a further expression for the function f'(t) was obtained :-

$$f'(t) = 0.441E_a\ (1 - t/t_x) \quad \text{--------- (2.8)}$$

where t_x is the time at which $E_2 = E_a$, see Figure 2.7.

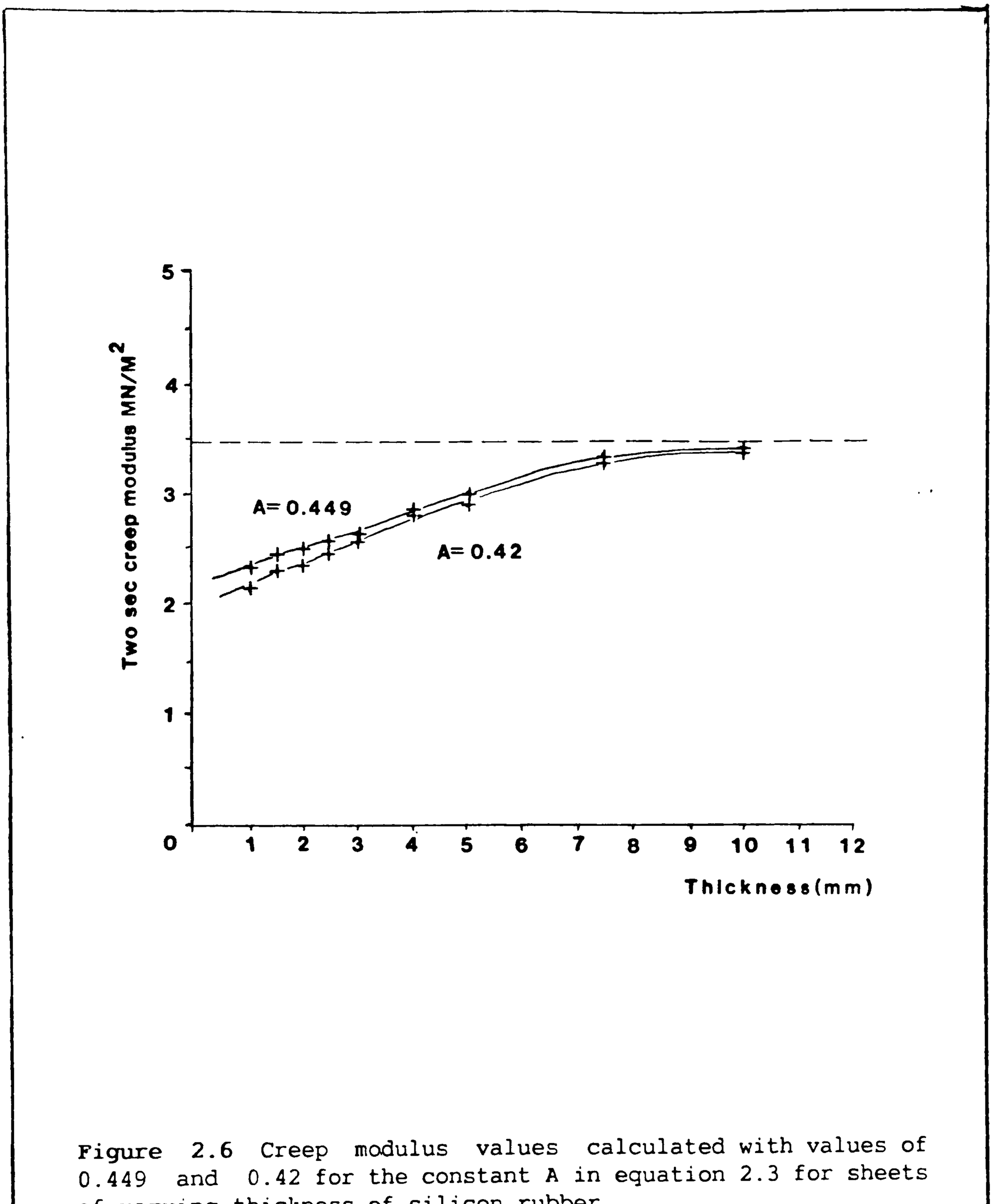


Figure 2.6 Creep modulus values calculated with values of 0.449 and 0.42 for the constant A in equation 2.3 for sheets of varying thickness of silicon rubber.

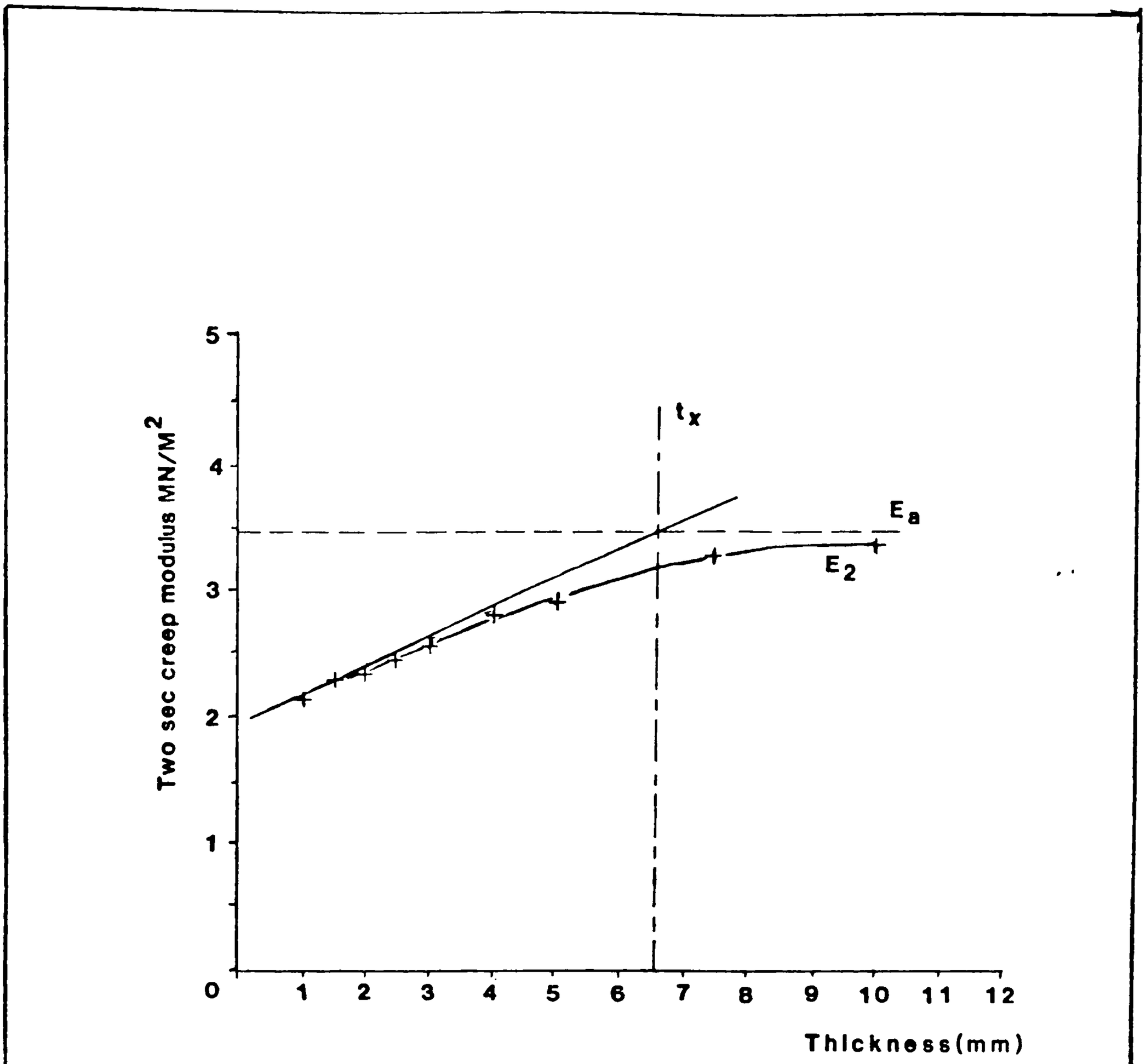


Figure 2.7 The graph of the two second creep modulus values plotted against the thickness of the silicon rubber sheets, indictaing how an additional correction function for finite sheet thickness was determined from the differences between the calculated modulus value E_2, and the asymptotic value E_a at each thickness.

As E_a is essentially equivalent to E', the further corrected creep modulus value, combining the equations 2.7 and 2.8 gives:-

$$E' = \frac{E_2}{0.559 + 0.06787t} \quad \text{---------- (2.9)}$$

As equation 2.9 is based upon the percentage change of E_2 with respect to E_a over a range of thicknesses, it may be applied to any material, irrespective of its stiffness, assuming that the percentage variation of E_2 with thickness, is similar to that of silicon rubber, ie the percentage variation of E_2 with thickness is independent of the stiffness of the material.

In reality, however, the effects of finite specimen thickness on the calculation of modulus values does depend on the stiffness of the material in question. The percentage variation of E_2 will decrease as the stiffness of the test specimen approaches that of the underlying material. It follows that if the thin layered material has the same stiffness as the underlying material, the effects on the measured modulus value will be zero.

As the stiffness of articular cartilage ranges from 3.0 MN/m^2 to 15.0 MN/m^2, which is soft in comparison with the stiffness of the underlying subchondral bone, it was reasonable to assume that the percentage variation in E_2 would be similar for cartilage of all stiffness. It was therefore appropriate to apply equation 2.8 to articular cartilage for further correction of calculated modulus values for finite thickness.

2.6 Poissons ratio and the calculated modulus value

Inherently assumed, in calculating modulus values using the formula given in equation 2.2, is a single value of 0.5 for the Poissons ratio of articular cartilage.

Experimentally, values ranging from 0.37 to 0.48 have been obtained for Poissons ratio (Kempson et al, 1971a; Hayes and Mockros, 1971; Hoch et al, 1983). As these estimated values depended as much on the experimental technique used to obtain them, as they did on the actual differences in the material properties of the cartilage, it was difficult to assess what was the best value to assume for Poissons ratio. A value of 0.5 was eventually chosen so that the data were comparable with the results of previous surveys of cartilage stiffness (Kempson et al, 1971b). It should be noted that as cartilage is an anisotropic material, a single value for Poissons ratio is insufficient to describe realistically the behaviour of the material, as Poissons ratio will vary from one mutually perpendicular direction to another.

As E is relatively sensitive to changes in Poissons ratio, it was worth examining the actual effect of a change in the value for Poissons ratio on the calculated modulus value. A change of 0.05 in the value for Poissons ratio would result in an approximate change of 4.5% in the calculated modulus value. It was apparent that other potential sources of error have a more significant effect on the calculated modulus values than the assumption of a single value of 0.5 for Poissons ratio.

2.7 Conclusions

Two main criticisms may be levelled at the theoretical basis behind the indentation test, when using it as a method for measuring the compressive stiffness of articular cartilage. First, the theory assumes the cartilage exhibits regular material characteristics, ie homogeneity, isotropy and elasticity. Clearly articular cartilage

does not possess such characteristics. A value of the Poissons ratio has also to be assumed.

The consequence of the anisotropy and non-homogeneity of articular cartilage on how it behaves when indented must also be considered. Variations of the mechanical properties of the various strata will alter the stress distribution underneath the indenter, producing greater variation in calculated modulus values. Quantifying these effects is extremely difficult and whilst being pertinent is not within the remit of this thesis. Hori and Mockros(1976) attempted to address this problem. Using indenters of different radii and varying the applied loads they soought to establish the effects of anisotropy and cartilage non-homogeneity on calculated shear modulus values. Although they reported variations of up to 50% this was rather misleading as they failed to establish the possible variations associated with varying the indenter radius and varying the applied load. As it has been shown in this study, thses variations are considerable. The effects of anisotropy and non-homogeneity are likely to be considerably smaller than the reported 50%.

The second criticism concerns the effect that finite specimen thickness has on the distribution of stress within the cartilage and hence the deformation of cartilage when loaded. In particular, empirical and theoretical attempts to account for such effects are suggested as being unsatisfactory. Coupled with the problem of finite thickness, is the questionable dependency of the calculated modulus values on experimental parameters such as the radius of the indenter and the size of the applied load.

With regard to the latter objection, there can be little doubt that calculated modulus values depend on the radius of the indenter and the size of the applied load employed. Particularly large variations were observed in calculated modulus values obtained from tests employing a number of indenters of different radii, section 2.4.1. As a direct consequence of this, the use of the indentation test to obtain precise values for the compressive stiffness of cartilage has to be seriously questioned.

Correction for thickness effects, using the formula of Waters, resulted in underestimates of the compressive stiffness, the magnitude of which remained a function of the thickness of the specimen. These variations were however, significantly less than corresponding variations in uncorrected modulus values calculated directly from Hertzian theory.

Attempts at further correction for finite thickness, as suggested in section 2.5.2, reduced variations in the calculated modulus values with thickness. The value of the correction factor for each specimen thickness decreased with increased material stiffness. Such an empirically derived correction factor was therefore not entirely satisfactory, although for a typical range of cartilage stiffness the assumption that variations in the correction factor with specimen stiffness were negligible was justifiable.

In the less rigorous confines of a comparative study, providing the radius of indenter and the applied load are not varied, modulus values calculated from indentation tests are still of great value. They can not be regarded as accurate values of the intrinsic stiffness of articular cartilage, but they constitute an extremely useful comparative measure of cartilage stiffness.

CHAPTER 3

EXPERIMENTAL APPARATUS

EXPERIMENTAL APPARATUS

Where large numbers of repeated tests are to be performed, as in surveying the compressive stiffness of articular cartilage using the indentation test, it is important that the experimental procedures involved are both quick and uncomplicated to perform. Techniques must be such that accurate and repeatable measurements can be made without being influenced by operator error.

A machine was developed capable of performing both indentation tests and the measurement of cartilage thickness. Indentations were made in a conventional manner; the cartilage was carefully loaded perpendicularly to the articular surface using a hemispherically ended indenter. Measurements of the cartilage thickness were made by replacing the indenter with a sharp needle and allowing it to pierce the cartilage layer into the subchondral bone. Both measurement techniques relied on a novel method for determining the position of the indenter/needle at specific moments during the actual testing of the cartilage. As a result, accurate measurements of both the depth of indentation and the thickness of cartilage were made quickly and easily with minimal effort.

3.1 The improved measurement techniques.

3.1.1 Indentation measurement.

The measurement of a depth of indentation requires a knowledge of the initial separation between the indenter and the cartilage surface so that it may be excluded from the eventual measurement. All previous techniques used in measuring depths of indentation have required the indenter to be rested on the cartilage surface whilst a static

measurement of the indenter position was made. This increased the time required to make each measurement and adding to the tedium and complexity of the technique. The proposed technique for measuring indentations eliminated the need for such a measurement. Instead, the position of the cartilage surface was determined from simultaneous records of the load applied to the cartilage and of the position of the indenter throughout the actual indentation test. For this purpose both a load and a displacement transducer were incorporated into the design of the machine.

By identifying the instant at which a load was first applied to the cartilage, it was possible to obtain from the corresponding displacement signal the position of the indenter as it touched the cartilage surface. The displacement reading taken at this moment thus provided a datum for measuring the depth of indentation. Figure 3.1 depicts graphically how the load and displacement signals were used to determine the position of the indenter.

After a specified time following this initial contact (in this case two seconds) the displacement reading was again noted. The difference between the indenter's position at this time and its position when surface contact occurred was the depth of indentation.

3.1.2 Cartilage Thickness Measurement.

Similar use was made of load and displacement signals simultaneously recorded during the penetration of the needle through the cartilage layer. The cartilage thickness measurement was obtained from the difference between the position of the needle as it touched the cartilage surface and its position as it reached the subchondral bone. The position of the needle on contact with the surface was

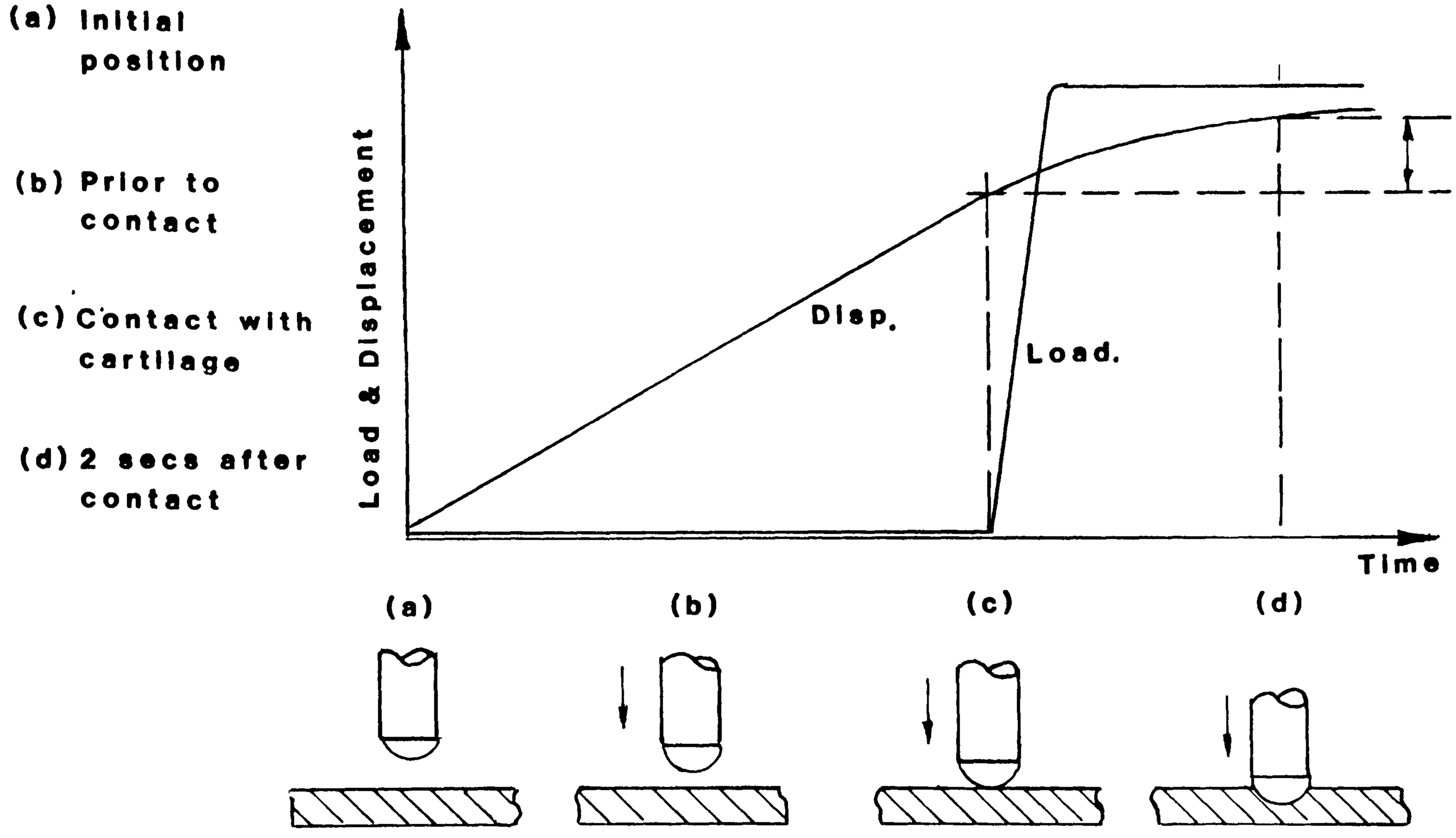


Figure 3.1 A typical record of the load and displacement signals taken during an indentation test showing graphically how measurements of the depth of indentation were obtained.

determined in an identical manner to that of the indenter. The event was denoted by an initial change in the load applied to the cartilage. The displacement reading which corresponded to this initial change in load was the position of the articular surface. The time at which the needle reached the cartilage-bone interface was again determined by identifying a distinct change in the load signal. As the needle penetrated through the cartilage layer, the resistance of the cartilage to its movement gradually increased, resulting in a constant increase in load being recorded.

Contact between the needle and the subchondral bone was marked by a rapid increase in the applied load. This reflected the increased resistance of the bone to the needle's penetration. The position of the needle at this time was obtained from corresponding displacement signals. Figure 3.2 depicts graphically how the two signals were used to obtain measurements of the cartilage thickness.

3.2 Design constraints.

3.2.1 Indentation measurement.

Load application.

Two aspects of load application were considered. First, loads needed to be applied perpendicular to the articular surface. As the geometry of the knee is complex, a sophisticated alignment device which allowed full adjustment of the specimen relative to the indenter was required. Attention was paid to the ease of alignment and to the accuracy with which it could be made.

Secondly, it was important to ensure that the loads applied to the cartilage were identical for each indentation test. With the proposed indentation measurement technique, the indenter was

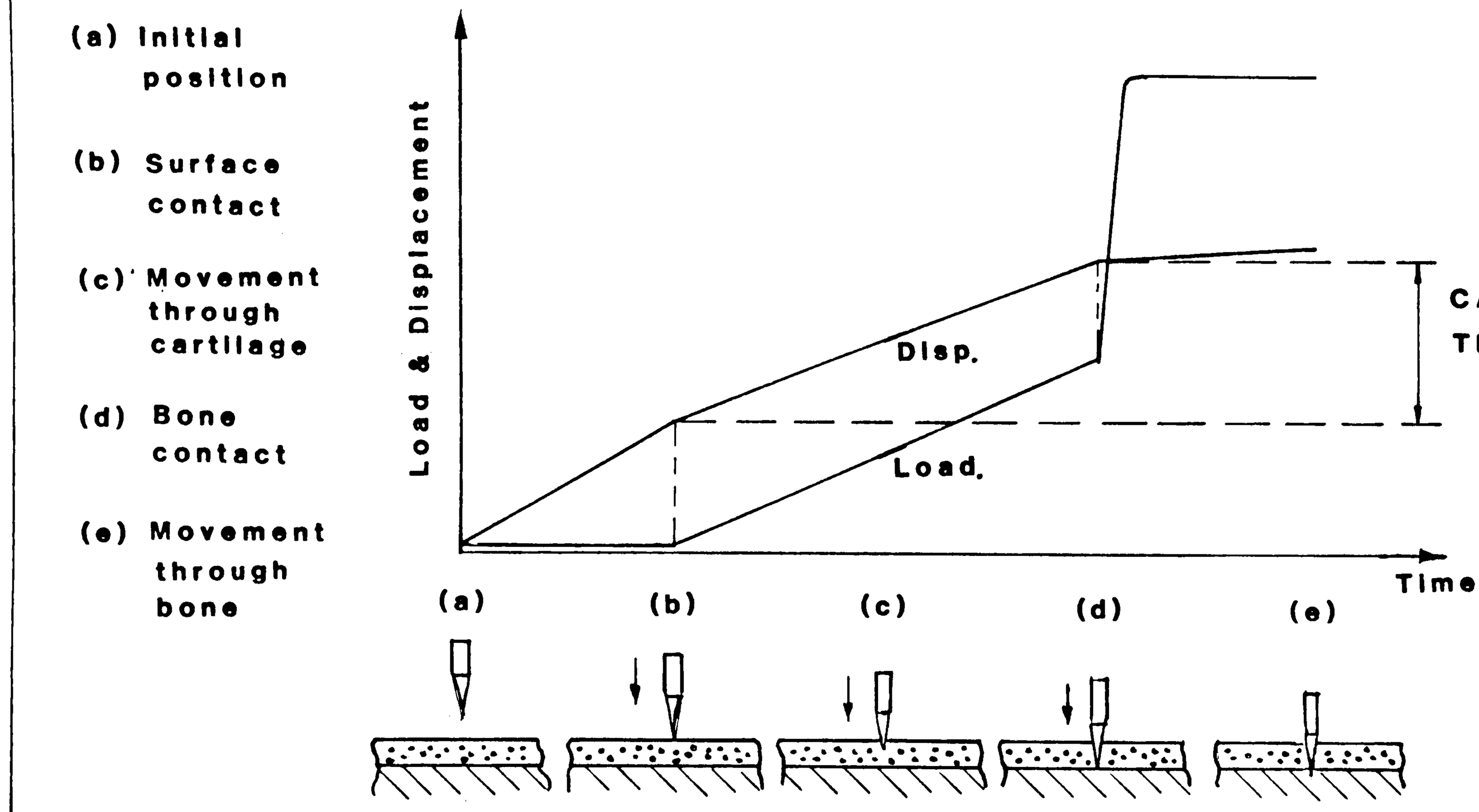


Figure 3.2 A typical record of the load and displacement signals taken during the measurement of the thickness of cartilage showing graphically how thickness measurements were obtained.

initially placed close to, but at an arbitrary distance from, the articular surface. Under the influence of the acceleration due to gravity alone, the velocity of the indenter on reaching the cartilage surface was dependent on the size of this initial separation. As a consequence of the interaction between the inertia of the indenter and the elastic component of the cartilage, transient loads of as much as twice the static load could be applied to the cartilage. Such loads had an undesirable effect on the repeatability of the depths of indentation.

The problem of indenter inertia was solved by including a dashpot in the apparatus to control the velocity of the indenter. By carefully choosing a fluid of suitable viscosity, the system was critically damped, eliminating altogether the possibility of transient loads occurring irrespective of the initial separation between the indenter and specimen.

Measurement requirements.

To measure the depth of indentations using the proposed technique, continuous records of both the load and displacement transducer signals throughout each complete test were required. Accurate recording of the rapid changes in load signal occurring at indenter cartilage contact required a data logging system with a sensitive and high frequency response. Utilizing a computer-based system eliminated the inertial effects associated with mechanical analogue recorders. Careful consideration was also given to the filtering of the load and displacement signals and to the digital sampling rates required so that undesirable signal distortion was avoided.

Environmental effects.

The mechanical properties of articular cartilage can change dramatically as a result of alterations in both the quantity and cationic concentration of the interstitial fluid in articular cartilage (Sokoloff, 1966). Tissue degeneration over prolonged periods following excision is also thought to have significant effects on cartilage properties (Black, 1976). A major criterion in the development of the apparatus and measurement techniques was thus to minimize the time required to perform a complete stiffness survey of a joint and to ensure that changes in dehydration and cationic concentration were avoided.

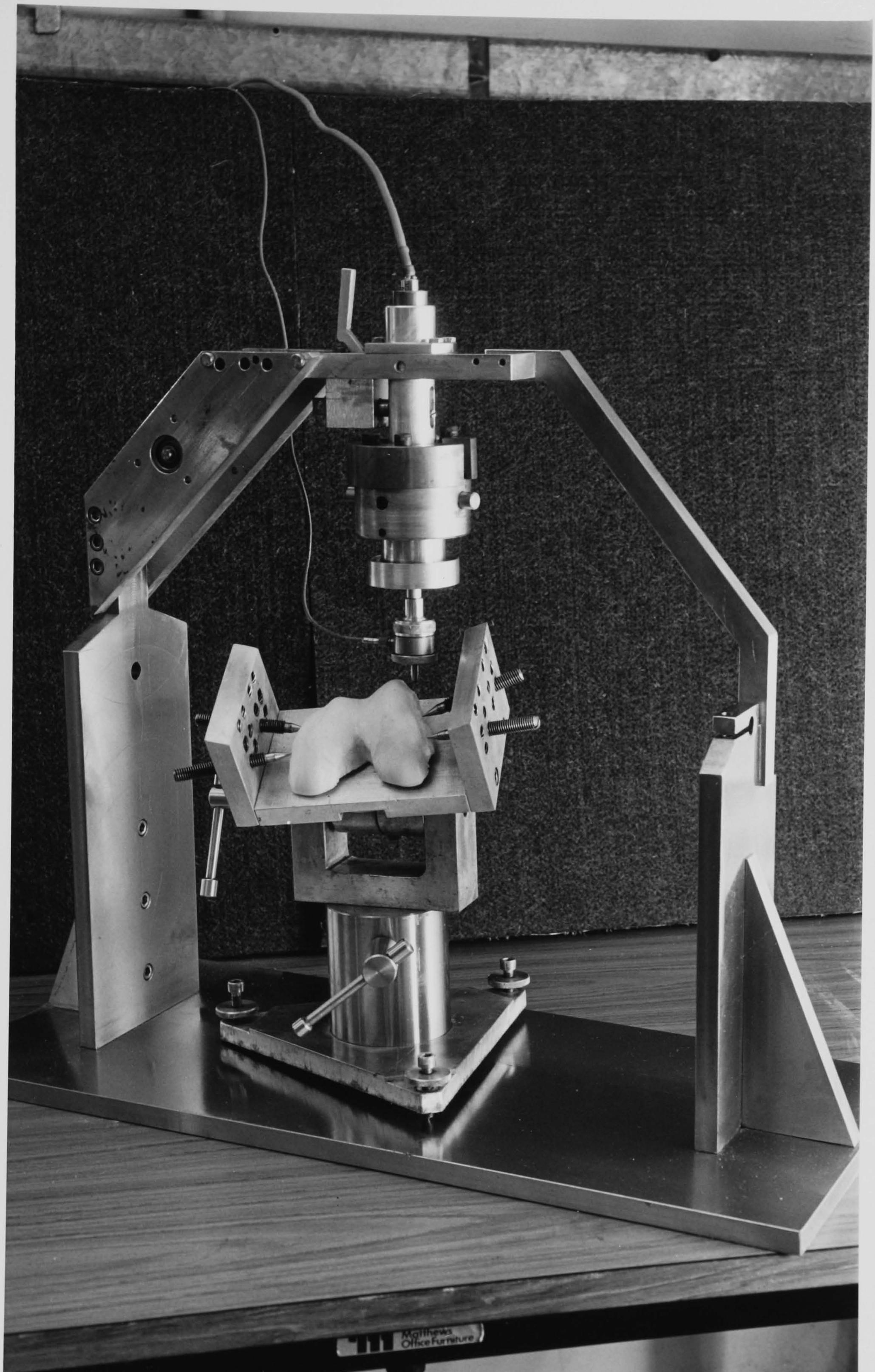
3.2.2 Cartilage thickness measurement.

The technique proposed for measuring the thickness of cartilage relied upon obtaining a load response from which the instances at which the needle encountered the surface and subsequently the bone could be identified. As the form of the load response was dependent on the profile of the needle, the damping effect of the dashpot and the load applied through the needle, the correct choice of these factors was necessary to accentuate the important features of the load response and thus simplify the indentification of the upper and lower bounds of the cartilage layer.

3.3 Apparatus.

The complete indentation/cartilage thickness measurement apparatus, Photgraph 3.1, is illustrated in section in Figure 3.3. Bracketed numbers in the text below refer to the part numbers shown in Figure 3.3.

Photograph 3.1 The indentation rig.


Matthews
Office Furniture

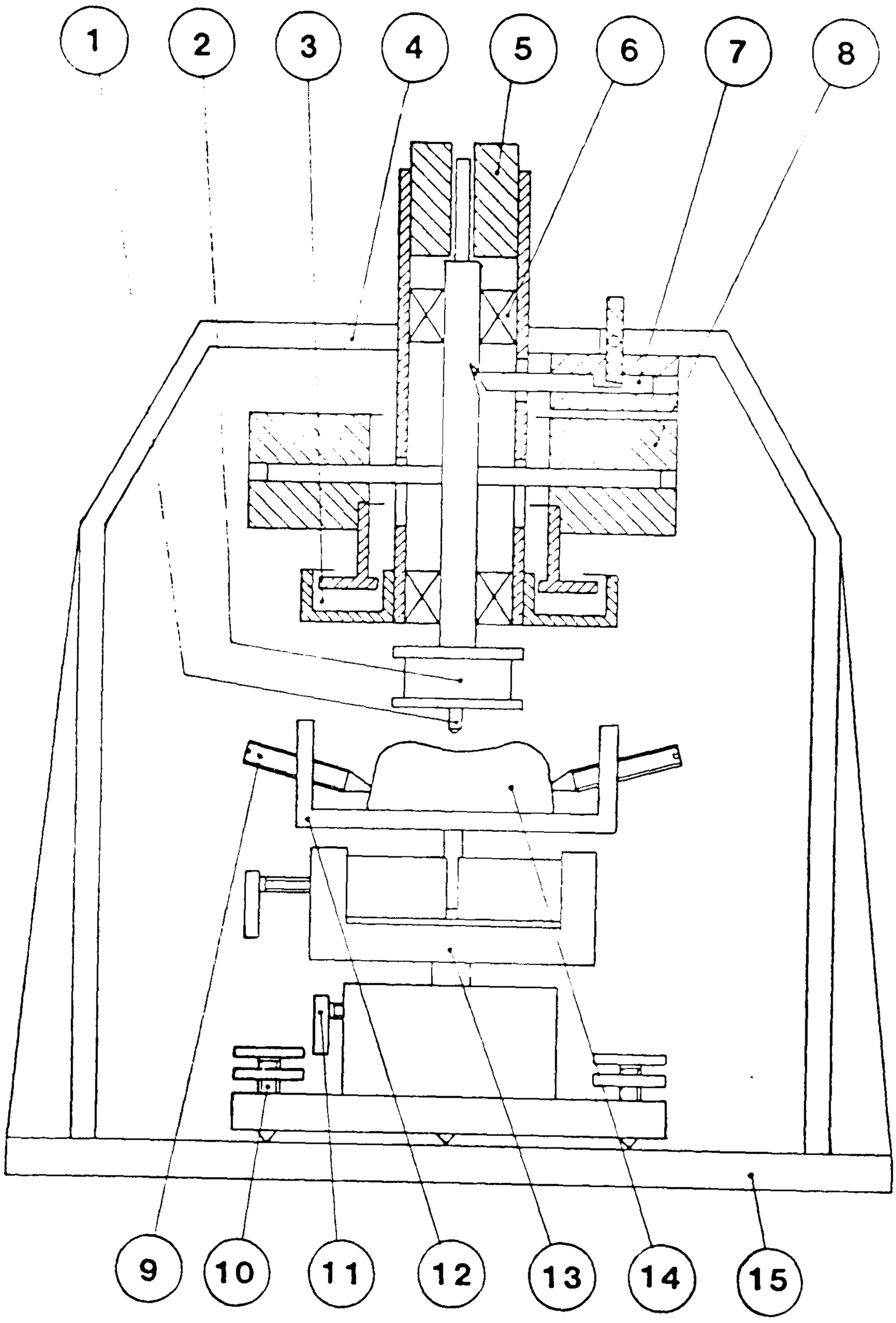


Figure 3.3 A sectioned view of the indentation machine.
(1) Indenter. (2) Load cell. (3) Dashpot. (4) Loading frame.
(5) Displacement transducer. (6) Linear bearing.
(7) Release mechanism. (8) Weight. (9) Securing screw.
(10) Leveling screws. (11) Clamp. (12) Specimen holder.
(13) Alignment device. (14) Specimen. (15) Base plate.

3.3.1 Loading mechanism and frame.

The apparatus consisted of a rigid frame(4) mounted on a flat base plate(15). Fixed to the frame was a small assembly, which comprised a vertically mounted tubular housing containing a shaft restrained by a pair of linear bearings(6) to move axially within the housing. Movement of the shaft could be initially restrained by engaging a manually-operated release mechanism(7) incorporated into the cross member of the frame.

A weight(8) was attached to the central shaft via two metal dowels extending from the shaft through slots cut along the length of the housing. The weight was external to the main housing and equally distributed about its vertical axis. A circular dashpot arm, screwed to the lower edge of the weight, moved freely within an oil-filled cup(3), fixed to the lower end of the main housing.

Attached to the lower end of the shaft, which protruded from the main housing, was a load transducer(2) and directly below this was a replaceable hemispherically ended indenter(1). For thickness measurements, the indenter was unscrewed and exchanged for a needle.

Attached to the top of the shaft was a thin soft iron displacement transducer armature, which was free to move axially within the body of the displacement transducer mounted inside the top of the main housing. Once released, velocity of the loading device (weight, indenter, displacement transducer armature, load transducer and central shaft) remained constant due to the damping effect of the dashpot.

3.3.2 Specimen alignment device.

Each specimen was firmly secured into a specimen holder (12) by advancing sharp-ended screws(9) into the epicondylar bone mass. The screws forced the specimen tightly onto the base of the specimen holder and prevented any movement of the specimen relative to the holder during load application.

The knurled spigot on the base of the specimen holder was located vertically between the two halves of a split cylinder pivoted horizontally between the arms of a U-shaped support(13). The specimen could be orientated relative to the indenter about two perpendicular axes of rotation and gripped securely in position by clamping the two halves of the cylinder together. The U-shaped support was located in the lower part of the alignment device by means of a further spigot which could again be clamped once correct positioning of the specimen in the vertical direction had been achieved.

Three levelling screws(15) situated at the corners of the triangular base of the alignment device allowed further fine adjustments of both the vertical position and angulation of the cartilage surface. Any slack in the threads of the levelling screws was taken up by tightening down three locking nuts prior to carrying out each test.

3.3.3 Measurement instrumentation.

Displacement transducer.

Movement of the indenter was monitored using a linear variable differential transformer (LVDT) type displacement transducer (RDP 05/100). The LVDT consisted of two identical windings on a common former. The primary winding, excited by an AC voltage supply, induced

a voltage in the secondary winding whose phase and amplitude were dependent on the position of an armature situated between the two windings. Induced AC voltages were demodulated and filtered through a low-pass filter with a cut-off frequency of 1330Hz using a E.M.I 1503 signal processing module. A smooth DC voltage, proportional to the armature displacement and of an appropriate sign according to the direction of the armature displacement was obtained.

Load transducer.

The loads applied to the cartilage were measured using a piezo-electric type load transducer (Kistler type 9011 quartz load washer). The load cell produced an electronic charge proportional in magnitude to the load being applied. The charge signals were then fed through a charge amplifier (Kistler type 5001) which converted the signal into DC voltages proportional to the applied load. This type of load transducer has an exceptionally high frequency response, the output signal being filtered at a frequency of 150KHz.

3.3.4 Data logging system.

The amplified transducer signals were monitored using a 12 bit analogue to digital (A/D) converter (Microlink) connected to a microcomputer (ACT Sirius). Data conversion was controlled by passing information regarding sampling frequencies and durations to the A/D converter from specially written control software run on the microcomputer. Figure 3.4 is a schematic block diagram of the component parts of the data logging system.

3.3.5 Computer software.

All programs written for the microcomputer and used in the

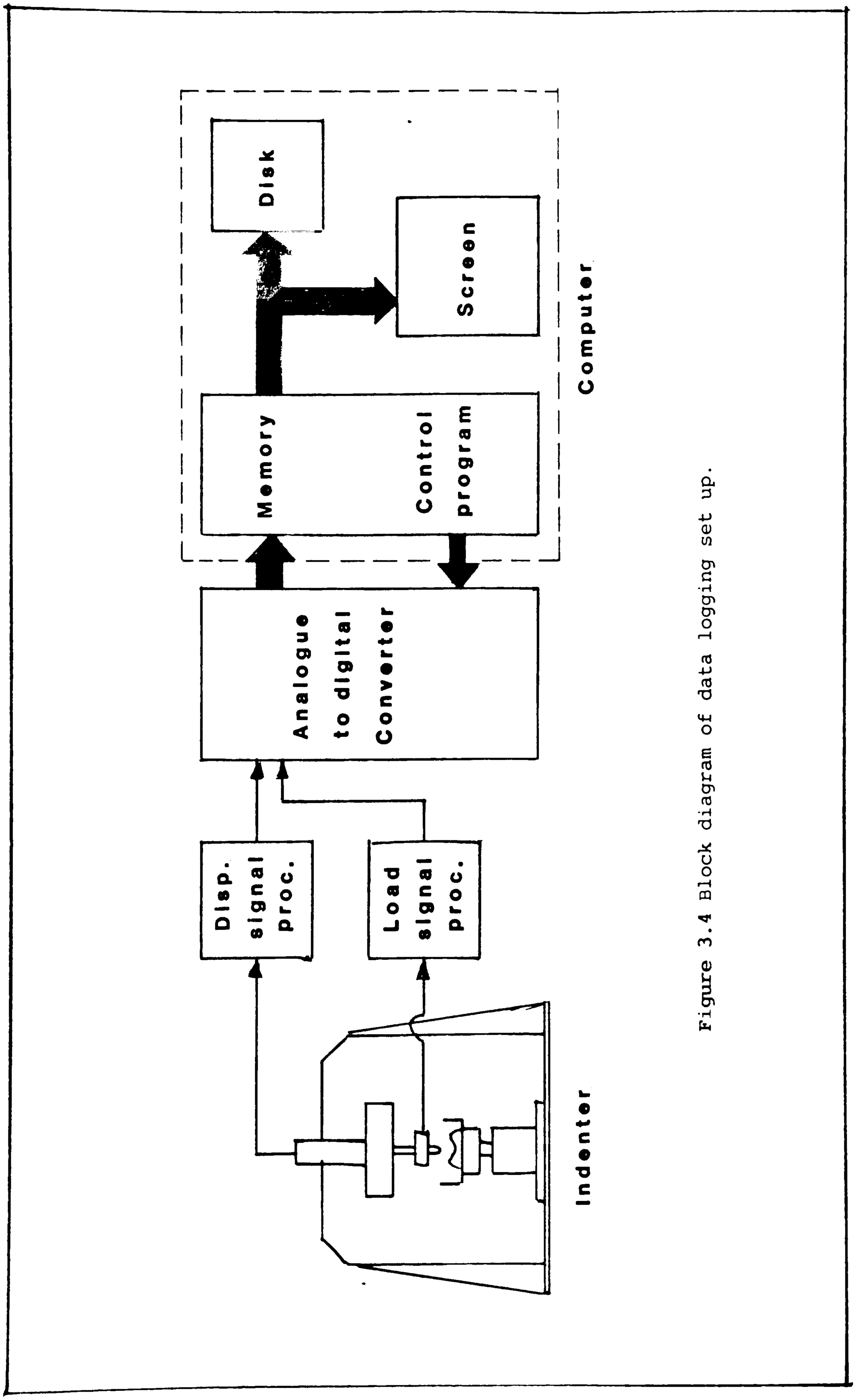


Figure 3.4 Block diagram of data logging set up.

acquisition of measurements required for calculating creep modulus values can be found in Appendix A. The flow chart in Figure 3.5, illustrates the sequence of use of the programs and gives a brief functional description of each.

Calibration.

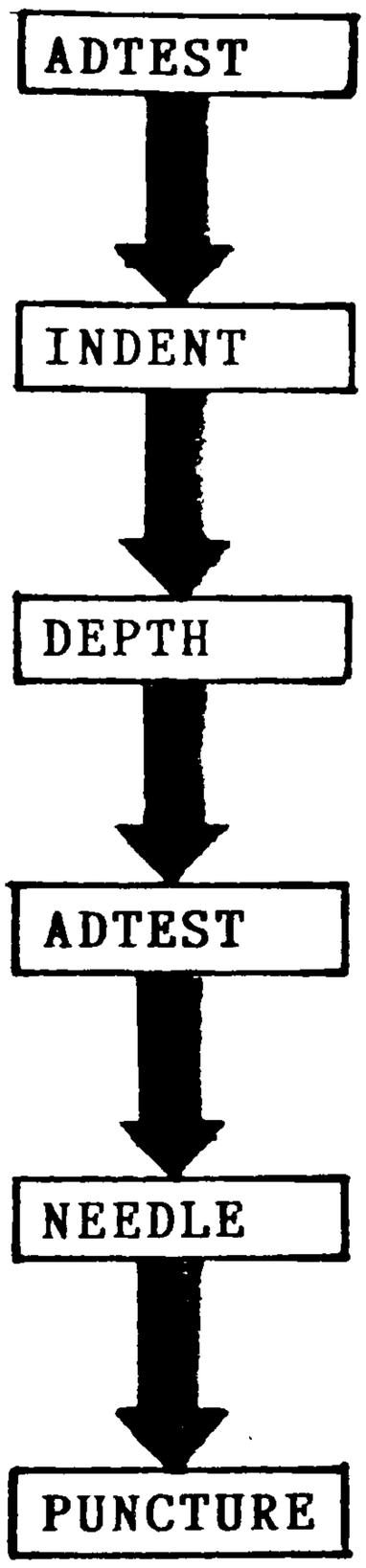
Monitoring of the load and displacement signals during calibration was performed using the program **ADTEST**. Converted digital values of both the transducers signals were fed continuously to the V.D.U. screen of the microcomputer. Output could be halted and restarted by use of predefined keys.

Data logging.

Data logging commenced by passing data regarding the modes of sampling to the A/D converter from a control program. The data logging carried out during indentation tests was controlled by the program **INDENT**. Two modes of sampling were performed. Initially both transducer signals were sampled simultaneously at a frequency of 1KHz for a period of 500 ms. Immediately following this, the two signals were sampled at a frequency of 10Hz for a period of 10secs. For each indentation test, a total of 600 readings were taken from each transducer.

Data-logging during the measurement of cartilage thickness was controlled by the program **NEEDLE**. Both transducer signals were sampled at a frequency of 1KHz for a period of 1.5 secs.

Triggering of the data-logging by both **INDENT** and **NEEDLE** was automatic: succesive readings taken from the displacement transducer at a frequency of 50HZ were continuously compared. A difference of



Calibration of load and dispacement transducers prior to indentation testing.

Data logging during indentation testing.

Determination of indenter/cartilage contact, calculation of depth of indentation at 2 sec.

Calibration of load and displacement transducers prior to thickness measurements.

Data logging during cartilage thickness measurement.

Determination of needle/cartilage contact, determination of bone/cartilage interface, calculation of 2 sec creep modulus.

Figure 3.5 Flow chart indicating the order of use of computer programs during indentation and thickness measurement.

five digits between successive readings was used as the threshold for triggering the data-logging. A change of five digits indicated a definite movement of the indenter and was enough to ensure correct triggering of the data-logging. Delays in the exchange of control from the detection of the trigger to data-logging were minimal, approximately 20 msecs. An initial separation between the indenter and cartilage surface of at least 0.1mm was thus required to ensure that data-logging commenced before contact between indenter and cartilage occurred. The transfer of recorded data between A/D converter and microcomputer was carried out during the actual data sampling.

File Handling.

Data obtained for each indentation test were saved in a separate file on a floppy disk. Each file name was uniquely coded enabling easy data identification. Coding incorporated a specimen identifier, a component identifier and the coordinates of the test site. A similar coding system was employed for the identification of stored data obtained from measurements of the cartilage thickness.

Detection of surface contact.

The time at which contact between the indenter and cartilage occurred was determined automatically using the program **DEPTH**. The program searched through the load data until five successive readings were identified which were all greater than the previous reading. Detection in this manner ensured correct surface contact identification. The displacement reading corresponding to the first of these five load readings was taken as the position of the indenter as contact occurred. The same algorithm was used in the program

PUNCTURE for determining the position of the needle as it made contact with the cartilage surface.

Detection of the cartilage-bone interface.

The change in gradient of the load signal associated with the needle encountering the subchondral bone was determined by plotting the load data on the high resolution (800 x 400) V.D.U. screen of the microcomputer. Orthogonal cross wires, generated by specially written software, were then positioned on the V.D.U. using predefined keys, at the intersection of the two gradients. The displacement reading, which corresponded to load reading indicated by the cross wires, was taken as the position of the needle as it first encountered the underlying subchondral bone.

3.4 Transducer Calibration.

3.4.1 Displacement transducer.

A series of combinations of slip gauges were placed on a horizontal platform and the indenter gently rested on each The position of the indenter was recorded in each case. Steps of 0.01mm were made over the entire working range (2.5mm) of the displacement transducer. The calibration curve is shown in figure 3.6. The gradient of the straight line fitted to the data was 1358 digits/mm. One recorded digit was equivalent to 0.0007mm. The calibration curve also exhibited exceptional linearity over the entire working range of the transducer. This was reflected in the Pearson coefficient, r = 0.99999.

A similar calibration of the displacement transducer prior to using the apparatus for measuring the thickness of cartilage was also

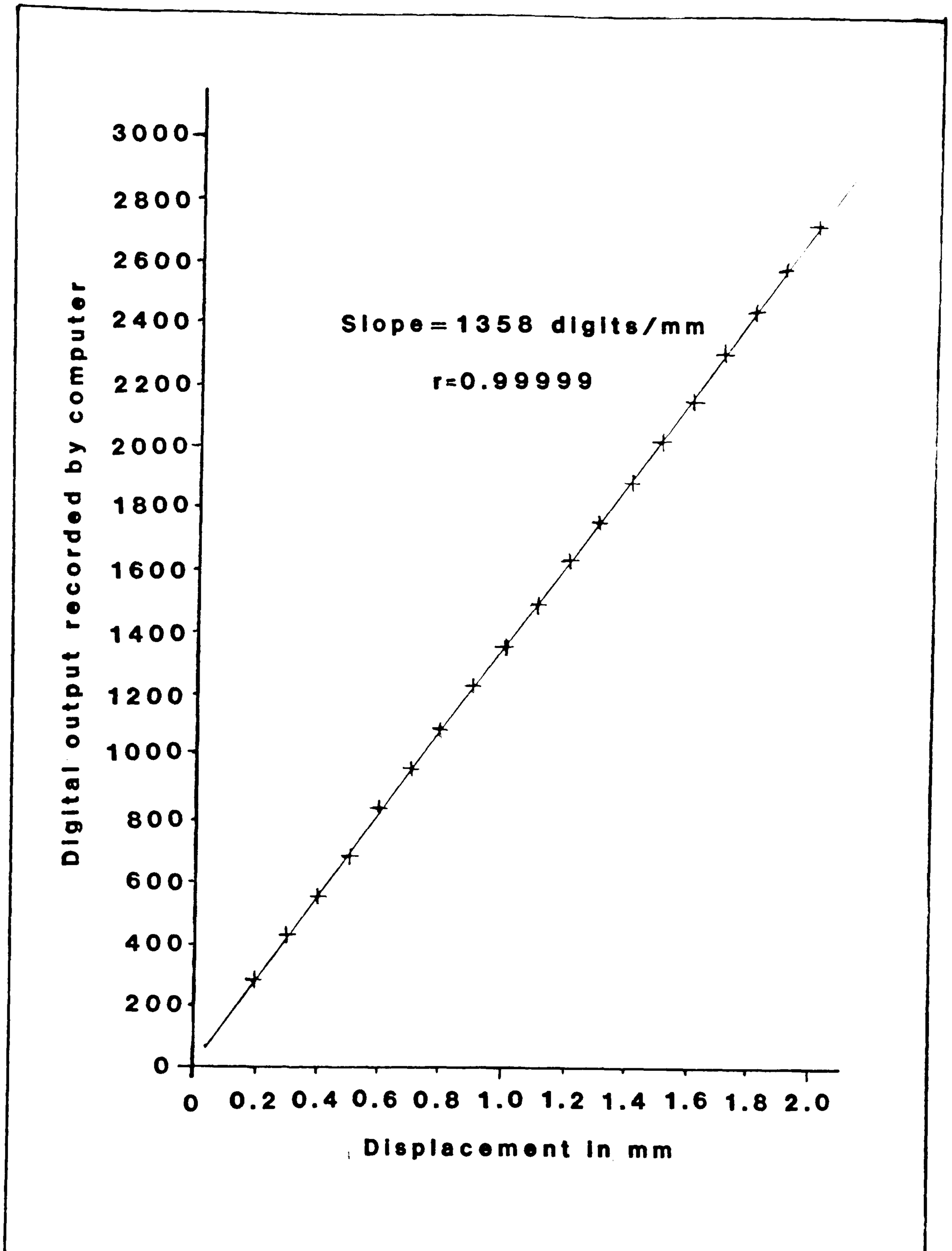


Figure 3.6 Displacement transducer calibration curve.

performed as the sensitivity of the displacement transducer was reduced, to allow an extended working range of 5.0mm. A calibration of 667 digits/mm was obtained. One recorded digit was equivalent to 0.0016mm.

3.4.2 Load transducer.

The loading device was dismantled and all the components which contributed to the effective load applied to the cartilage were weighed. The total mass was 1.115Kg (10.92 Newtons). Following re-assembly, the indenter was allowed to rest on a rigid horizontal platform. Load signals were recorded before and after the release of the indenter. A series of 10 such tests gave a mean difference of 1780 digits (s.d. = $\pm$5.33 digits). Changes in the applied load of 0.006 N could be detected.

3.5 Apparatus Performance.

3.5.1 Accuracy of indentation and thickness measurement techniques.

Specimen alignment.

Assessment of specimen alignment prior to each indentation and measurement of cartilage thickness were made by viewing the indenter and specimen surface from two mutually perpendicular directions. If necessary, further adjustment of the specimen was made followed by a second visual assessment. To investigate the likelihood of misalignment, a plastic model of a femur was secured in the specimen holder and aligned relative to the indenter, using the proposed technique. Once positioned, the indenter was allowed to rest on the surface of the model, trapping a thin steel strip between itself and the surface. The steel strip naturally assumed a position tangential

to the articular surface at the point of contact between the indenter and model, Figure 3.7. Measurements of the angle between the steel strip and the axis of the indenter were made directly from enlargements of photographs taken of the apparatus. Similar measurements were made with the steel strip rotated through 90° about the vertical axis of the indenter. The misalignments obtained are given in Table 3.1. The mean misalignment calculated by taking the absolute values of those shown in Table 3.1 was 1.60° (s.d. = $\pm$ 1.22°). The accuracy to which alignment could be made with the aid of only the eye was comparable with the accuracies obtained using the more involved procedures employed by Hirsch(1944) and Kempson et al(1971a). Visual assessment was regarded as a satisfactory method for attaining perpendicular indenter specimen alignment.

Detection of the cartilage surface.

The reliability of algorithm used in detecting the point of surface contact was assessed by comparing the results of computerized surface detection and those made by manually examining print-outs of the data recorded during the indentation tests. The data from 30 indentations were examined. The differences between the two assessments are summarized in Table 3.2. The majority of the computed assessments (27 out of 30) were either identical or late by one reading. As contact between the indenter and cartilage occurred, data were recorded at a rate of one reading per millisec. As the velocity of the indenter once released was approximately 5mm/sec, such an error in the detection of the contact with the cartilage would correspond to an underestimation of the depth of indentation of 0.005mm. As the depth of indentations were likely to be more than 0.3mm, this is equivalent to an error of approximately 1.5% in the overall indentation

Table 3.1 Summary of misalignment data.

Location of test site on model femur.	Misalignment of indenter.	Misalignment of indenter at 90°
A	2°	1°
B	-1°	-3°
C	-2°	0°
D	1°	2°
E	1°	3°
F	2°	-4°
G	1°	-2°
H	0°	0°
I	1°	-2°
J	4°	-

Table 3.2 A comparison of assessments of the position of the cartilage bone interface made manually and automatically by the computer.

Difference between two assessment in millisecs.	Frequency
+1	1
0	13
-1	14
-2	2

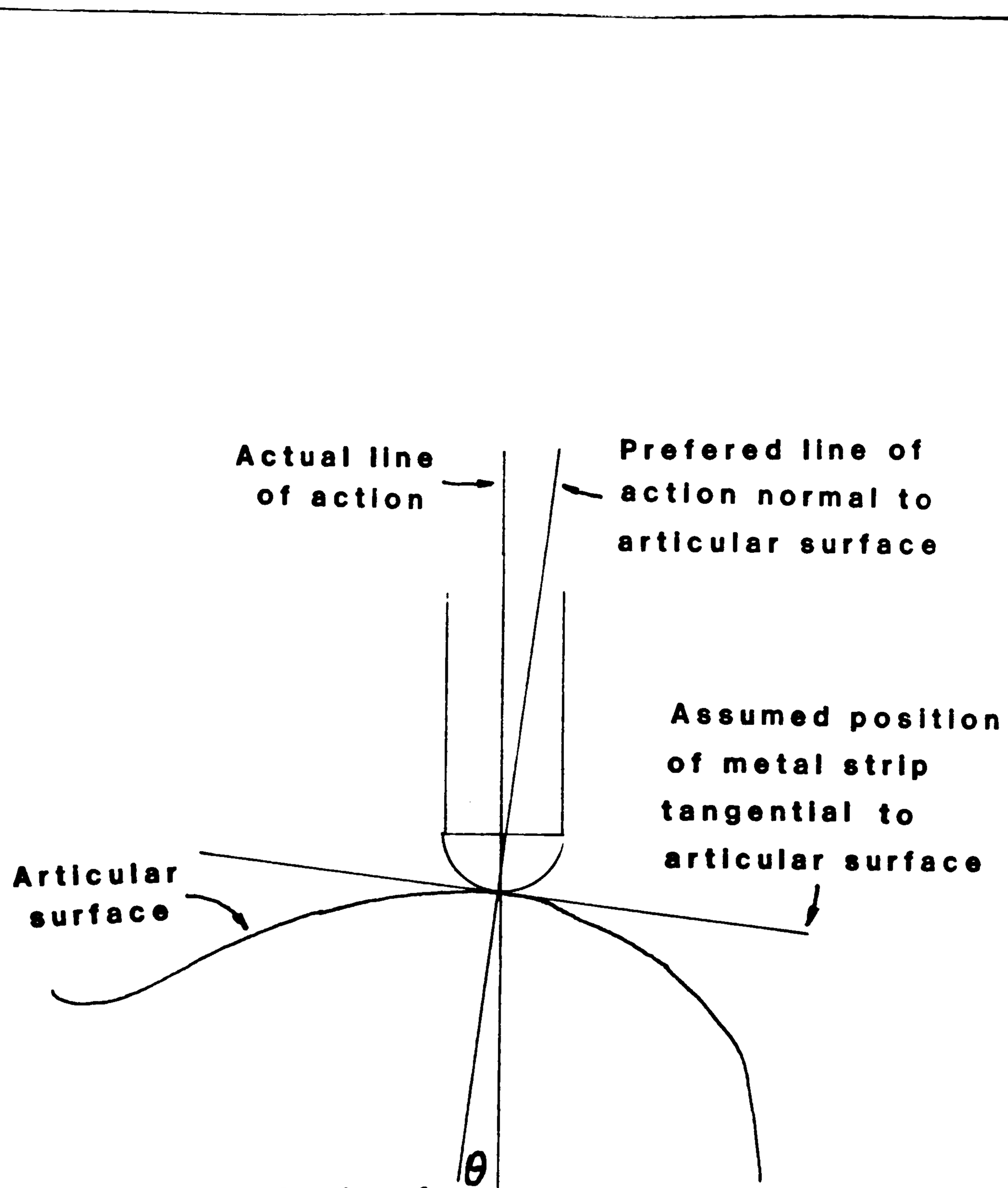


Figure 3.7 The measurement technique used for assessing the errors in aligning the indenter to the articular surface.

measurement. Similar absolute errors could also occur when determining the position of the needle as it touched the cartilage surface. In terms of a percentage of the overall measurement, however, this corresponds to a much smaller error, since the thickness of cartilage is considerably larger than the depths of the indentation measured.

Detection of the bone-cartilage interface.

The load and displacement records from 20 cartilage thickness measurement tests were examined and the point at which the needle reached the subchondral bone was identified with the aid of the computer software described in Section 3.3.3. Each record was assessed twice and the results compared. The maximum difference between the two positions of the needle corresponding to the cartilage bone interface was 0.0073mm. As cartilage is typically 1mm to 5mm thick, such a difference would constitute an error in the measurement of the cartilage thickness of less than 1%.

Signal filtration.

The difference between the cut-off frequencies of the filters used for conditioning the two transducer signals was examined as a possible source of measurement error. The load signal was filtered at an exceptionally high frequency, 150KHz, and thus the time lag between the filtered and unfiltered signals was negligible. The displacement signal however was filtered at a significantly lower frequency, approximately 1330Hz. The time lag between the filtered and unfiltered signals was estimated by considering the signal as a summation of a series of sine waves of varying frequencies. For a sine wave of frequency w, the phase lag Φ introduced by filtering at

cut off frequency of w_c is given by equation 3.1 :-

$$\Phi = \tan^{-1} (w/w_c) \quad \text{----------} \quad (3.1)$$

The highest frequency component of the displacement signal was estimated by Fourier analysis to be in the region of 100Hz, and hence from the above equation, a phase lag of 4.2° could be expected. This corresponded to a time lag in the filtered displacement signal of approximately 0.1 msec. Given a maximum indenter velocity of about 5mm/sec such a lag in the displacement signal could result in errors in the position of the indenter of 0.0005mm. In comparison with other likely errors these were small.

3.5.2 Measurement repeatability.

Indentation.

The repeatability of the complete measurement system was assessed by performing a series of identical indentation tests on a sheet of vulcanized rubber of an approximate stiffness to that of cartilage. The rubber sheet was glued to a flat steel plate to simulate the subchondral bone.

Initially 20 indentation tests were performed. A period of 15 mins was allowed between each test to ensure full recovery of the rubber. Once positioned perpendicular to the indenter, no realignment of the test specimen was made.

A further 20 indentation tests were conducted using the same experimental protocol, with the exception of realignment. Deviations of up to 4° from the perpendicular were deliberately produced. The

results of both tests are summarized in Table 3.3. In both cases, the coefficients of variation for the depths of indentation were small, 1.48% and 2.14%, and well within acceptable experimental limits of 5%. The increased spread in the depths of indentation in the second series was attributed to the effects of specimen realignment.

Thickness.

The complete thickness measurement technique was assessed by conducting a series of 17 separate tests on a sheet of vulcanised rubber of known thickness. The lower cartilage bone interface was simulated by glueing the rubber onto a flat lead plate. The load and displacement responses obtained from this combination compared favourably with those obtained from cartilage on bone. A mean thickness of 1.44mm was obtained (s.d. = ±0.017mm). The low coefficient of variation, 1.20%, indicated good measurement repeatability. The mean thickness value obtained was also within 3% of the actual thickness of the rubber, measured directly with a screw micrometer.

3.5.3 Indentation measurement reproducibility

The reproducibility of indentation measurement reproducibility was assessed by performing a repeated series of indentation tests on normal articular cartilage, using the apparatus described in Section 3.3. Thirty two indentation tests were performed on the femoral surfaces of a knee joint prepared accordingly (see Section 4.2.1). After each test, the exact test site was marked with haematoxyline acid.

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Table 3.3 Mean depths of indentation produced in thin sheets of rubber with and without realignment of the rubber specimen between tests.

	n	Mean depth of Indentation	s.d.	Coeff. of variation
		mm	mm	
Without specimen alignment	20	0.6069	0.0090	1.48%
With specimen alignment	20	0.5960	0.0128	2.14%

Following a 30 minute period of re-equilibration in physiological saline, 32 further indentation tests were performed at the marked sites. The paired indentation measurements were then compared. 72% (23 out of 32) of the repeated measurements were then ±3% whilst all but two of the pairs (91%) were within ±5%. A Wilcoxon signed rank test indicated that the second series of measurements were significantly smaller ($p<0.005$) than the first. This finding suggested that a minimal amount of damage occurred when the cartilage was indented.

3.5.4 Effects of freezing on measurement reproducibility

A similar repeated series of indentation tests were carried out on normal articular cartilage. In all, 27 tests were performed. Following a 30 minute period of re-equilibration in physiological saline, the test specimen was frozen and stored for a period of 28 days.

Following rethawing, a further 27 tests were performed. The indentation measurements were then compared as above. Of the repeated measurements, 74% (20 out of 27) were within ±3%, whilst all but three pairs (89%) were within ±5%. The second series again proved to be significantly larger ($p< 0.05$) than the first set. Both sets of reproducibility data are summarised in Table 3.4.

Freezing the specimen appeared to have little effect on the mechanical properties of the articular cartilage as measured with this indentation technique.

Table 3.4 Summary of the reproducibility data obtained from repeated indentation tests performed on fresh and defrosted normal articular cartilage.

	Without freezing	Following Freezing
No. of Repeated Tests	32	27
Mean % difference	±1.23%	±1.48%
s.d.	±2.40%	±2.80%
Largest Difference	5.84%	8.00%
< ±3%	72%	74%
< ±5%	94%	89%

3.6 Measurement error and the calculated creep modulus.

As the formula used to calculate the creep modulus value is a complex function of the depth of indentation d and the cartilage thickness t, errors in these measurements will have a varying effect on errors in the calculated modulus values. To demonstrate these effects, errors in the modulus value were calculated using equation 3.2 for a range of errors in the depth of indentation for two values of d, 0.25mm and 0.50mm.

$$\text{Error in } E = \frac{E_{d+\Delta d} - E_d}{E_d} \quad \text{--------- (3.2)}$$

These calculations were performed for two values of the cartilage thickness, 1mm and 3mm. Figure 3.8 shows the family of curves generated in this manner. A similar family of curves generated for errors in t are shown in figure 3.9. This time, errors in the modulus value were calculated for a range of errors in the cartilage thickness for the two values of thickness mentioned above, and for the same two values of d. As can be seen in figures 3.8 and 3.9, the calculation of E is more sensitive to errors in the indentation than it is to errors in the cartilage thickness. An over-estimation of 0.05mm of an indentation 0.3mm in depth, taken at site where the cartilage thickness is 2mm, results in a 2.55% reduction in the value calculated for E. The same error of 0.05mm made in the measurement of the cartilage thickness results in only a 2.28% increase of the value of E. The calculation of E is also more sensitive to underestimations of d than it is to similar over-estimations. An under-estimation of 0.05mm of an indentation which measures 0.3mm at a site where the cartilage thickness is 2mm would result in a 4.16% increase in the value calculated for E.

As the errors in the calculated creep modulus E, resulting from measurement errors, also depend on the actual size of d and t, it was difficult to relate directly measurement errors to errors in E. Table 3.5 contains possible percentage errors in the calculated modulus value for a variety of combinations of d and t for the errors in d and t which could be expected when using the apparatus described above. In the worst situation, the maximum percentage error in the calculated modulus value due solely to an error in d was 4.2 %. Likewise, for errors solely in t, the worst possible percentage error was 2.9 %.

Table 3.5(a) Percentage errors in the creep modulus value E which result for errors of ±0.005 mm in the depth of indentation d, calculated for a range of values for the depth of indentation d and the cartilage thickness, t.

	d = 0.25 mm	d = 0.30 mm	d = 0.50 mm
t = 1.00 mm	-3.94% +4.18%	-3.30% +3.47%	-1.97% +2.03%
t = 2.00 mm	-3.70% +3.91%	-3.11% +3.26%	-1.88% +1.94%
t = 3.00 mm	-3.50% +3.70%	-2.95 +3.09%	-1.81% +1.86%

Table 3.5(b) Percentage errors in the creep modulus value E which result for errors of ±0.012 mm in the cartilage thickness t, calculated for a range of values for the depth of indentation d, and the cartilage thickness, t.

	t = 1.00 mm	t = 2.00 mm	t = 3.00 mm
d = 0.25 mm	±2.90%	±1.09%	±0.54%
d = 0.30 mm	±2.96%	±1.14%	±0.58%
d = 0.50 mm	±3.11%	±1.27%	±0.68%

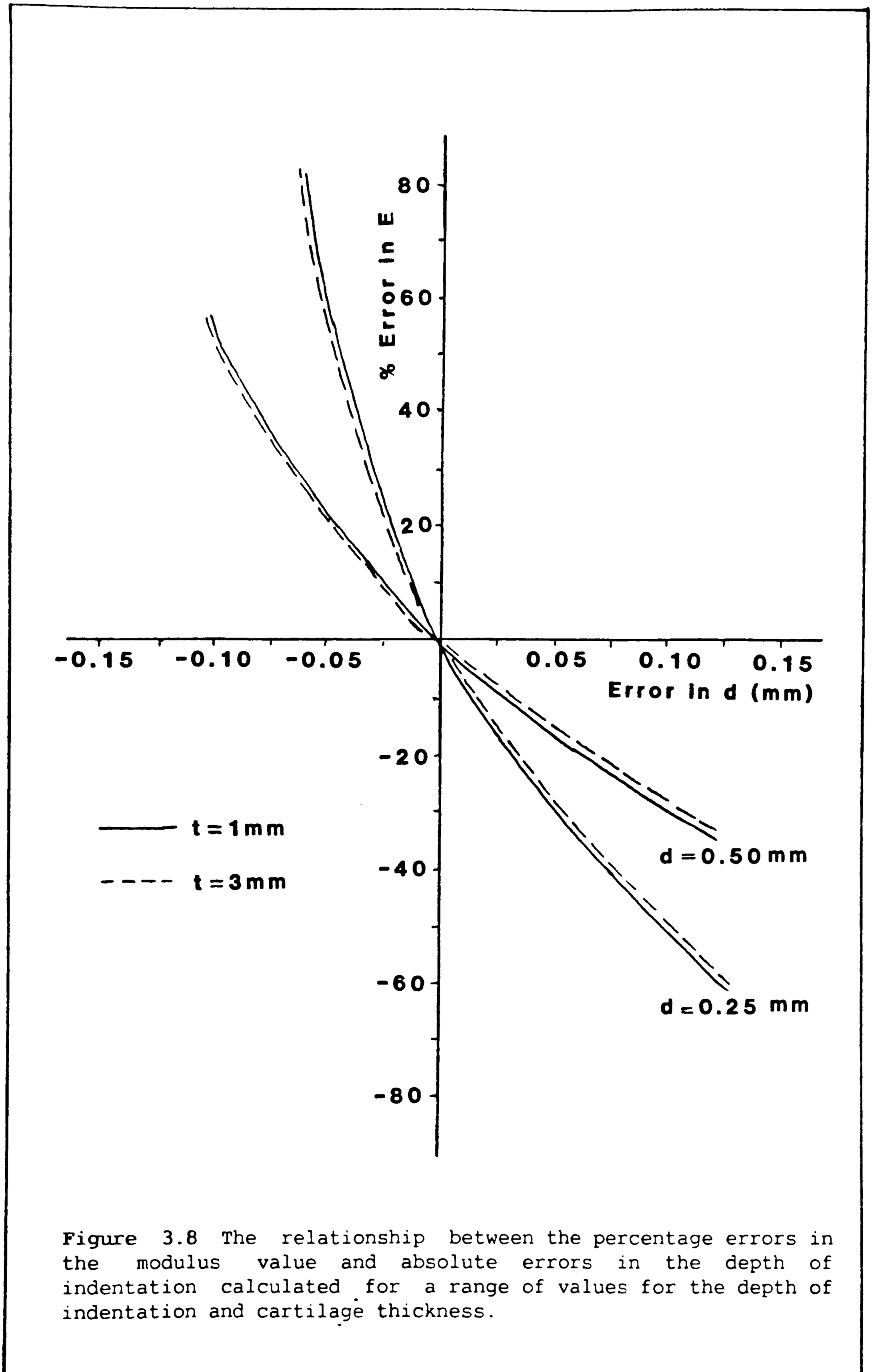


Figure 3.8 The relationship between the percentage errors in the modulus value and absolute errors in the depth of indentation calculated for a range of values for the depth of indentation and cartilage thickness.

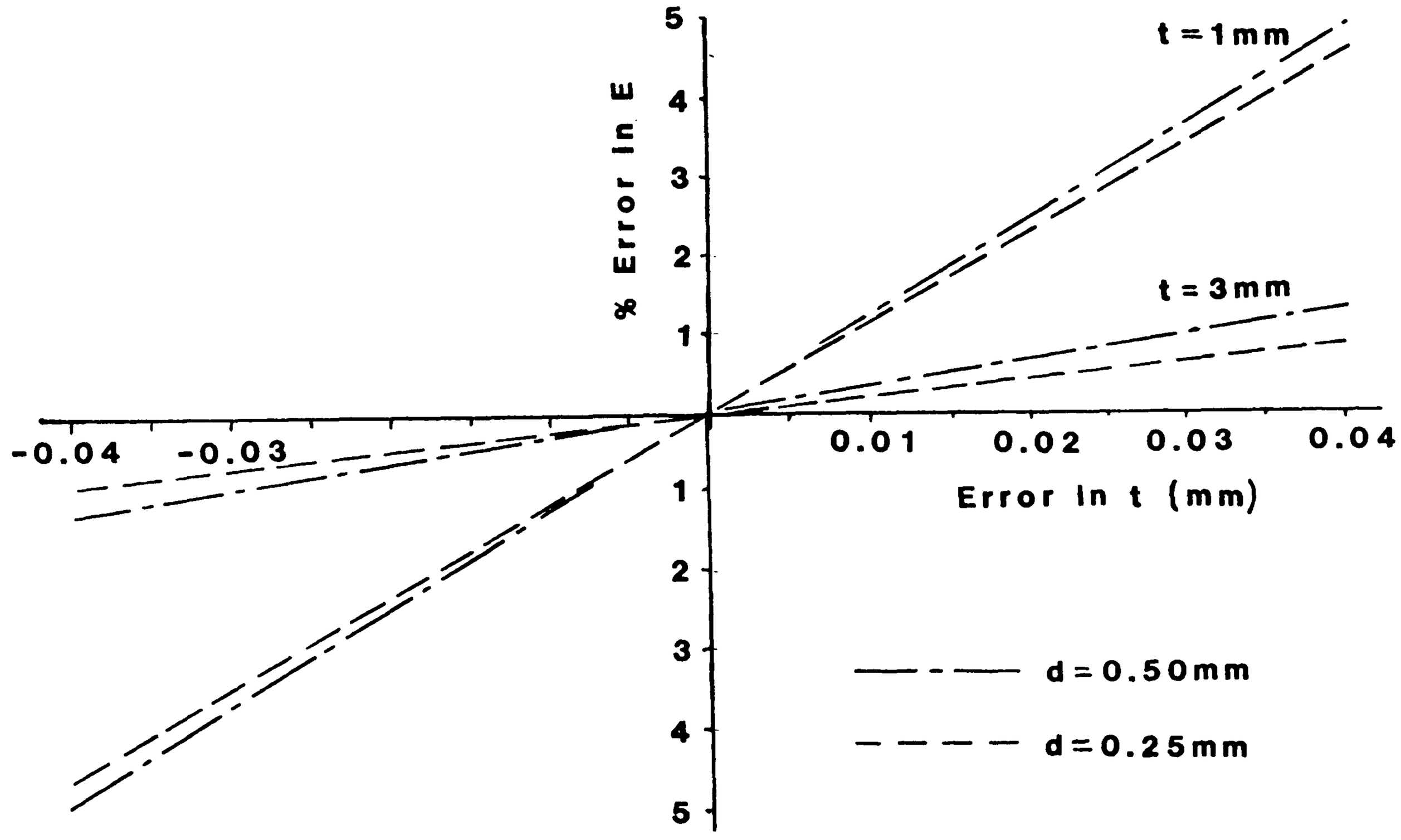


Figure 3.9 The relationship between the percentage errors in the creep modulus value and absolute errors in the cartilage thickness calculated for a range of values for the depth of indentation and cartilage thickness.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

4.1.1 Cartilage stiffness survey.

4.1.2 Biochemical assaying.

4.2. Specimen preparation.

4.2.1 Cartilage stiffness survey.

4.2.2 Biochemistry.

4.3. Analysis techniques.

4.3.1 Topographical variations in cartilage stiffness.

4.3.2 Quantitative assessments of variations in cartilage stiffness.

4.3.3 Cartilage stiffness and stress.

4.3.4 Age dependency.

4.3.5 Cartilage thickness.

4.3.6 Correlation of the mechanical properties and biochemical constituents of cartilage.

MATERIALS AND METHODS

4.1 Materials

4.1.1 Cartilage stiffness survey.

The knee

Surveys of the stiffness of visibly normal articular cartilage from the knee were carried out on 13 unembalmed cadaveric femora and 11 of the opposing tibial articular surfaces. Where available, details of the age, sex, height and weight were recorded, Table 4.1. For a complete survey of the femoral surfaces of a knee joint, approximately 50 sites were tested, whilst for the tibial surfaces, approximately 35 sites were tested.

The ankle

Visibly normal cartilage from 10 talar surfaces and 8 of the opposing tibia were subjected to stiffness surveys. The majority of the ankle specimens were obtained from below knee amputations, and it was not possible to obtain details regarding their donors. It was reasonable to assume however that the donors were middle aged or older as they suffered mostly from ischaemic diseases. For a complete survey of the talar surfaces of an ankle joint, approximately 25 sites were tested. For the opposing tibial surfaces, approximately 20 sites were tested.

4.1.2 Biochemical Analysis

A total of 214 full depth cartilage plugs 2.5 mm in diameter were obtained from the articular surface of knee specimens 7, 16 and 17, and ankle specimens 6 and 7, from sites previously subjected to both

Table 4.1 Age, sex, height and weight details regarding the specimens used in surveying the compressive stiffness of articular cartilage from the knee joint. A dash (-) denotes that information regarding the donor of the specimen was un-available

Specimen No.	Sex (M/F)	Age (Yrs)	Height (M)	Weight (Kg)
1	-	-	-	-
2	M	48	1.82	63
4	F	48	1.78	56
5	F	41	1.73	44
6	-	-	-	-
7	F	41	1.80	49
8	M	14	1.73	40
10	-	-	-	-
11	M	21	1.88	58
14	F	59	1.73	58
15	F	34	1.63	40
16	M	39	1.78	57
17	M	40	1.93	80

an indentation test and a measurement of the cartilage thickness. Only visibly normal cartilage was selected for biochemical assaying.

4.2 Specimen preparation

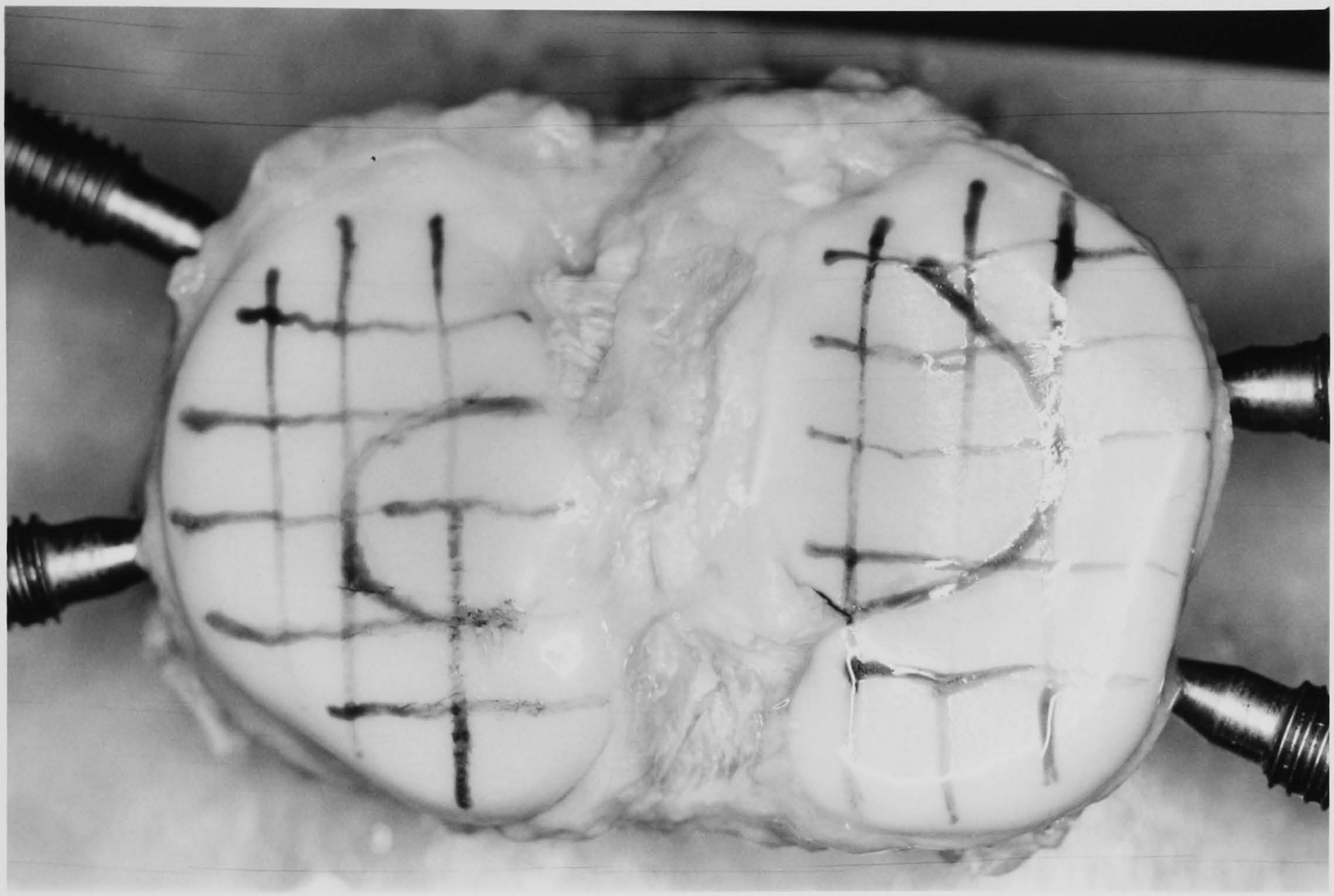
4.2.1 Cartilage stiffness survey

Cadaveric specimens were received within two days post mortem. Amputation specimens (primarily ankle specimens) were obtained on the day of the operation. In all cases, the specimens were frozen at -17°C and stored for periods of up to 28 days.

When required for mechanical testing, the specimens were thawed at room temperature whilst sealed in air tight polythene bags to prevent specimen dehydration. Once defrosted, the specimens were disarticulated and all surrounding tissue removed. The condylar bone mass was removed from the main bone shaft by sawing perpendicular to the long axis of the bone, approximately 5cm from the joint line, leaving a convenient flat surface for mounting the specimen onto the specimen holder. The tali were mounted complete. The specimens were then soaked in physiological saline (PH. 7.4) for at least 30 minutes, allowing the cartilage to equilibrate fully.

An orthogonal grid identifying potential test sites was drawn on the articular surfaces using a fibre tipped pen containing haematoxyline acid (Photograph 4.1). Areas of the tibial condyles exposed to direct articular contact (at full extension) were also marked with haematoxyline acid, prior to removing the menisci. Sites within the grid which exhibited visual signs of degeneration were noted and subsequently excluded from surveys of the cartilage properties. An indication of the integrity of the cartilage surface was obtained by

Photograph 4.1 Orthogonal grids drawn using haematoxyline acid on the articular surfaces of specimen No.10 indictaing potential test sites. The circular lines on the tibial condyles separate the areas covered and uncovered by the menisci at full extension.



observing the cleaness of the haematoxyline acid lines drawn on the cartilage. Where the cartilage was fibrillated, the lines were badly defined as the acid had a tendency to seep along fissures in the surface.

4.2.2. Biochemistry

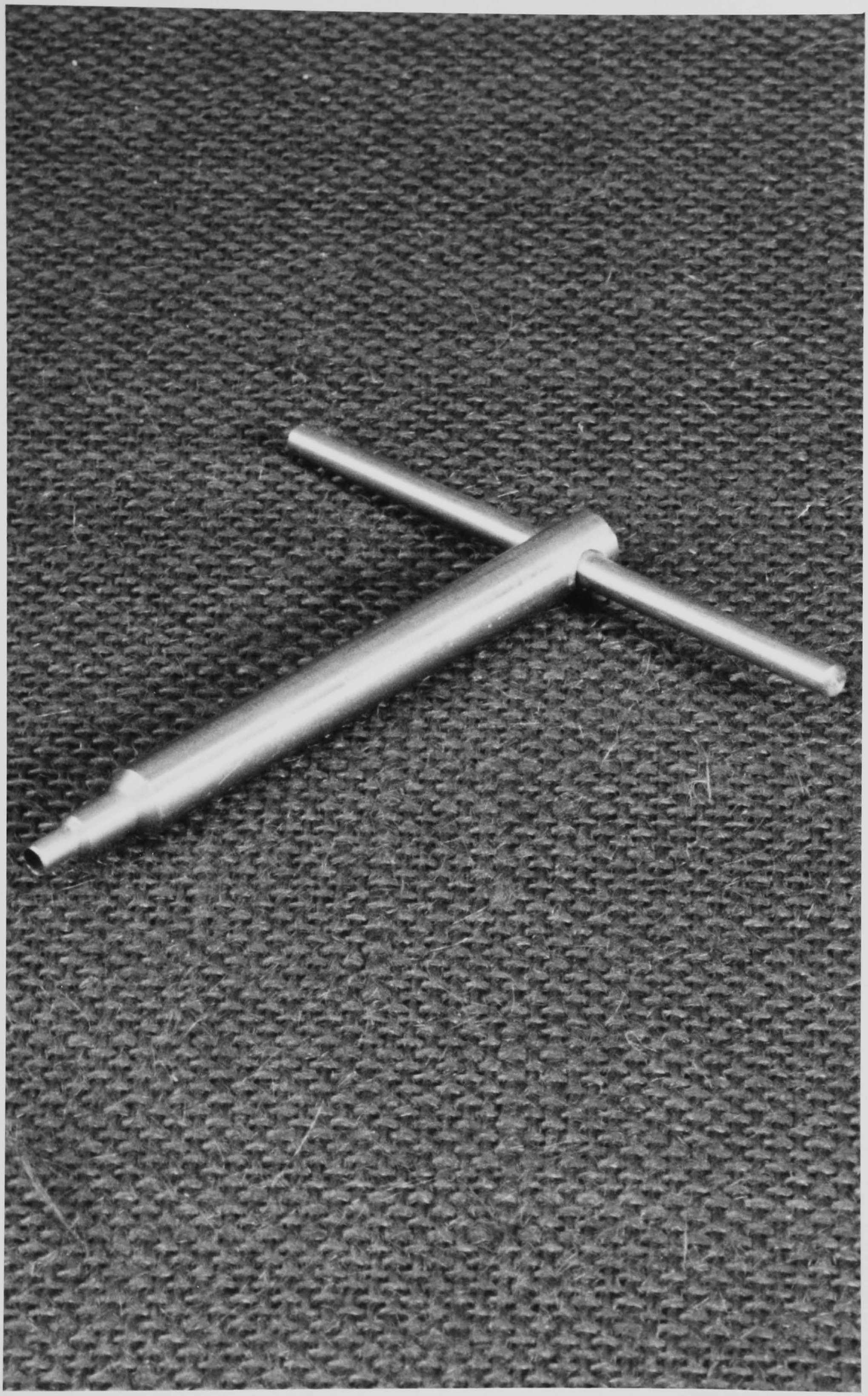
Cartilage plug removal

Following a 30 minute period of equilibration in physiological saline, full depth cartilage plugs were removed from the articular surfaces using a purpose-designed reamer (Photograph 4.2). Plugs were removed by pushing the reamer through the cartilage layer into the subchondral bone. The force required to cut through the cartilage was minimized by ensuring the circular cutting edge of the reamer was well honed. Once into the subchondral bone, the reamer was gently levered from side to side until the subchondral bone fractured. The composite plug was extracted from the reamer by pushing it from the bone side through the length of the reamer using a special ram. This avoided any unnecessary compression of the cartilage layer and hence avoided any possible losses of water from the plug. Separation of the cartilage from the subchondral bone was made using a scalpel, as close as possible to the cartilage-bone interface. Special attention was given to ensure that calcified material was excluded from the cartilage plug.

Papain digestion

Before placing the cartilage into empty, pre-weighed, screw-topped phials, they were lightly swabbed with tissue paper to remove any surplus surface water. . The phials were again weighed and the wet weight of each cartilage plug determined. The dry weight and thus the

Photograph 4.2 The specially designed reeming tool used for the extraction of cartilage plugs prior to biochemical assaying.



water content of each plug was obtained by dehydrating the plugs under vacuum for 48 hours using potassium hydroxide pellets and reweighing the phials. A nominal volume (2ml) of a papain digestion solution was then added to each phial. The exact weight of the digest was determined by a further weighing of each phial. The phials were then placed for 16 hours in an oven at 65°C allowing full cartilage digestion to occur. The digests were kept at 4°C until required for assaying.

Hydroxyproline

A small quantity of each papain digest solution was placed separately into sealable ampules and mixed with an equal amount of concentrated hydrochloric acid. The ampules were then sealed by flaring the necks in the flame of a bunsen burner. The samples were hydrolyzed at 110°C for 16 hours, dehydrated under vacuum using potassium hydroxide pellets and then stored at 4°C. When required for assaying each sample was re-suspended in a measured amount of distilled water.

4.3 Analysis techniques

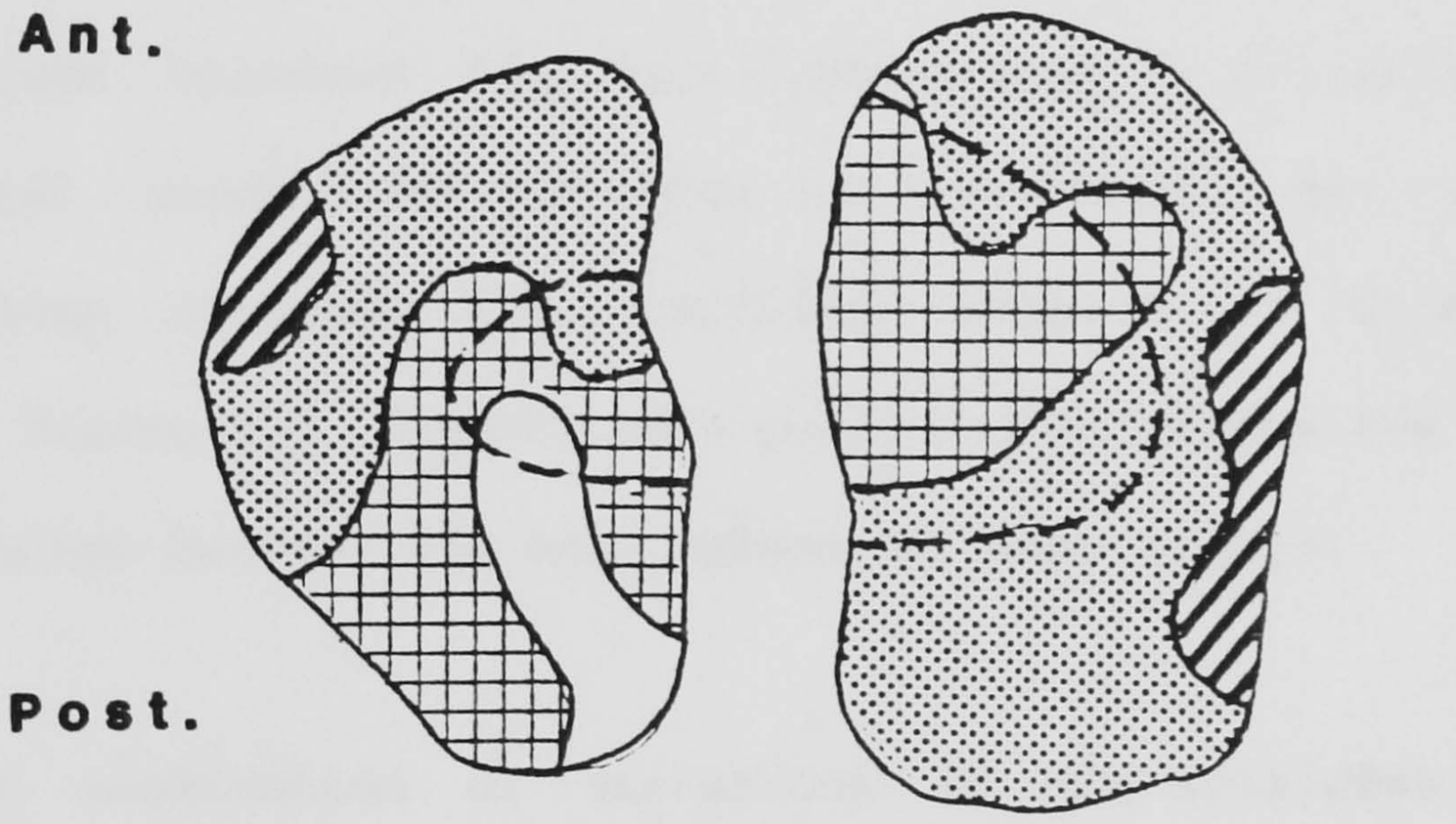
4.3.1 Topographical variations in cartilage stiffness.

Creep modulus values obtained from both the knee and the ankle joints were topographically arranged and used to construct contoured cartilage stiffness maps. In each case, the modulus values were separated into four distinct ranges of stiffness. Figure 4.1 illustrates a typical contoured stiffness map, together with the creep modulus values from which it was constructed. The dotted lines on the contour map indicate the areas of the tibial condyles which were covered and uncovered by the menisci at full extension. Osteoarthrotic areas of cartilage excluded from the survey have been marked with the

	7.29	6.35	
10.12	9.04	7.61	8.92
7.39	5.84	5.48	8.41
7.51	4.16	3.94	5.08
	4.86	3.92	4.69
		4.90	3.90

	7.78	7.12	
5.04	6.53	5.22	8.62
6.95	5.59	7.13	12.75
5.82	6.09	7.73	9.82
	8.92	8.84	10.96
	8.93	8.70	

(a)



(b)

Figure 4.1 (a) Topographical arrangement of modulus values obtained from the tibial surfaces of specimen No.10
(b) The corresponding contoured map of cartilage stiffness constructed from the modulus values above.

letters OA. The contoured stiffness maps were then examined. Special attention was given to identifying trends suggesting intra-articular and inter-articular variations in the cartilage stiffness. Details regarding the prevalence and location of osteoarthrotic lesions were also recorded.

4.3.2 Quantitative assessments of variations in cartilage stiffness.

The knee

For each knee joint, means and standard deviations were calculated for creep modulus values grouped according to their topographical location. Modulus values obtained from the femoral surfaces were divided into three groups; one for the patellar surfaces of the femur, and one for each of the two femoral condyles. Modulus values obtained from the tibia were divided into four groups; two groups for those values obtained from cartilage covered by the menisci (one for the lateral condyle and the other for the medial), and two groups for those values obtained from cartilage exposed to direct articular contact, figure 4.2. T-tests were performed to assess the significance of differences between the mean values of these groups.

A further examination of variations in the stiffness of cartilage from the knee was carried out by ranking the modulus values from each joint surface in descending order. The topographical locations of the lowest and highest 25% of the values were then marked on maps of the joint, Figure 4.3. Any tendency for either the high values or the low values to appear in any one specific area was noted.

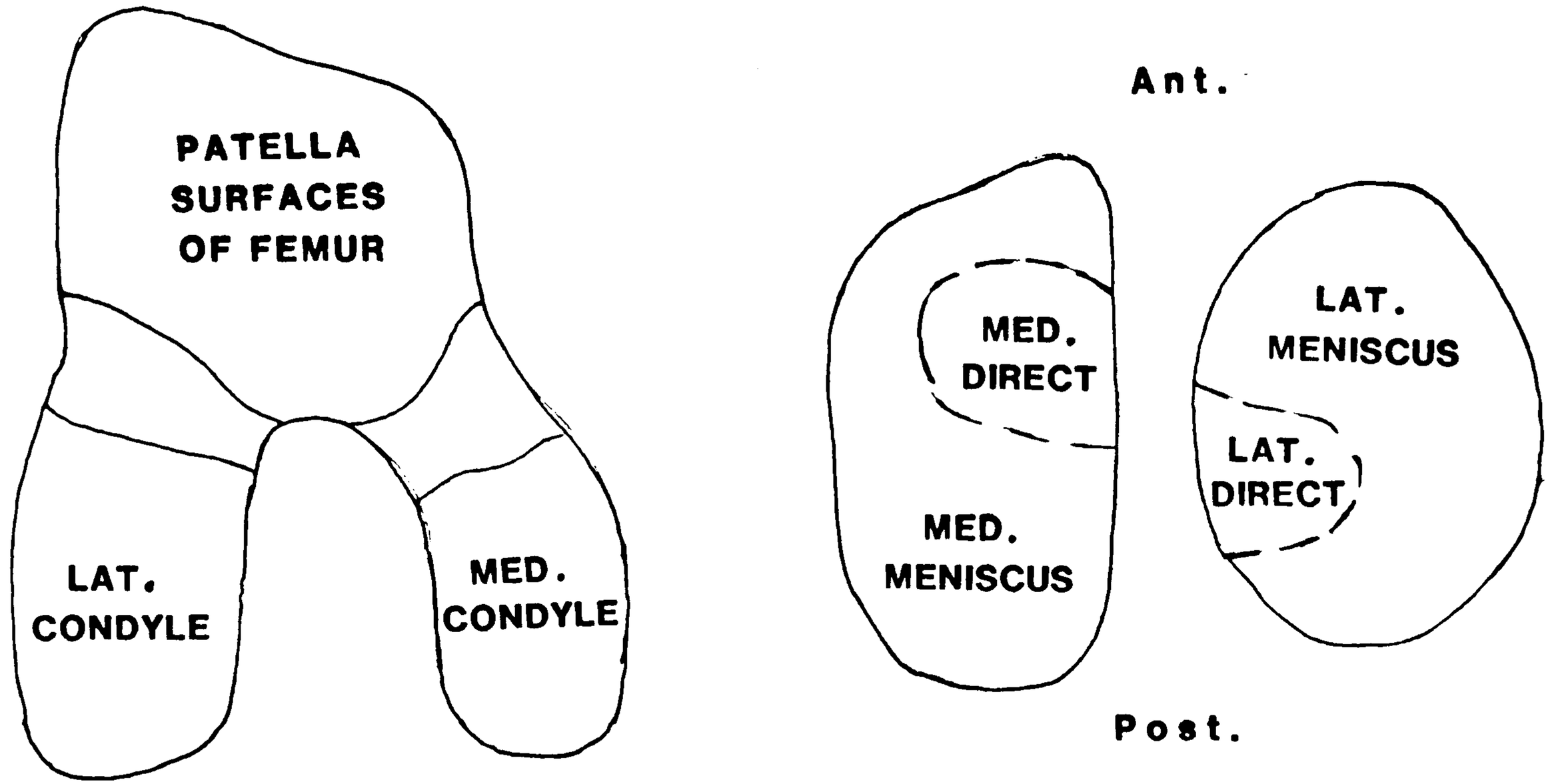


Figure 4.2 Areas of the femoral and tibial surfaces of the knee for which mean stiffness and thickness values were calculated.

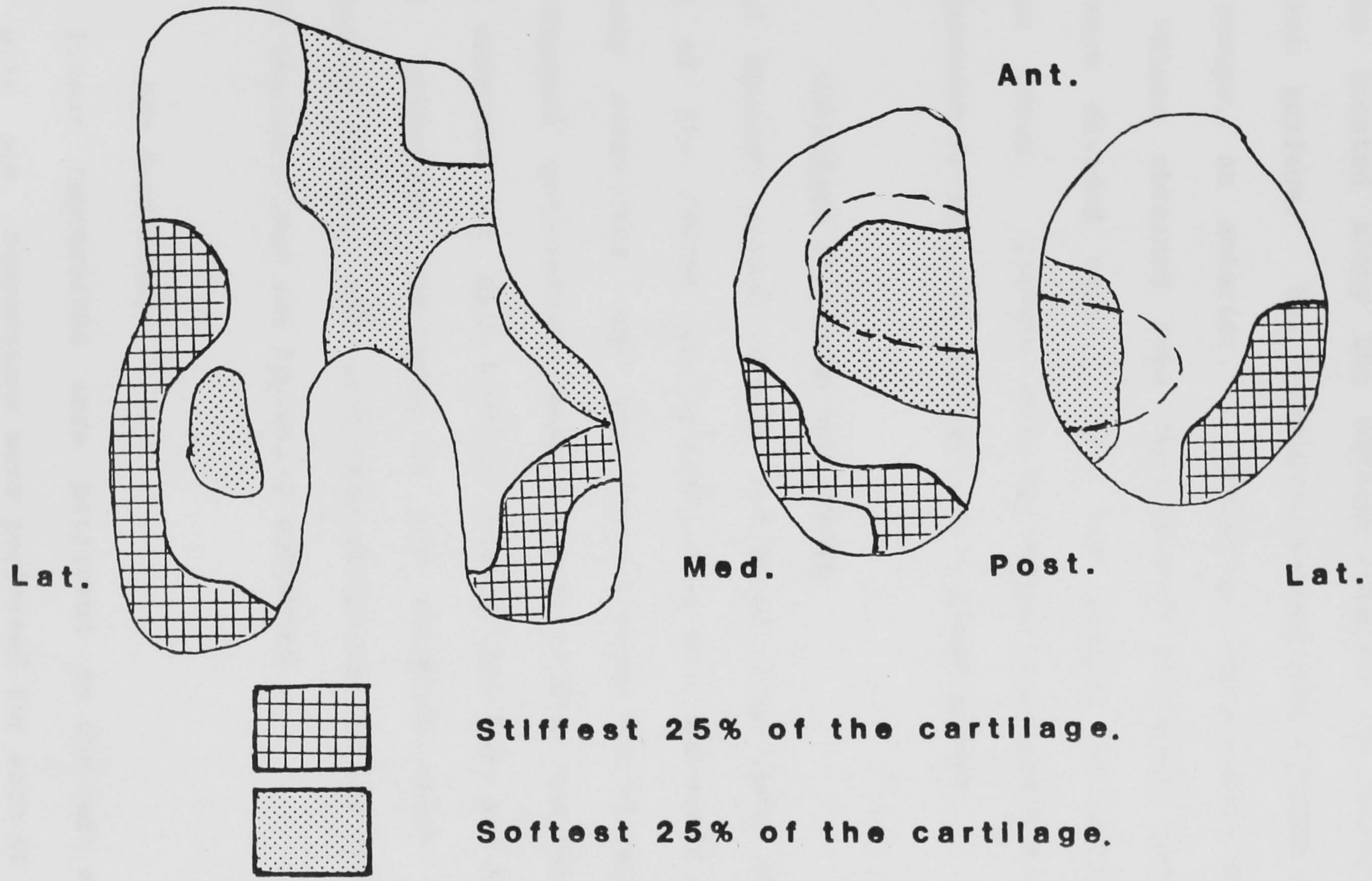


Figure 4.3 Map of the articular surfaces of knee specimen No. 5 indicating the topographical location of the largest and smallest 25% of the modulus values.

The ankle

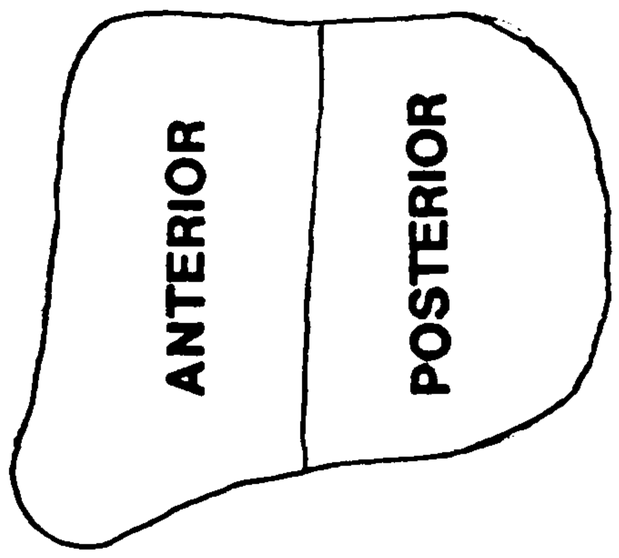
Means and standard deviations were also calculated for groups of creep modulus values obtained for cartilage from the ankle joint. Modulus values obtained from the talar surfaces were divided into four groups. The first group was made up of those values obtained from cartilage located along the extreme anterior and posterior edges of the talar surface. The remaining values were divided equally into three groups, an anterior, a posterior and a middle group, figure 4.4. Values obtained from cartilage on the tibial surfaces of the ankle were divided equally into two groups, an anterior and a posterior group. T-tests were performed to assess the significance of differences between the mean values of these groups.

4.3.3 Cartilage stiffness and stress

Averaged applied stress levels estimated from data obtained from studies of the forces acting within the knee and ankle joint during ambulatory activities, and the contact areas during such activities were compared qualitatively with the overall mean compressive modulus values obtained for each specific area of the knee and ankle joints. Special attention was paid to any evidence which suggested a relationship between the cartilage stiffness and the level of stress to which the cartilage was typically subjected.

4.3.4 Age dependency

Simple linear regressions were performed on the mean creep modulus values with age. Regressions were performed for each of the areas of the knee shown in Figure 4.2. Multiple linear regressions were also performed with the mean creep modulus as the dependent variable and the age, sex, height and weight as the independent variables.



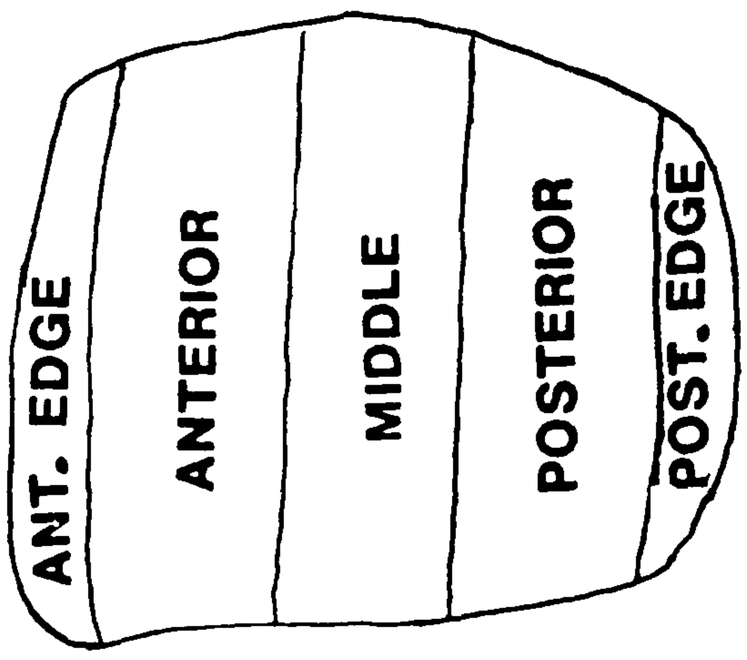


Figure 4.4 Areas of the tibial and talar surfaces of the ankle for which mean stiffness and thickness values were calculated.

4.3.5 Cartilage thickness

Examinations of the variations in cartilage thickness followed similar lines to the examination made of cartilage stiffness. Contoured thickness maps were constructed and used to make preliminary assessments of the variations in cartilage thickness. Quantitative assessments of these variations were made by statistically comparing mean thickness values calculated from the data obtained from each of the areas defined in Figures 4.2 and 4.4. The technique of ranking the thickness values in order of magnitude and plotting the location of the largest and smallest 25% of the values on maps of the articular surfaces was again employed.

4.3.6 Correlation of the mechanical properties and biochemical constituents of cartilage.

The possibility of any relationship existing between the mechanical properties of articular cartilage (compressive modulus and cartilage thickness) and the biochemical constituents of the cartilage (water, proteoglycan and collagen) was examined using simple linear regression techniques. In some instances, more complex relationships were examined.

CHAPTER 5

RESULTS

5.1 Topographical variations in the compressive stiffness of articular cartilage.

5.1.1 The knee joint

5.1.2 The ankle joint

5.2 Topographical variations in cartilage thickness.

5.2.1 The knee joint

5.2.2 The ankle joint

5.3 Location of osteoarthrotic lesions

5.4 Correlates of Stiffness

5.4.1 Stress

5.4.2 Age

5.4.3 Age, Sex, Height and Weight

5.4.4 Cartilage thickness

5.5 The biochemistry of cartilage

5.5.1 Proteoglycan

5.5.2 Collagen

5.5.3 Water

5.6 The biochemistry of cartilage and stiffness

5.6.1 Proteoglycan

5.6.2 Water

5.7 The effects of a further empirical correction for finite thickness

5.7.1 Stiffness variations

5.7.2 Stiffness and thickness

5.7.3 Stiffness and biochemistry

RESULTS.

5.1 Topographical variations in the compressive stiffness of articular cartilage.

5.1.1 The knee joint

The femur

In general, cartilage from the femoral condyles was stiffer than that from the patellar surface of the femur, although half the femora examined had areas of stiff cartilage situated on the lateral edge of the patellar surfaces. The central area of the patellar surfaces of the femur were softer than adjacent areas of cartilage on both the lateral and medial sides.

No apparent difference between the stiffness of medial and lateral femoral condylar cartilage was observed, although there was a tendency for the cartilage on both condyles to be stiffer in the more posterior areas. The variations in stiffness over the femoral surfaces of Specimen No.8, Figure 5.1(a), typified these trends. A complete set of contoured stiffness maps can be found in Appendix B.

Statistical comparisons of mean creep modulus values, calculated for specific areas of each of the femora, Table 5.1, indicated (a) that cartilage from the femoral condyles was significantly stiffer ($p<0.001$) than cartilage from the patellar surfaces and (b) that there were no significant medial-lateral differences in cartilage stiffness ($0.2>p>0.1$).

An indication of the topographical variations in cartilage stiffness was also obtained by identifying the location of those areas where the

Table 5.1 Mean creep modulus values obtained for cartilage situated on the femoral surfaces of the knee.

Specimen No.	Lateral condyle	Medial condyle	Patellar surfaces
	MN/m^2	MN/m^2	MN/m^2
1	9.15	9.57	6.17
2	4.89	6.20	4.48
4	7.80	11.32	5.97
5	9.19	10.56	6.90
6	9.45	8.80	7.42
7	7.86	7.43	6.03
8	9.63	11.29	5.13
10	10.27	10.64	6.88
11	8.37	7.77	5.41
14	7.43	10.09	7.43
15	9.58	9.29	6.71
16	6.84	10.06	5.98
17	9.69	10.30	6.92
Overall Mean Creep Modulus	8.47	9.48	6.26

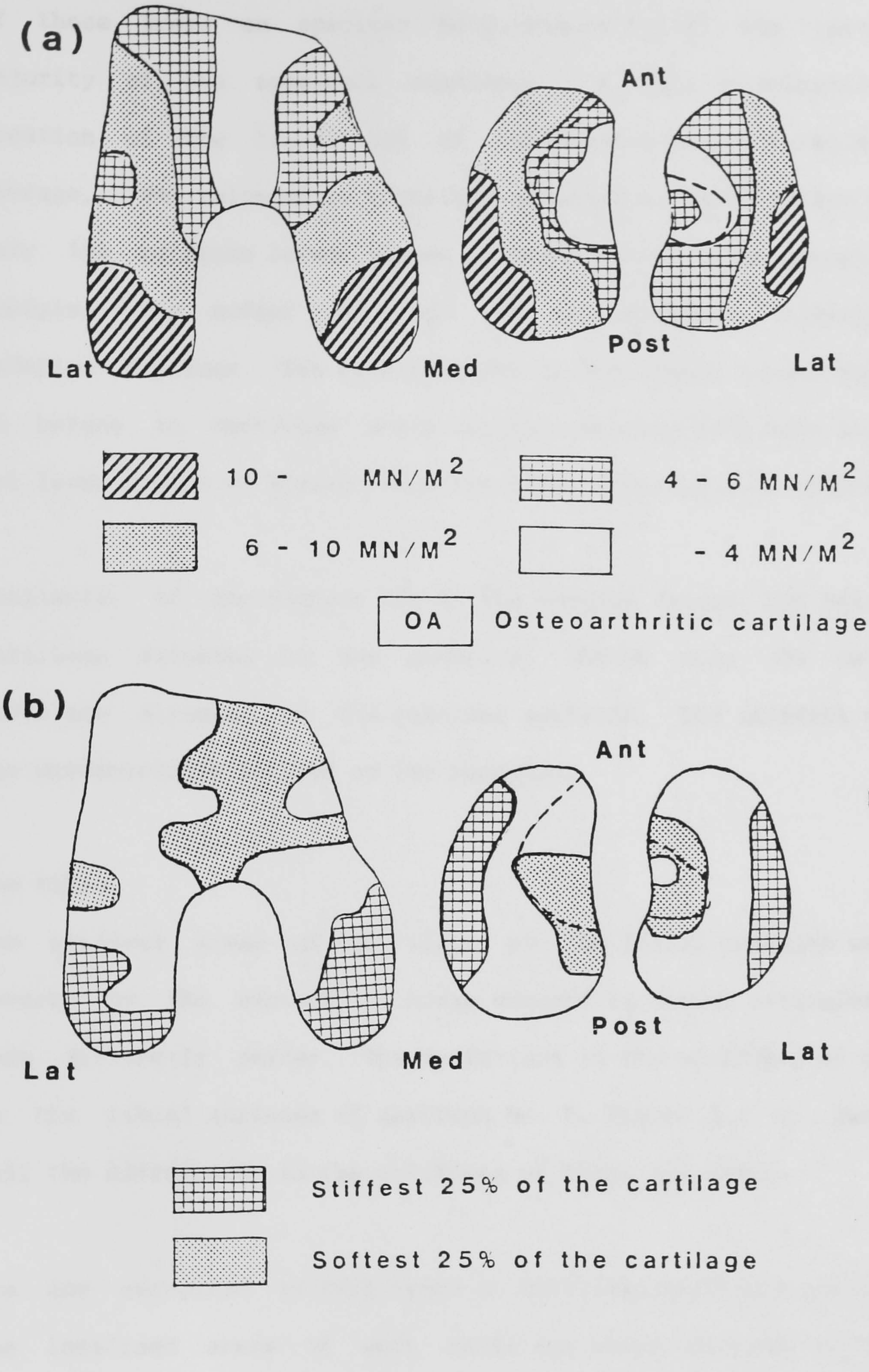


Figure 5.1 (a) Contoured stiffness maps of specimen No.8 and (b) corresponding maps indicating the locations of the lowest and highest 25% of the modulus values.

highest and lowest 25% of the modulus values were found. The location of these areas on specimen No.8, Figure 5.1(b), was typical of the majority of the specimens examined. A full examination of the location of the lowest 25% of the modulus values revealed that on average, 70% belonged to cartilage situated on the patellar surfaces. Only 10% of these lowest values belonged to cartilage situated on the condyles. The softer cartilage was predominantly located on the patellar surfaces. The remaining 20% of the lowest values were deemed to belong to cartilage where contact occurred with both the patella (at large angles of flexion) and the tibia (nearing full extension).

Similarly, of the highest 25% of the modulus values, 80% belonged to cartilage situated on the condyles, whilst only 12% belonged to cartilage situated on the patellar surfaces. The stiffest cartilage was predominantly located on the condyles.

The tibia

The stiffest areas of cartilage on the tibial condyles were those covered by the menisci. Areas exposed to direct articular contact were distinctly softer. The variations in the stiffness of cartilage on the tibial surfaces of specimen No. 8, Figure 5.1 (a), demonstrate well the differences in the stiffness of these two areas.

The one exception to this trend in cartilage stiffness variation was the localised areas of soft cartilage which occurred in the medial posterior sections of the areas underlying the lateral meniscus of specimens Nos. 1, 10 and 16, Figure 5.2. No corresponding softness was observed in the lateral posterior sections of the medial condyle. Whilst there was no significant medial lateral difference in the

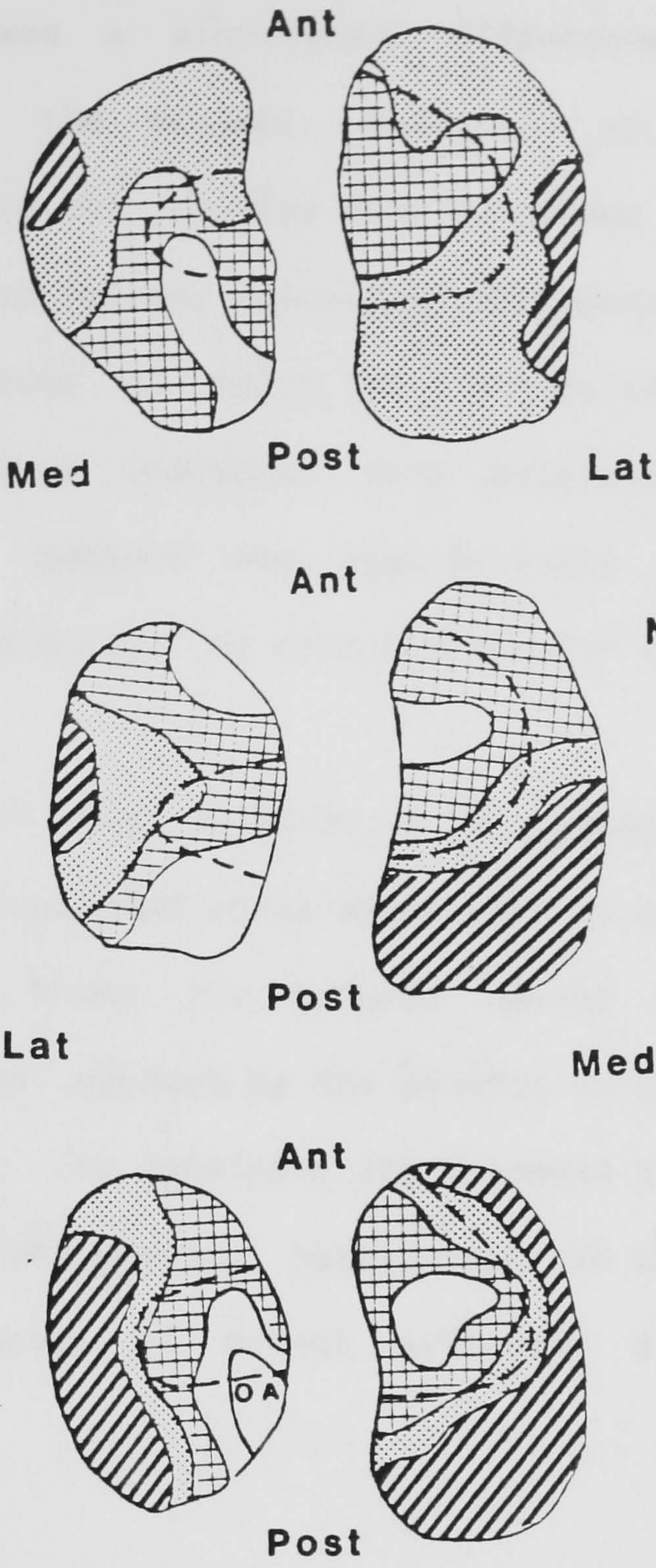


Figure 5.2 Contoured stiffness maps of the tibia of specimen Nos 10,16 and 17. All exhibited soft cartilage in the medial posterior corner of the lateral condyle.

stiffness of cartilage in areas of the tibial uncovered by the menisci, there was a significant difference in stiffness of those areas underlying the menisci. Cartilage on the medial condyle was significantly ($p=0.02$) stiffer than cartilage on the lateral condyle. Mean creep modulus values, calculated for specific areas of the tibiae examined, are given in Table 5.2. Statistical comparisons of these mean modulus values indicated that articular cartilage in areas underlying the menisci was significantly stiffer ($p<0.001$) than cartilage in areas subject to direct articular contact.

An examination of the location of the highest and lowest 25% of the modulus values confirmed these variations in cartilage stiffness. On average, 72% of these low modulus values belonged to cartilage situated in areas exposed by the menisci to direct articular contact with the femur. The remaining 28% belonged to cartilage situated in areas underlying the menisci, particularly in areas situated posterior to the marked areas of direct articular contact, on the lateral condyle.

5.1.2 The ankle joint

The talus

The stiff est areas of cartilage on the talus, extended medially from the lateral edge along the central portion of the articular surface. The contoured stiffness maps of specimens No. 4 and No. 7, Figure 5.3, illustrate this variation in cartilage stiffness. A complete set of contoured stiffness maps for the ankles examined can be found in Appendix B. Similar patterns in cartilage stiffness were observed on 8 out of 10 of the specimen examined. On the fringes most anterior and posterior to the stiffest areas, the cartilage gradually became

Table 5.2 Mean creep modulus values obtained for cartilage situated on the tibial surfaces of the knee. A dash (-) denotes that the articular surface was not tested.

	Cartilage exposed to direct contact		Cartilage covered by menisci	
Specimen No.	Lateral condyle	Medial condyle	Lateral condyle	Medial condyle
	MN/m^2	MN/m^2	MN/m^2	MN/m^2
1	6.64	5.53	7.60	9.52
2	-	-	5.79	-
5	3.54	4.54	7.50	8.80
7	-	3.19	-	6.95
8	3.78	4.10	6.83	8.63
10	5.72	6.36	6.43	8.87
11	-	4.31	6.83	7.48
14	4.21	3.51	5.56	6.78
15	3.70	4.31	6.38	7.13
16	4.34	4.31	6.52	9.69
17	4.96	4.61	8.66	12.07
Overall Mean Creep Modulus	4.61	4.47	6.81	8.59

Table 5.3 Mean creep modulus values obtained for cartilage situated on the talar surfaces of the ankle.

Specimen No.	Anterior area	Middle area	Posterior area	Anterior/ Posterior fringes
	MN/m^2	MN/m^2	MN/m^2	MN/m^2
1	11.19	11.07	9.24	8.54
2	11.09	14.16	12.91	5.40
3	12.61	11.12	10.86	9.26
4	8.77	11.10	10.94	5.77
5	10.15	12.59	11.15	5.62
6	10.91	11.29	8.98	5.16
7	12.46	11.96	11.03	6.12
8	12.64	13.23	12.90	8.04
9	9.50	12.49	11.49	6.92
10	12.64	12.95	13.58	11.24
Overall Mean Creep Modulus	11.19	12.19	11.20	7.20

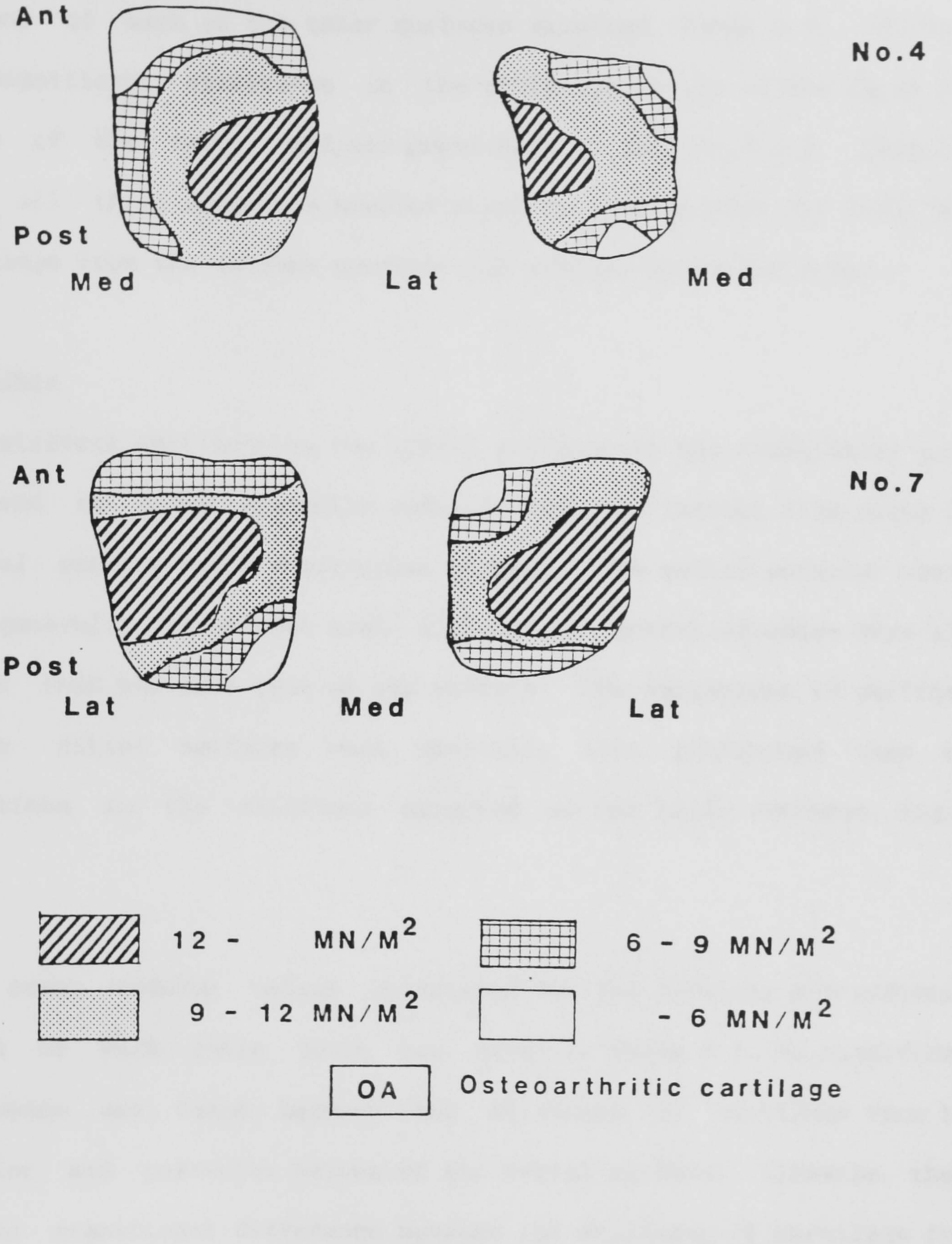


Figure 5.3 Contoured **stiffness** maps of ankle **specimen** No.4 and No.7, exhibiting **typical** cartilage stiffness **variations**.

softer. The medial posterior and anterior corners seemed to be the softest areas, although in three instances, specimen Nos 2,4 and 6, the softest areas extended along the entire anterior edge. Statistical comparisons of mean creep modulus values, calculated for specific regions of each of the talar surfaces examined, Table 5.3, indicated no significant difference in the stiffness of any of the three main areas of the talus, defined previously in section 4.3.2. Cartilage from all three areas was however significantly stiffer ($p < 0.01$) than cartilage from the extreme anterior and extreme posterior areas.

The tibia

The stiffest cartilage on the tibial surfaces of the tibio-talar joint occurred in areas extending medially from the lateral side along the central portion of the articular surface. The medial anterior corner was generally the softest area, although the posterior edges were also softer than the main bulk of the surface. The variations in stiffness across tibial surfaces were generally less pronounced than the variations in the stiffness observed on the talar surfaces, Figure 5.3.

Mean creep modulus values calculated for the anterior and posterior halves of each ankle joint are given in Table 5.4. No significant difference was found between the stiffness of cartilage from the anterior and posterior halves of the tibial surface. Likewise, there was no significant difference between the stiffness of cartilage from opposing anterior and posterior areas of the talar and tibial surfaces.

Table 5.4 Mean creep modulus values obtained for cartilage situated on the tibial surfaces of the ankle.

Specimen No.	Anterior surfaces	Posterior surfaces
	MN/m^2	MN/m^2
3	10.78	11.12
4	8.68	10.77
5	12.02	12.44
6	9.56	9.14
7	9.78	10.21
8	14.03	14.62
9	8.02	11.05
10	4.43	4.50
Overall Mean Creep Modulus	9.66	10.48

Table 5.5 Mean thickness values obtained for cartilage situated on the femoral surfaces of the knee.

Specimen No.	Lateral condyle	Medial condyle	Patellar surfaces
	mm	mm	mm
1	2.32	1.76	2.52
2	2.81	2.61	2.35
4	1.93	1.77	1.98
5	2.93	1.75	2.26
6	2.27	2.57	2.16
7	2.70	2.90	2.67
8	2.33	2.57	2.82
10	2.53	1.96	2.48
11	3.24	2.76	3.62
14	2.14	1.81	2.25
15	2.02	1.48	2.15
16	3.09	2.16	2.74
17	2.85	2.25	2.97
Overall Mean Thickness	2.55	2.18	2.54

5.2 Topographical variations in cartilage thickness.

5.2.1 The knee joint.

The femur

The thickness of cartilage across the femoral surfaces varied from 1 mm to 5 mm. In general, cartilage situated on the patellar surfaces was thicker than that situated on femoral condyles. There was also some indication that the lateral condyle was covered with slightly thicker cartilage than the medial condyle. The variations in the cartilage thickness on specimen No. 16 and No. 17, Figure 5.4, were most typical of the majority of the femora examined. A complete set of thickness maps for the femora and tibiae examined can be found in Appendix C.

There were however considerable differences in the thickness of cartilage from one joint to another. This can be clearly seen in the mean cartilage thickness values calculated for specific areas of each of the femora examined, Table 5.5. The cartilage on specimen No. 11 and No. 17 was particularly thick, whilst the cartilage on specimen No. 4 and No. 15 was easily the thinnest. It was interesting to note that no extreme mean values for cartilage stiffness were obtained for any of these joints.

Statistical comparisons of the mean thickness values showed that cartilage situated on both the lateral condyle and on the patellar surfaces of the femur was significantly thicker ($p<0.05$) than cartilage situated on the medial condyle. There was no significant difference in the thickness of cartilage from the lateral condyle and the patellar surfaces of the femur.

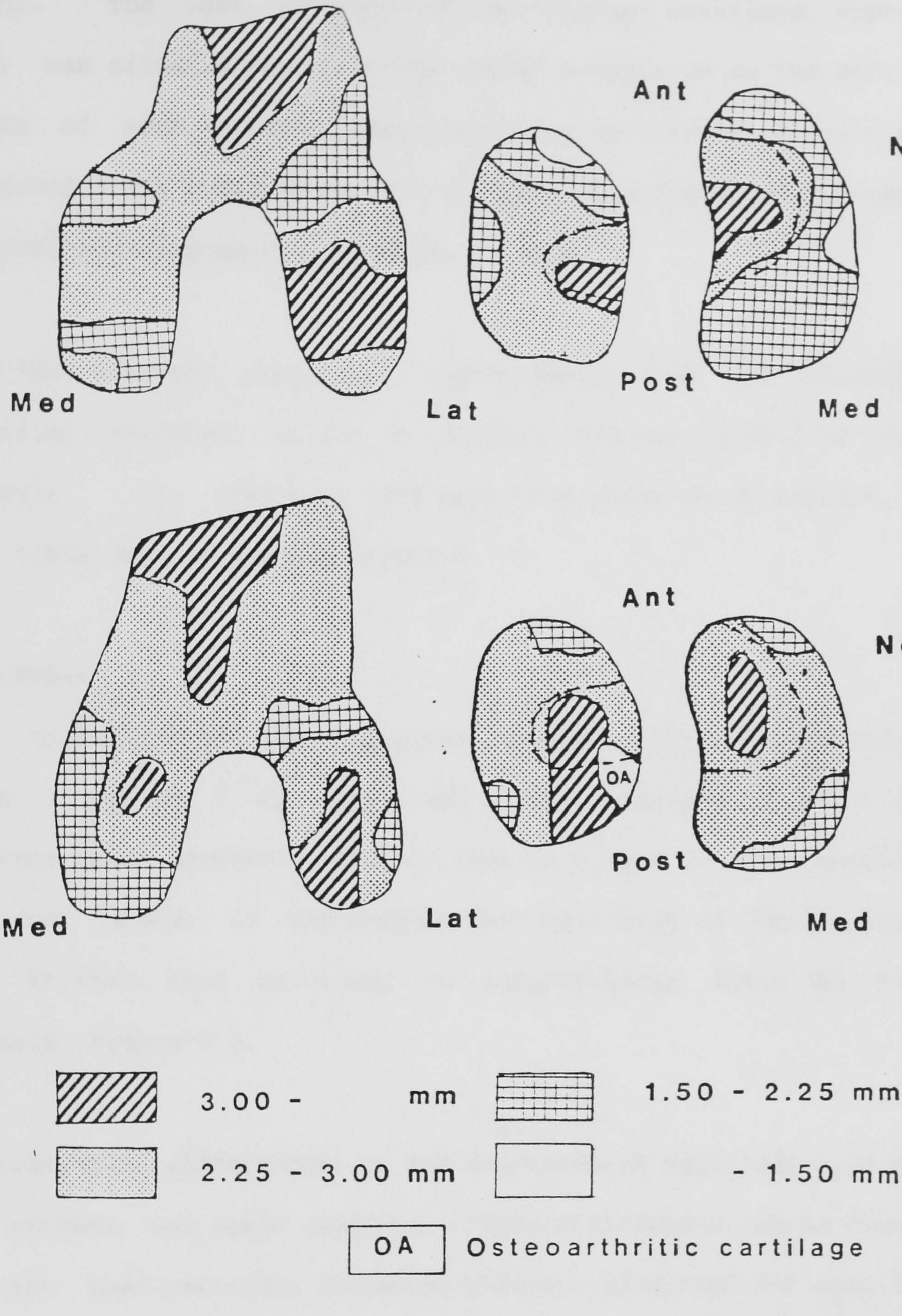


Figure 5.4 Contoured thickness maps of specimen No.16 and No.17, both exhibiting typical cartilage thickness variations.

Similar variations in cartilage thickness were indicated by noting the location of the thickest and thinnest 25% of the cartilage from each joint. The vast majority of the thinnest cartilage, approximately 72%, was situated either on the medial condyle or on the more anterior areas of each condyle, where contact with both the patella and tibia occurred. The remainder of the thinnest cartilage was situated on the lateral condyles and the patellar surfaces.

Of the thickest cartilage, approximately 51% was situated on the patellar surfaces, whilst a further 28% was located on the lateral condyle. The remaining 21% were from areas where contact with both the tibia and the patella occurred.

The Tibia

The thickness of the cartilage on the tibial surfaces again ranged from 1 mm to 5 mm. Cartilage in areas exposed to direct articular contact was generally thicker than cartilage in areas underlying the menisci, whilst it was evident that cartilage on the lateral condyle was thicker than cartilage in corresponding areas on the medial condyle, Figure 5.4.

Considerable differences in the thickness of cartilage from one joint to another was again observed. These differences can be clearly seen in the mean cartilage thickness values, calculated for specific areas of each of the tibial surfaces examined, given in Table 5.6. It was also interesting to note that the opposing femoral components of these tibiae exhibiting the thickest cartilage (specimen No. 11 and No.17) also exhibited thick cartilage. A similar pattern emerged for the tibial and femoral components which were covered with the thinnest layers of cartilage.

Table 5.6 Mean thickness values obtained for cartilage situated on the tibial surfaces of the knee. A dash (-) denotes that the articular surface was not tested

	Cartilage exposed to direct contact		Cartilage covered by menisci	
Specimen No.	Lateral condyle	Medial condyle	Lateral condyle	Medial condyle
	mm	mm	mm	mm
1	2.81	2.54	2.46	1.86
2	-	-	2.83	-
5	3.73	2.84	2.71	2.09
7	-	3.06	-	2.33
8	3.98	2.54	2.93	1.95
10	3.11	2.09	2.63	1.34
11	-	3.04	3.39	2.47
14	3.23	2.33	2.05	1.89
15	3.17	1.92	1.98	1.69
16	3.14	3.16	2.23	1.80
17	3.30	2.90	2.64	2.11
Overall Mean Thickness	3.30	2.66	2.58	1.95

From statistical comparisons of these mean thickness values, it was evident that cartilage in areas exposed by the menisci, and hence subject to direct contact with the femur, was significantly thicker than cartilage in areas underlying the menisci ($p < 0.01$). On the whole, cartilage in both areas of the lateral condyle was significantly thicker ($p<0.01$) than cartilage in the corresponding areas of the medial condyle.

An examination of the location of the thickest and thinnest 25% of the cartilage indicated similar variations in cartilage thickness across the tibia. The majority of the thinnest 25% of the cartilage (approximately 71%) was situated on the medial condyle in areas underlying the menisci, whilst 24% of the thinnest cartilage was situated on the lateral condyle in areas underlying the menisci. Only 5% of the thinnest cartilage was situated in areas exposed to direct articular contact.

5.2.2 The ankle joint

The talus

The main trends in cartilage thickness variation across the talar surfaces were demonstrated by calculating mean thickness values for each row of measurements made on the tibiae examined. Mean thickness values calculated for specimen Nos 1, 2, 7 and 9 are plotted against their anterior posterior location in Figure 5.5. The thickest cartilage occurred about two-thirds the way back from the anterior edge of the articular surface. Both anterior and posterior to this position, the cartilage gradually decreased in thickness, the thinnest cartilage being situated along the anterior edge. No significant medial-lateral variations in the thickness of cartilage from the talar

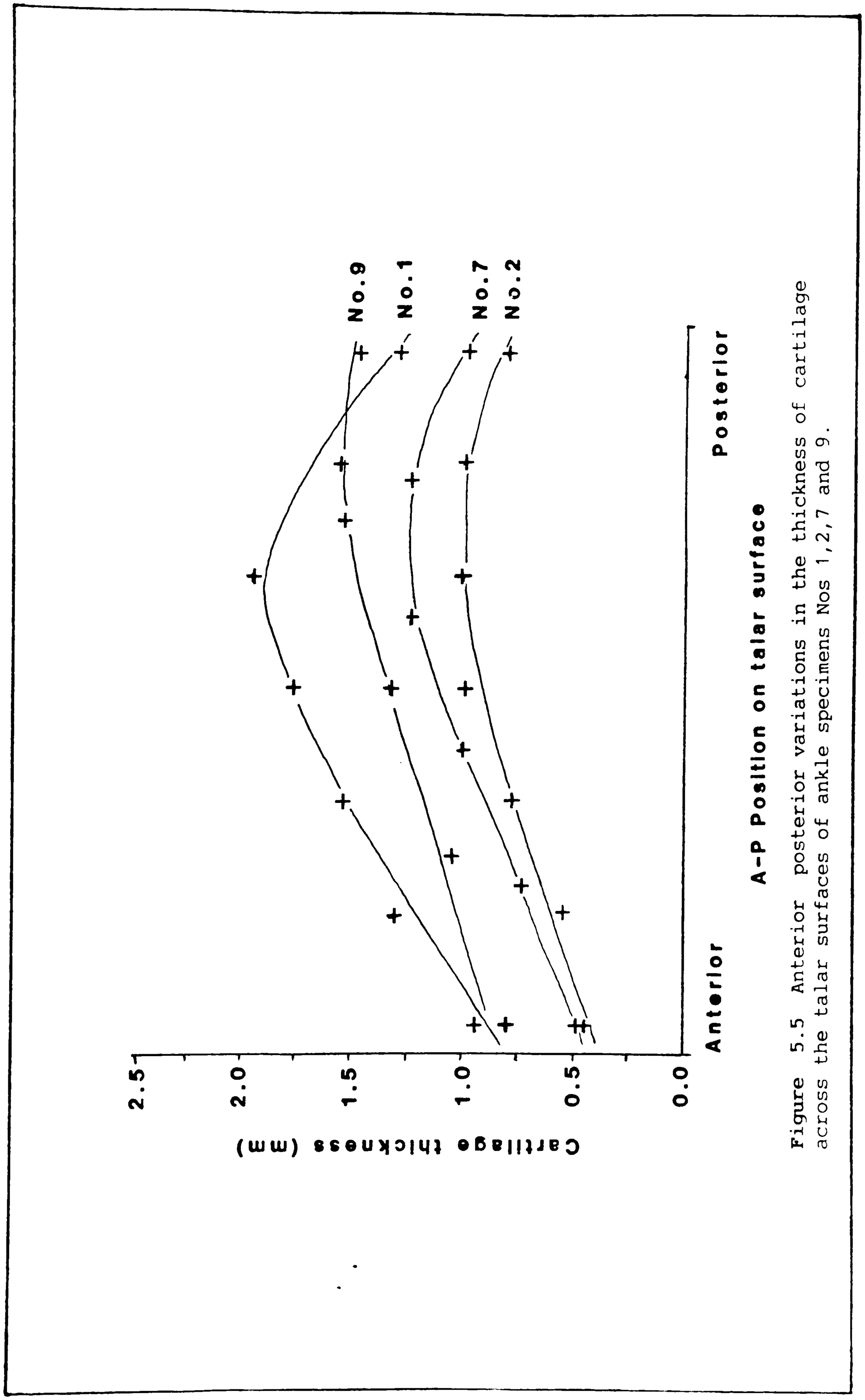


Figure 5.5 Anterior posterior variations in the thickness of cartilage across the talar surfaces of ankle specimens Nos 1,2,7 and 9.

surfaces were found. Thickness maps obtained for the ankles examined can be found in Appendix C.

Mean thickness values, calculated from thickness measurements made in each of the divisions of the talar surface defined in 4.3.2, are given in Table 5.7. The anterior area was significantly thinner than both the posterior area ($p < 0.01$) and the middle area ($p < 0.05$).

The tibia

Cartilage on the tibial surfaces of the ankle was similar in thickness to cartilage on the opposing talar surfaces, ranging from 0.5 mm to 2.5 mm. There were no significant topographical variations in the thickness of tibial cartilage, although cartilage from certain joints (Specimen No.3) was generally thicker than cartilage from others. Mean thickness values calculated for each joint, for the anterior and posterior halves of the tibial surfaces, are given in Table 5.8.

5.3 The location of osteoarthrotic lesions

The criterion for specimen selection for this study was that they should have visibly normal cartilage. Nevertheless, localized areas of osteoarthrotic damage were found on some specimens. Although not subjected to indentation tests, the location of these areas were recorded.

The patellar surfaces of three femora had areas of cartilage exhibiting localized fibrillation or other more progressive signs of osteoarthrotic damage. These areas appeared to correspond with the areas shown to be in contact with the patella at angles of 40°- 80° flexion (Seedhom et al,1979). No osteoarthrotic damage was found on any of the femoral condyles examined.

Table 5.7 Mean thickness values obtained for cartilage situated on the talar surfaces of the ankle.

Specimen No.	Anterior area	Middle area	Posterior area	Anterior/ Posterior fringes
	mm	mm	mm	mm
1	1.41	1.71	1.71	0.94
2	0.67	0.92	0.97	0.54
3	0.37	1.60	1.76	1.11
4	1.10	1.71	1.71	0.87
5	0.50	1.03	1.24	0.72
6	1.48	1.50	1.53	1.15
7	0.70	1.11	1.18	0.70
8	0.75	1.12	1.06	0.63
9	1.07	1.39	1.56	1.22
10	1.15	1.52	1.67	1.29
Overall Mean Thickness	0.92	1.36	1.43	0.91

Table 5.8 Mean thickness values obtained for cartilage situated on the tibial surfaces of the ankle.

Specimen No.	Anterior surfaces	Posterior surfaces
	mm	mm
3	1.85	1.55
4	1.55	1.38
5	1.05	0.93
6	1.54	1.27
7	1.17	1.03
8	1.02	0.88
9	1.57	1.35
10	0.73	0.69
Overall Mean Thickness	1.31	1.13

Of the tibial surfaces examined, only one specimen (No.14) had any visible signs of fibrillation on the medial plateau, namely, the area exposed to direct articular contact with the femur. On the other hand, six tibiae had localized areas of fibrillation on the lateral plateau. In two cases, the fibrillated cartilage occurred in areas of direct articular contact, whilst in the other four cases, the fibrillated cartilage was situated in the medial posterior corner of the lateral condyle, in areas covered by the meniscus at full extension. No osteoarthrotic damage was found on any of the surfaces of the ankles examined.

Two otherwise healthy tibial specimens exhibited surface 'blisters' approximately 6 mm in diameter, in areas exposed to direct articular contact with the femur, Photograph 5.1, which bore ressemblance to the 'blisters' described previously by Goodfellow et al(1976).

5.4 Correlates of stiffness

5.4.1 Stress

The nature of techniques available for assessing the stresses acting within articular joints has limited such investigations to the estimation of averaged stress levels which occur during the most frequent of ambulatory activities. Nevertheless, such data are a good indication of the predominant levels of stress to which cartilage is likely to be subjected. The studies of Seedhom et al (1979), Seedhom and Hargreaves (1979) and Jones and Metcalfe (1985) were concerned with estimating the level of stress occurring within the knee and the ankle joints during walking. The levels of stress which act typically during level walking are summarized in Table 5.9. As level walking is the most frequent of the ambulatory activities of man, these stress

Photograph 5.1 A 'Blister' on the articular surface of what otherwise was a completely healthy tibia.

Table 5.9 Overall mean creep modulus values obtained for articular cartilage and the predominant acting stress levels.

	Overall Mean Creep Modulus value MN/m^2	Predominant Stress level MN/m^2
The ankle		
Talar surfaces	11.20	2.0 - 5.0
Tibial surfaces	11.02	2.0 - 5.0
The knee		
Femoral condyles	9.48	1.5 - 2.0
Tibial condyle covered by menisci	8.59	0.6 - 2.8 (mean = 1.57)
Patellar surfaces of femur	6.28	1.0
Tibial condyle exposed by menisci	4.47	0.1 - 2.7 (mean = 0.97)

levels can therefore be considered as being representative of the predominant levels acting within the knee and ankle.

In the case of the tibial condyles, mean values have been calculated from the average stress levels obtained from a series of eight joints. The distribution of stress over the tibial condyles is largely dependent on the geometry and mechanical properties of the menisci and therefore are subject to considerable variation from joint to joint.

For example, direct contact between the femoral and tibial surfaces in areas not covered by the menisci may be prevented if the menisci are of sufficient thickness. In this situation, the cartilage in these areas of the tibia will remain unstressed. The areas underlying the menisci, however, will be subjected to high stress since the menisci are transmitting all the load.

On the other hand, if the menisci are sufficiently thin (or ruptured), only direct contact between the two condyles will occur. The stresses acting on these areas will therefore be substantial whilst cartilage in areas underlying the menisci will be unstressed. More likely, contact will occur in both areas, with a proportion of the load being transmitted through each. The predominant levels of stress acting in each area will thus depend on how the menisci distribute the load between the two areas. This will depend on their thickness and how they deform when loaded.

Table 5.9 also includes the corresponding overall mean cartilage stiffness values. These values were calculated from the mean stiffness values presented in Tables 5.1, 5.2, 5.3 and 5.4. The data

in table 5.9 have been arranged in descending order, with the stiffest areas of cartilage at the top and the softest at the bottom.

The most striking feature of these data was the marked consistency in the order of the cartilage stiffness and predominant stress values obtained for each area, when ranked. Where the predominant stresses are high, cartilage is stiff and where the predominant stresses are low, cartilage is soft. This relationship applies not only to the differences observed in the knee joint, but also to differences found between the knee and ankle joints. The difference in the level of stress to which the two joints are predominantly subjected are matched by a similar difference in the overall stiffness of the articular cartilage. It was also interesting to note the similarity in the stiffness values of cartilage from the tibial and talar surfaces of the ankle. These opposing surfaces are subjected to indentical levels of stress.

A simple regression performed on the data in Table 5.9 indicated good correlation between the stiffness of cartilage and the stress to which it is predominantly subjected. The Pearson coefficient of $r=0.934$ ($n=6$) was significant at the $p<0.01$ level.

5.4.2 Age

As many believe the degeneration of articular cartilage is directly related to age, it was interesting to examine the available cartilage stiffness data for any age related variations.

Simple linear regressions were carried out on the mean creep modulus values obtained from the main areas of cartilage in the knee (the patellar and condylar surfaces of the femur and the areas of the

tibial condyles, covered and uncovered by the menisci) with age. The results are presented in Table 5.10. In every case, the correlation coefficients were not significant (NS), indicating no direct relationship between age and stiffness.

5.4.3 Age, Sex Height and Weight.

A further examination of the data, using a multiple linear regression analysis (MLRA) to include three other variables: sex, height and weight, was also performed. The analysis enabled the effects of correlations between the three extra variables with stiffness to be eliminated prior to correlating age with stiffness. The partial correlation coefficients obtained from the analyses are summarized in Table 5.10. Once again, no significant partial correlation was found between age and the stiffness of any of four areas of cartilage. No significant correlations between sex, height and weight and stiffness were found either.

5.4.4 Cartilage thickness

As with the stiffness data, overall mean thickness values were calculated for each area of the knee and ankle joints. The two sets of overall mean values are presented in Table 5.11.

At first glance, comparisons of these overall mean values suggest an inverse relationship between the stiffness and thickness of articular cartilage, ie stiff cartilage was thin and soft cartilage was thick. Data obtained from the ankle joint also suggested an inverse relationship between stiffness and thickness. Cartilage from the ankle was both stiffer and thinner than cartilage from the knee joint.

Table 5.10 Correlation coefficients obtained from simple linear regressions of cartilage stiffness from the knee with age, and multiple regressions with age, sex, weight and height.

	n	Simple Reg Coeff.	Multiple Regression Coeffs.			
			Age	Sex	Height	Weight
Femoral condyles	10	-0.30 N.S.	-0.15 N.S.	-2.82 N.S.	-17.8 N.S.	0.19 N.S.
Patellar surfaces of knee	10	0.42 N.S.	-0.05 N.S.	-1.95 N.S.	-10.4 N.S.	0.12 N.S.
Exposed areas of tibia	10	-0.12 N.S.	-0.02 N.S.	0.09 N.S.	-5.15 N.S.	0.05 N.S.
Covered areas of tibia	10	-0.21 N.S.	-0.05 N.S.	0.81 N.S.	-7.45 N.S.	0.12 N.S.

Table 5.11 Overall mean creep modulus and thickness values obtained for cartilage from the knee joint.

	Overall Mean Thickness	Overall Mean Creep Modulus
	mm	MN/m^2
Medial femoral condyle	2.18	9.48
Medial tibial condyle covered by menisci	1.95	8.59
Lateral femoral condyle	2.55	8.47
Lateral tibial condyle covered by menisci	2.58	6.81
Patellar surfaces of the femur	2.54	6.26
Lateral tibial condyle exposed by menisci	3.30	4.61
Medial tibial condyle exposed by menisci	2.66	4.47

A more specific examination of this relationship was made using the stiffness and thickness values obtained from each cartilage site tested. The significance of the correlations coefficients obtained for each joint, are given in Table 5.12. It was particularly interesting to note that all the significant correlations found for cartilage from the surfaces of the knee were negative correlations, ie the stiffest cartilage was thinner than the softer cartilage, whilst all the significant correlations found for ankle cartilage were positive, ie the stiffer cartilage was thicker than the softer cartilage.

5.5 The biochemistry of cartilage

5.5.1 Proteoglycan

Measurements of the proteoglycan content of 214 cartilage samples were made by estimating the concentration of uronic acid present in each cartilage sample. Estimates of the proteoglycan content of cartilage ranging from from 15 to 80 μg/mg dry weight of cartilage. The values are indicated on maps of the articular surfaces in Figures 5.6(a) and 5.6(b).

There were no apparent trends in the topographical variation of the proteoglycan content of articular cartilage from the knee. Values appeared to be reasonably consistent over all the surfaces of both joints examined. There was also little difference in the proteoglycan content of corresponding areas of different joints. Likewise, proteoglycan levels across the two talar surfaces examined varied little. There was however, a marked posterior-anterior increase in the proteoglycan content of cartilage on the tibial surfaces of ankle No. 7, although there was no corresponding variation observed in the

Table 5.12 A summary of the significance of coefficients resulting from correlations made between cartilage stiffness and thickness from the surfaces of the knee and ankle joints tested. The number of each type of joint surface on which correlations were performed is indicated by n. The values in the body of the tables indicate how many were significant at each particular level, whilst the bracketed signs denote the direction of these correlations, + indicates a direct relationship, - indicates an inverse relationship.

	Knee		Ankle	
Level of significance	Femur n = 13	Tibia n = 9	Talus n = 7	Tibia n = 6
p<0.001	4 (-)	3 (-)	1 (+)	-
p<0.01	2 (-)	3 (-)	2 (+)	-
p<0.02	-	1 (-)	1 (+)	-
NS	7	2	3	6

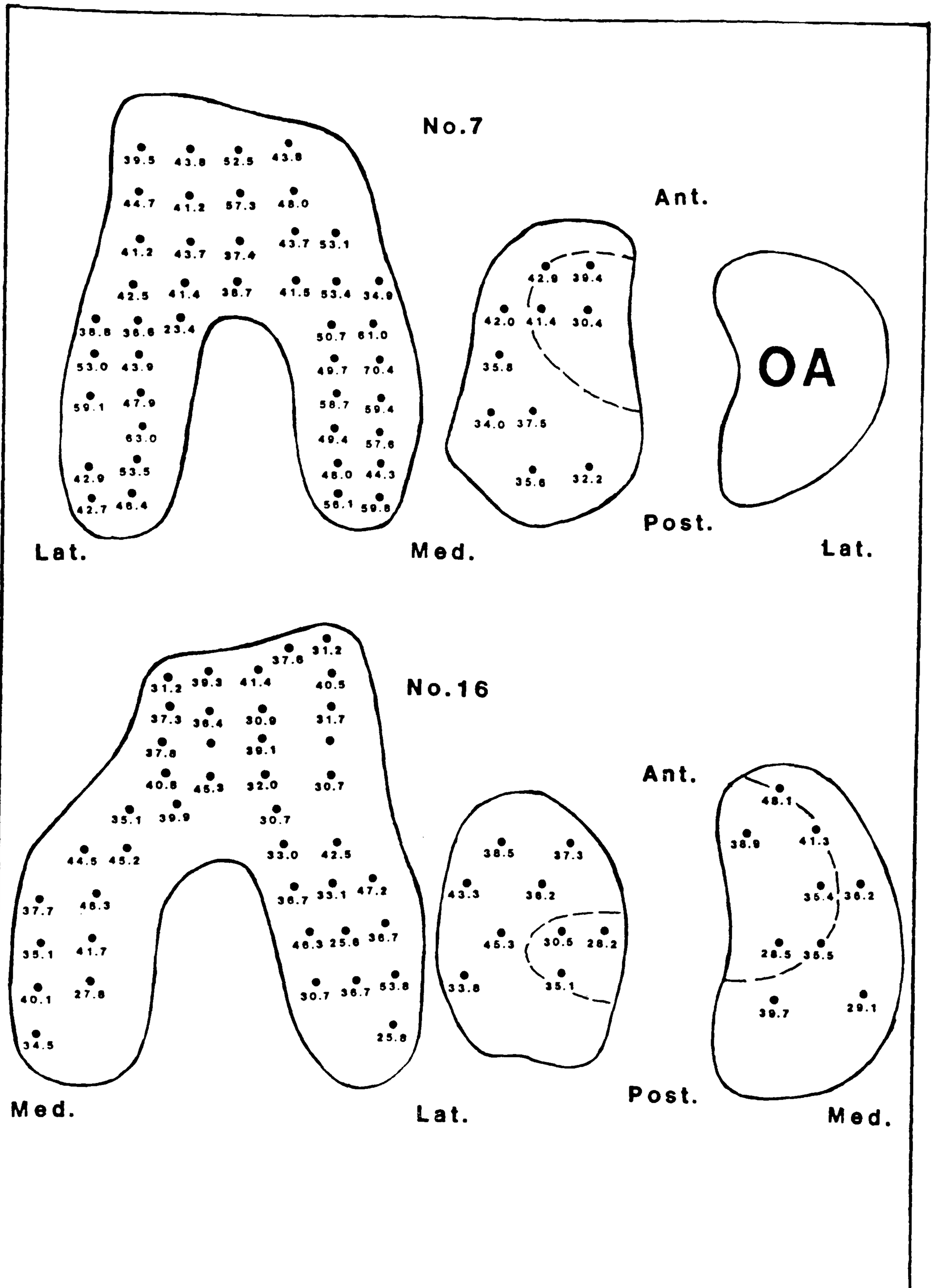


Figure 5.6(a) Maps of specimen No.7 and No.16, indicating proteoglycan content in µg of uronic acid per mg dry weight of cartilage.

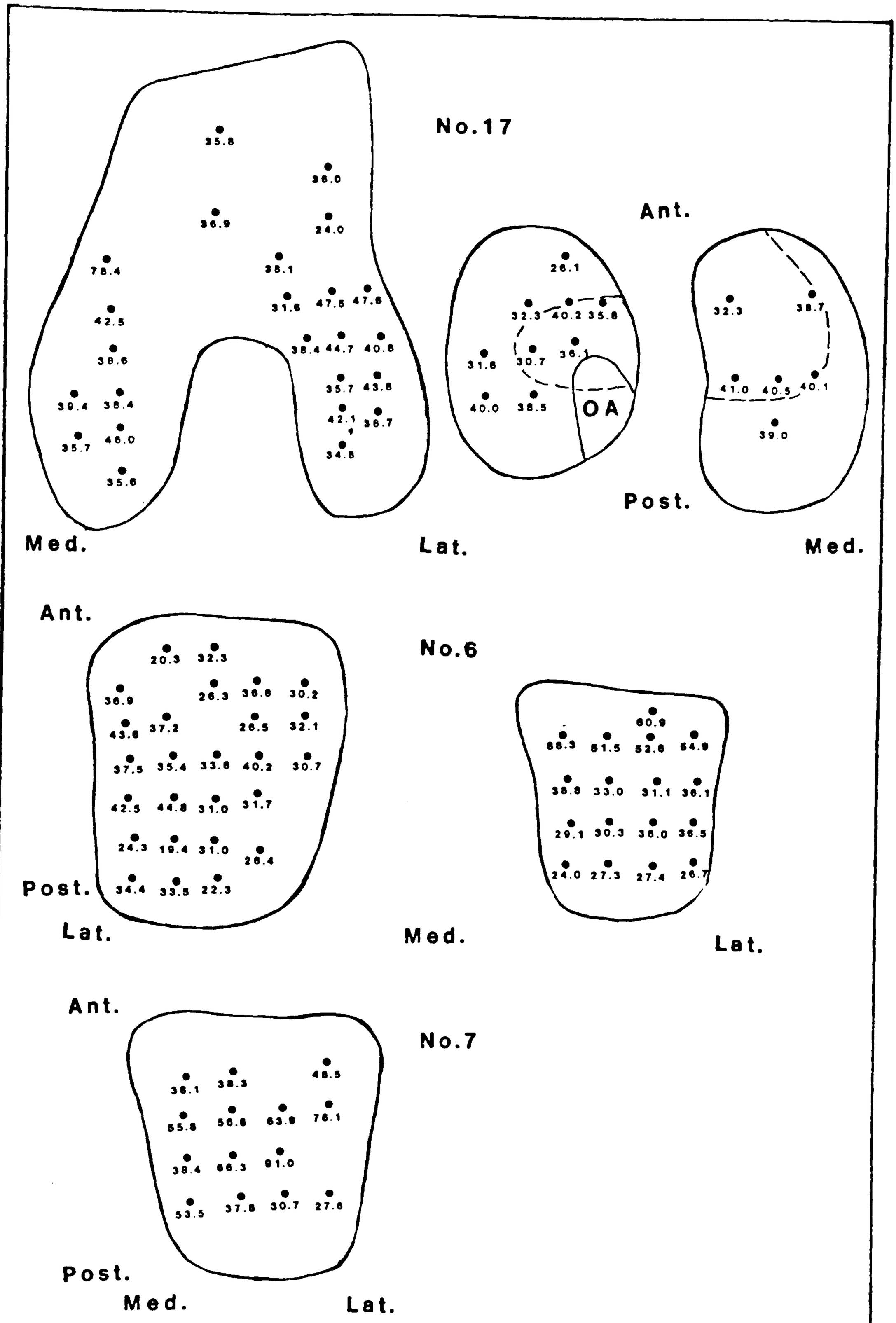


Figure 5.6(b) Maps of specimen No.17 and ankle specimen No.6 and No.7, indicating the proteoglycan content in μg of uronic acid per mg dry weight of cartilage.

cartilage stiffness of the same joint.. The proteoglycan content of ankle cartilage appeared marginally greater than the proteoglycan content of knee cartilage.

5.5.2 Collagen

Measurements of the collagen content made by assessing the hydroxyproline content of the same 214 cartilage samples. The values ranged from 10 to 140 µg/mg dry weight of cartilage. As hydroxyproline accounts for approximately 14% by weight of mammalian collagen (Jackson and Cleary, 1967), which in turn accounts for approximately 50% to 60% of the dry weight of cartilage, it should account for about 7% or 8% of the dry weight of the cartilage.

The measurements suggested that the hydroxyproline content by dry weight of cartilage was between 1% and 14%. As estimates of 1% were unrealistic of the hydroxyproline content of articular cartilage, doubt was cast on the accuracy of the measurements. Calibration of the analyser prior to and immediately following the assaying of the prepared samples, showed it to be working perfectly throughout the duration of the experiment. Problems with inadequate volumes of the samples may have contributed to the lack of consistency with which measurements were made.

5.5.3 Water

Water contents, calculated by weighing the specimen prior to and following dehydration, ranged from 55% to 75% (mean = 65.1% ± 4.7%) of the total wet weight of cartilage. In comparison with the values of approximately 70% obtained by Maroudas(1976) and Muir(1980), and 70% to 80% obtained by Armstrong and Mow (1982), these values were

slightly lower. Estimates of water content are indicated on maps of the articular surfaces from which the cartilage samples were taken in Figures 5.7(a) and 5.7(b). No distinct variations in the water content of cartilage from either the ankle or the knee joint were observed.

5.6 The biochemistry of cartilage and stiffness

5.6.1 Proteoglycan

Correlations performed on the uronic acid contents of the 214 cartilage samples with the corresponding creep modulus values revealed no significant direct relationship between the two variables. Although no direct correlation was found, the data which are shown in Figure 5.8, suggested there were definite bounds to the variations of proteoglycan content with cartilage stiffness.

The form of data suggested that the two variables could be exponentially related in the form:-

$$E = A\, e^{Pg} \quad \text{---- (5.1)}$$

Where E the calculated creep modulus, Pg the proteoglycan content and A a constant of proportionality.

The possibility of such a relationship was examined by taking natural logarithms of both sides of Equation 5.1 and plotting $\log_e E$ against Pg, the proteoglycan content, Figure 5.9. A correlation performed on the modified data indicated no significant exponential relationship between cartilage stiffness and its proteoglycan content.

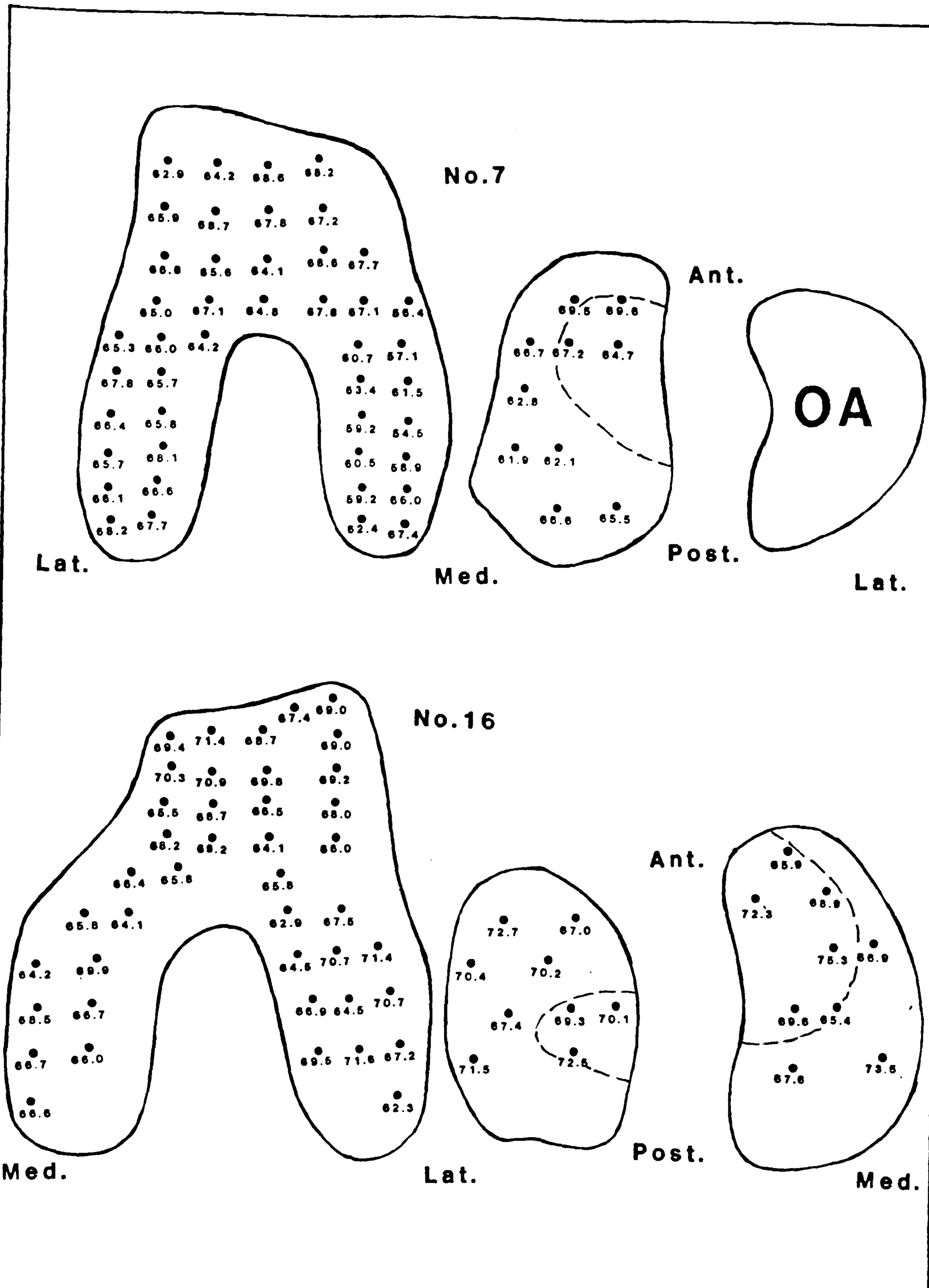


Figure 5.7(a) Maps of the water content of cartilage from specimen No.7 and No.16, expressed as a percentage of the total wet weight of cartilage.

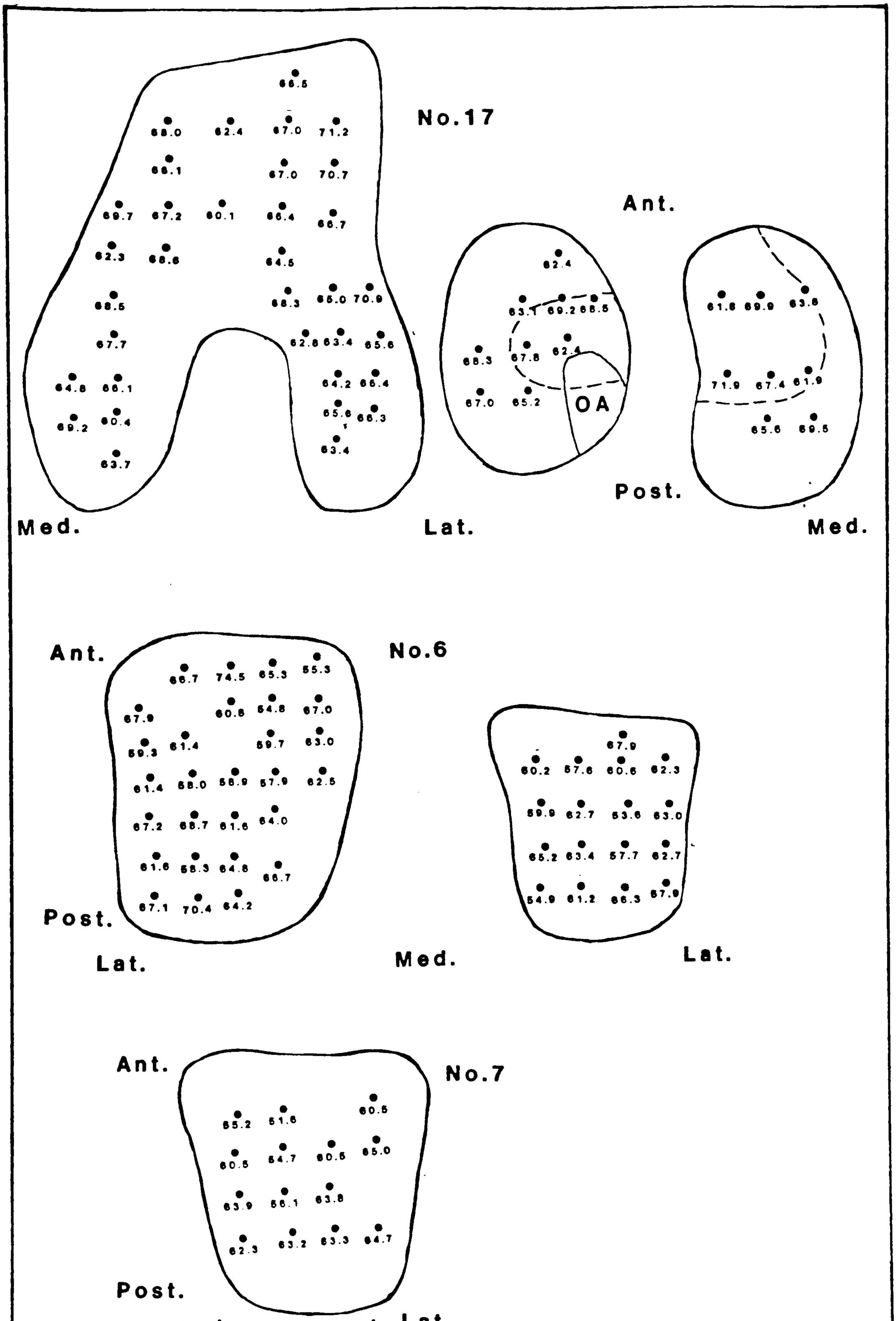


Figure 5.7(b) Maps of the water content of cartilage from specimen No.17 and ankle specimens No.6 and No.7, expressed as a percentage of the total wet weight of cartilage.

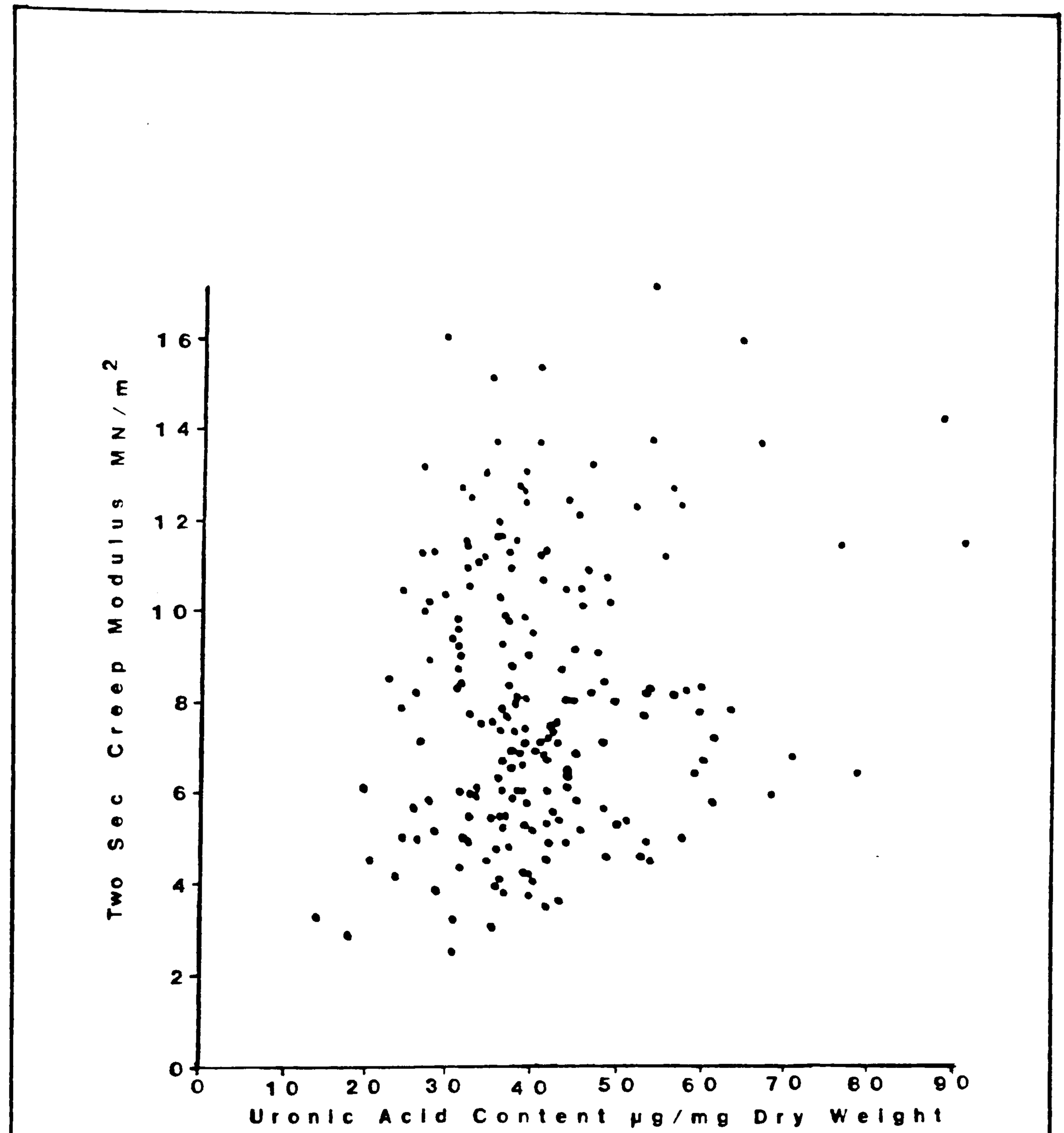


Figure 5.8 Proteoglycan content versus two second creep modulus.

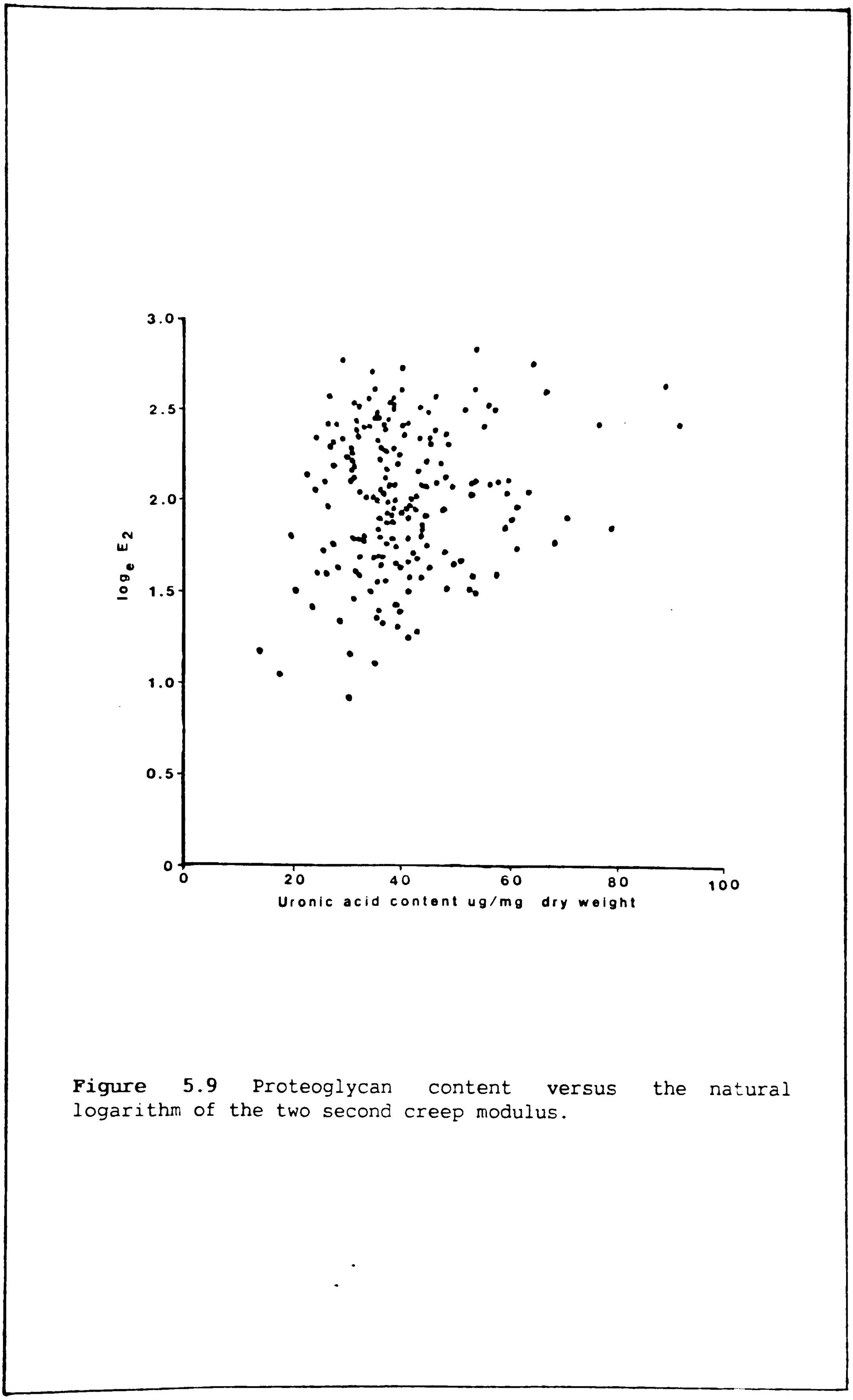


Figure 5.9 Proteoglycan content versus the natural logarithm of the two second creep modulus.

5.6.2 Water

Correlation of the water content with the two second creep modulus values, Figure 5.10, revealed no significant relationship between the two variables. This indicated that the stiffness of articular cartilage was not related to the quantity of water present.

5.7 The effects of a further empirical correction factor for finite thickness

5.7.1 Stiffness variations

In conclusion to Chapter 2, it was suggested that a further correction for finite thickness would result in modulus values which were less dependent on the thickness of the specimen being tested. Investigations showed that the formula of Waters's tended to over-correct for the thinner specimen thus resulting in underestimations of the modulus values.

The results of correlations performed on the creep modulus and thickness data have indicated that for cartilage from the knee, there was a tendency for the thinner areas of cartilage to be stiffer than the thicker areas (Section 5.4.3). Conversely, for the ankle joint the relationship between stiffness and thickness was the reverse, the thicker areas of cartilage were stiffer than the thinner areas.

Correcting the modulus values in the manner suggested (Section 2.5.2) would therefore have two effects. First, the variations in stiffness found across the surfaces of the knee would be more exaggerated as the stiffest areas of cartilage tended to be the thinnest and the softest areas tend to be the thickest. The stiffness values predicted for the thinner cartilage would be greater whilst the values for thicker cartilage would be smaller. Secondly, the variations in the stiffness

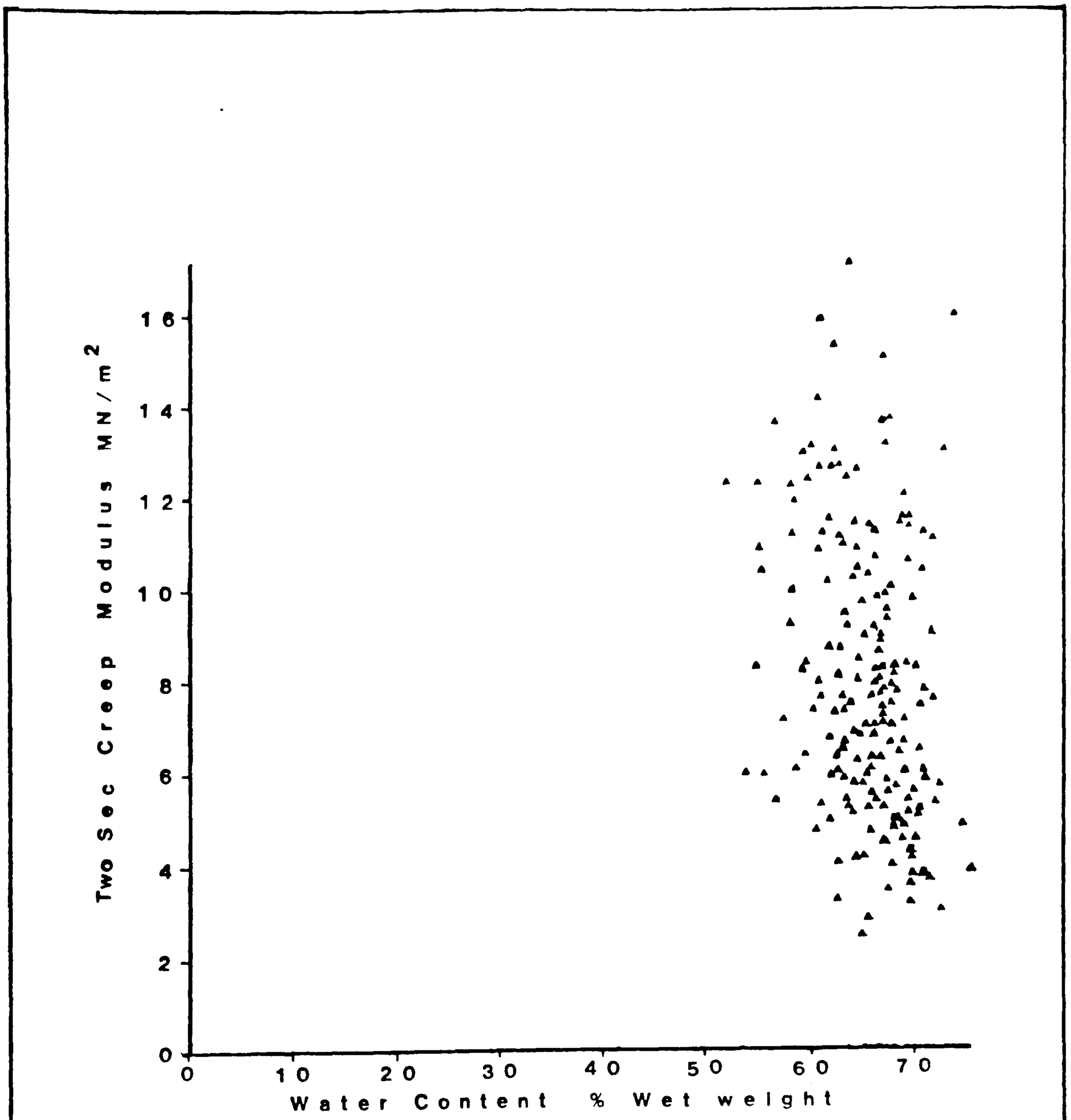


Figure 5.10 Water content versus two second creep modulus.

across the ankle would be reduced. In the ankle, the stiffest areas of cartilage were also the thickest and therefore the modulus values obtained for the thinner and softer areas would be corrected proportionately more than the modulus values obtained for the thicker and stiffer areas.

The effects of this further correction on the variations in stiffness across the knee are demonstrated by comparing the contoured maps of cartilage stiffness constructed from the original and the recalculated data, obtained for specimen Nos. 16 and 17. fgure 5.11(a) and 11(b).

The softer areas of femoral cartilage on both maps were located on the patellar surfaces whilst the stiffest areas were both situated on the condyles. The same can be said of the stiffest and softest areas of cartilage on the tibial surfaces. Both maps indicate similar locations for each. There were however one or two interesting differences worth noting. The maps constructed from recalculated data indicated that cartilage on the medial femoral condyle was stiffer than that on the lateral condyle. Such a difference was not apparent from maps of the original data. This difference reflects the significant difference found in the thickness of cartilage on the medial and lateral femoral condyles.

Similar comparisons of maps of the ankle, constructed from the original and recalculated data, Figure 5.12, demonstrate a reduction in the anterior-posterior variation in the stiffness of cartilage. Cartilage in areas along the anterior and posterior edges of the talus was still softer than cartilage from the main areas of the talar surfaces, although the variation in stiffness was reduced.

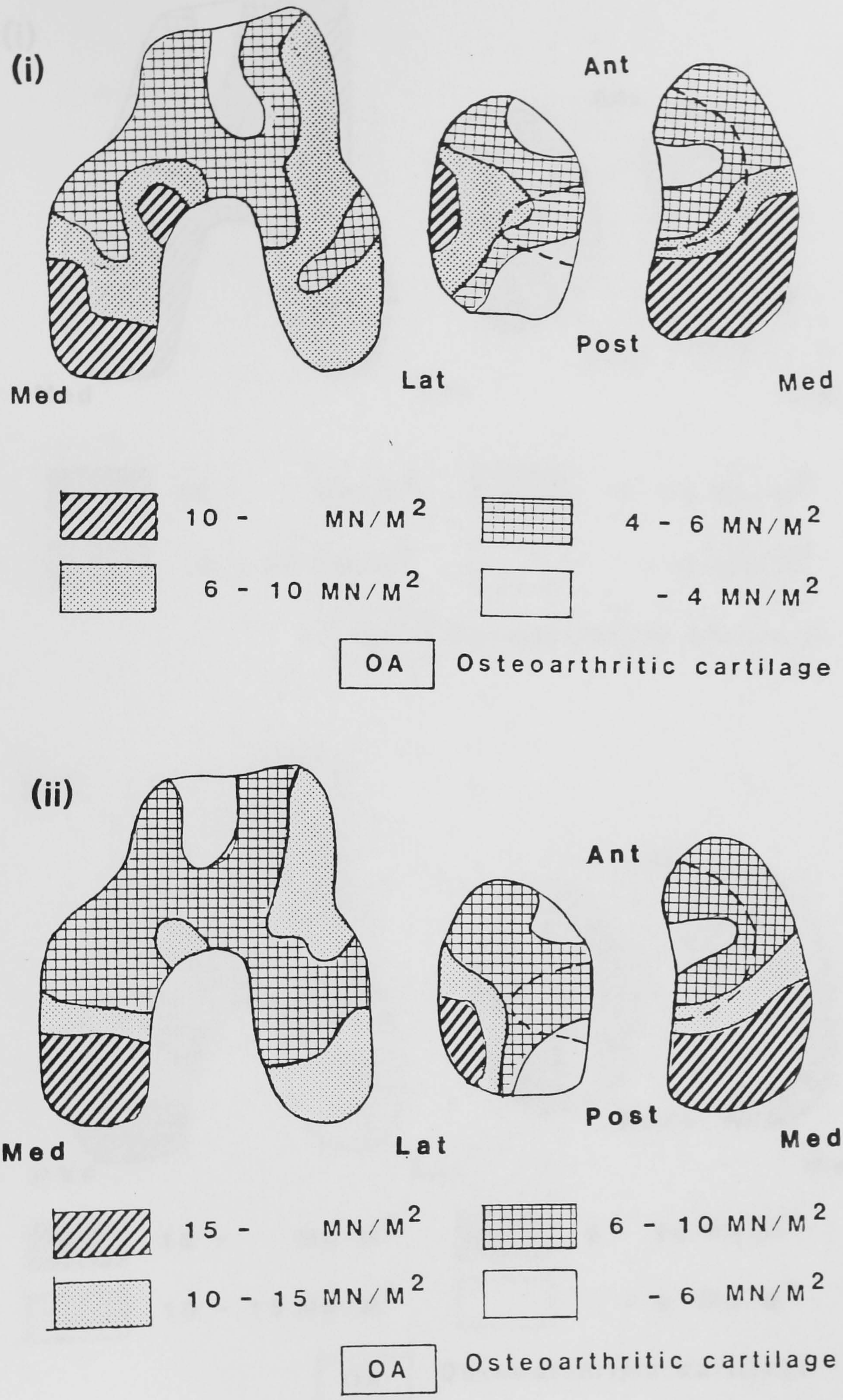


Figure 5.11(a) Comparison of the contoured stiffness maps constructed from (i) the original and (ii) the recalculated creep modulus values obtained from specimen No.16.

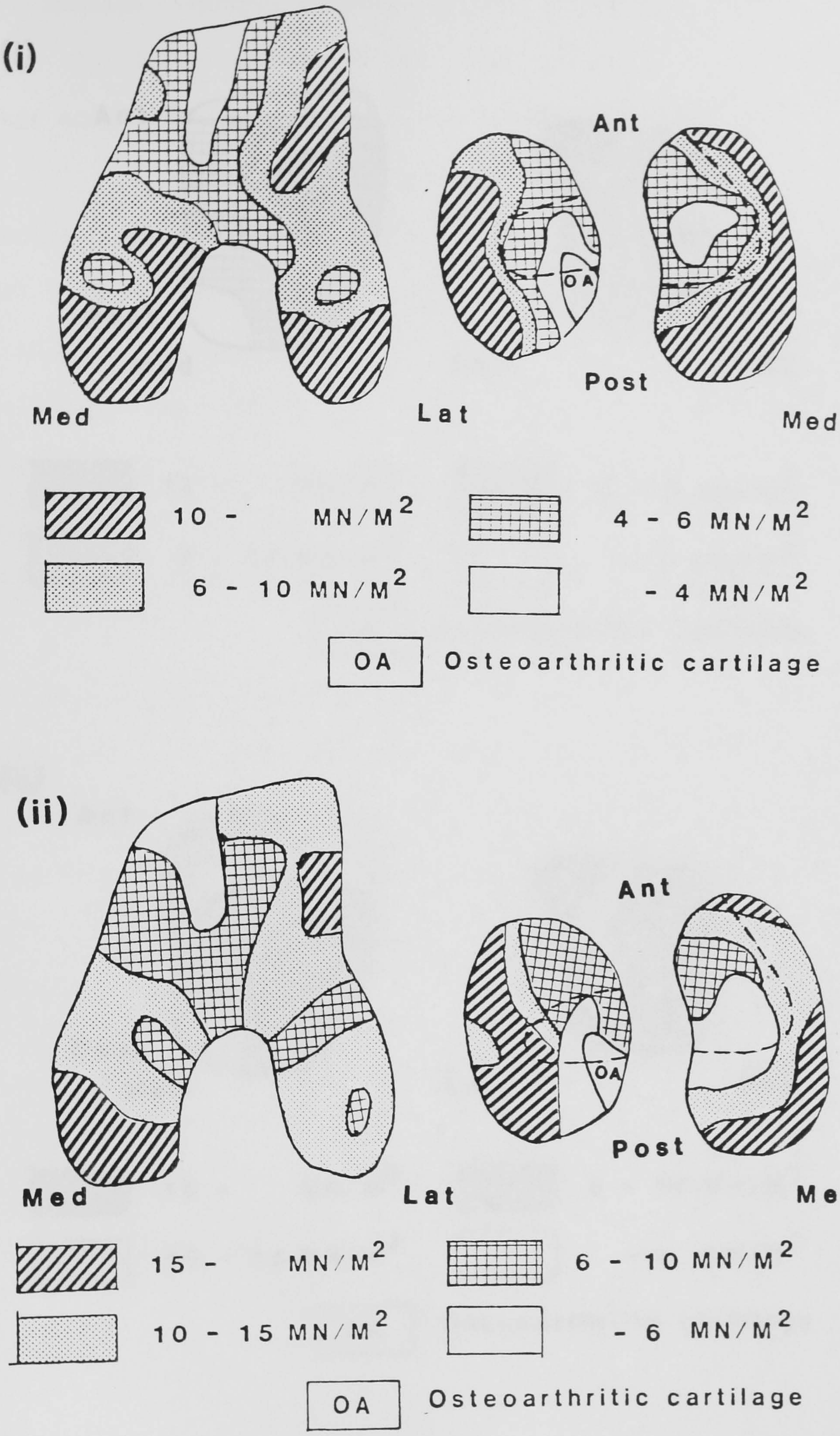


Figure 5.11(b) Comparison of the contoured stiffness maps constructed from (i) the original and (ii) the recalculated creep modulus values obtained from specimen No.17.

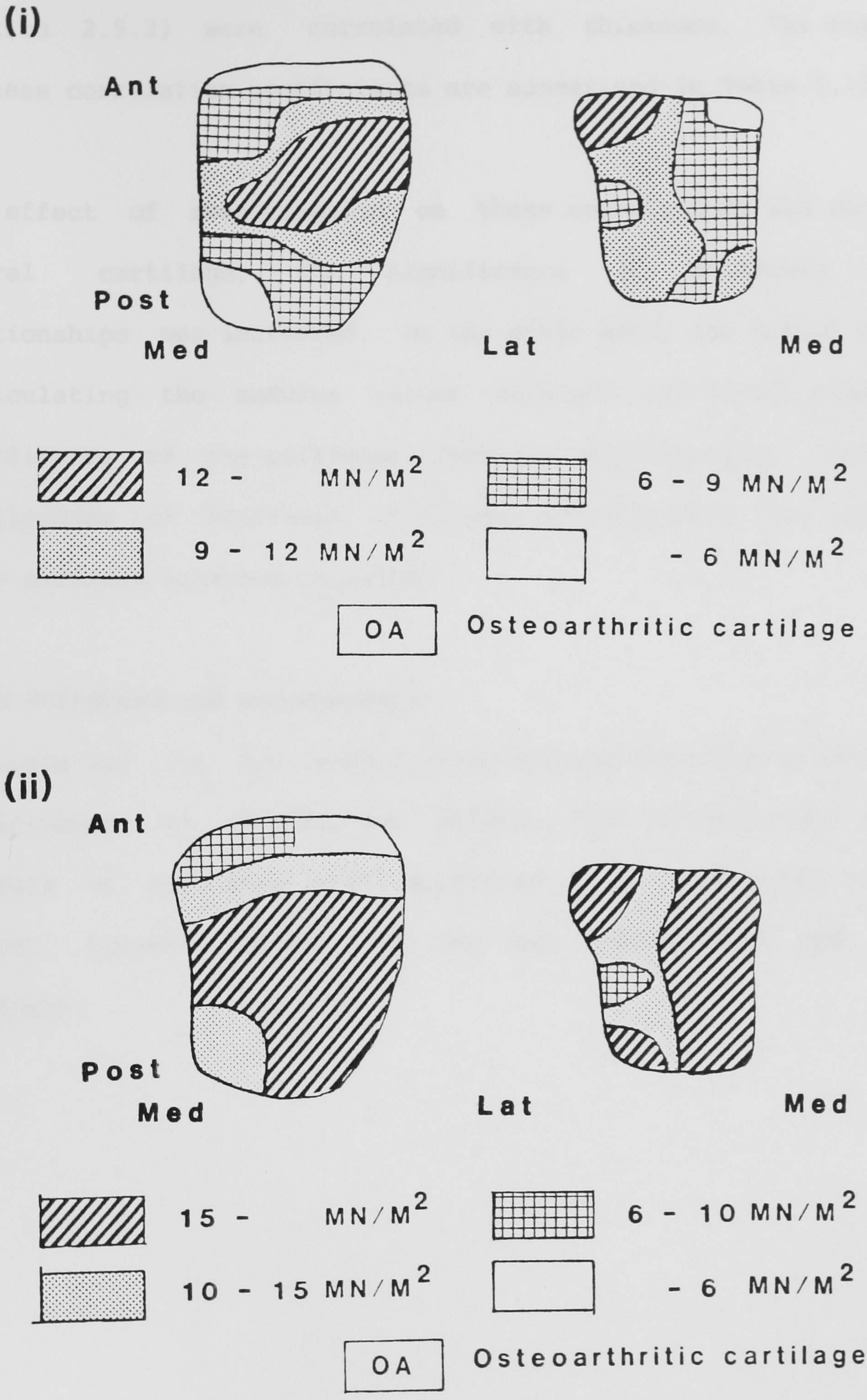


Figure 5.12 Comparison of the contoured stiffness maps constructed from (i).the original and (ii) the recalculated creep modulus values obtained from ankle specimen No.6.

5.7.2 Stiffness and thickness

Creep modulus values, recalculated in the manner suggested (Section 2.5.2) were correlated with thickness. The significance of these correlation coefficients are summarized in Table 5.13.

The effect of recalculation on these correlations was varied. For femoral cartilage, the significance of stiffness thickness relationships was increased. On the other hand, for tibial cartilage, recalculating the modulus values obtained had little effect on the significance of any stiffness thickness relationships. Likewise, the significance of stiffness thickness relationships for cartilage on talar surfaces remained unchanged.

5.7.3 Stiffness and biochemistry

Recalculating the two second creep modulus data had no effect on the significance of correlations between the proteoglycan and water contents of cartilage with stiffness. No significant correlation existed between either of the two constituents and cartilage stiffness.

Table 5.13 A summary of the significance of coefficients resulting from correlations made between the recalculated cartilage stiffness data and thickness. The number of each type of joint surface on which correlations were performed is indicated by n. The values in the body of the tables indicate how many were significant at each particular level, whilst the bracketed signs denote the direction of these correlations, + indicates a direct relationship, - indicates an inverse relationship.

	Knee		Ankle	
Level of significance	Femur	Tibia	Talus	Tibia
	n = 11	n = 10	n = 9	n = 8
p<0.001	4 (-)	6 (-)	-	-
p<0.01	3 (-)	-	2 (+)	-
p<0.02	-	-	1 (+)	-
p<0.05	3 (-)	2 (-)	-	2 (+)
NS	1	2	6	6

CHAPTER 6

DISCUSSION

6.1 The indentation test

6.2 Cartilage stiffness and stress

6.3 Stress and osteoarthrosis

6.4 Mechanical hypothesis of osteoarthrosis

6.5 Biochemical factors and cartilage stiffness

6.6 Suggestions for further investigation

(a) A model of osteoarthrosis

(b) Load distribution in the tibio-femoral compartment

DISCUSSION

The major concern of the present study was to examine an hypothesis regarding the role of mechanical stress in the aetiology of osteoarthrosis. The hypothesis in question (Seedhom et al,1979) suggests that osteoarthrosis is triggered by a peculiar pattern of mechanical stressing. The pattern of stressing is one whereby cartilage is subjected predominantly to one particular level of stress, but for less frequent periods is subjected to much larger stresses. It was suggested that in such circumstances, cartilage is mechanically conditioned, enabling it to transmit the predominant stress level without sustaining damage and that in the event of large and infrequent stresses cartilage suffers damage.

As a corollary to this hypothesis, it was suggested that the compressive stiffness of normal articular cartilage is related to the predominant level of stress to which it is subjected. Accordingly, cartilage predominantly subjected to high stresses should be stiffer than cartilage predominantly subjected to low stresses. Examination of this hypothesis in the present study has concentrated on this corollary. The primary objective was therefore to establish whether or not the mechanical properties of normal articular cartilage were related to the predominant levels of stress to which it was subjected.This was accomplished by surveying the compressive stiffness of visably normal articular cartilage from the knee and ankle joints and comparing mean values for the stiffness of cartilage with the predominant levels of stress to which cartilage is subjected during various normal ambulatory activities. Following these comprehensive surveys of the stiffness of knee and ankle joint

cartilage, a study of the biochemical constituents of cartilage was made. Proteoglycan, collagen and water contents were assessed. It was hoped to further elucidate the relationship between the mechanical properties of cartilage and its biochemical composition and perhaps suggest a mechanism through which cartilage may adapt to mechanical stress in the way the above mentioned hypothesis has suggested.

6.1 The indentation test

Values for the compressive stiffness of articular cartilage were obtained using a radically improved indentation technique. The indentation test was chosen as it provides a speedy and convenient non-destructive method for testing cartilage in situ on its subchondral bone substratum.

Measurements of indentation depths could be made to an accuracy of 0.005 mm with a coefficient of variation of 2.4%. Cartilage thickness measurements could be made to an accuracy of 0.012 mm with a coefficient of variation of 1.2%. These figures represent a dramatic improvement in the accuracy and repeatability with which both the indentation and cartilage thickness could be measured.

The formula of Waters (1965) relating indentation depth and undeformed cartilage thickness to a time dependent elastic modulus was used to obtain approximate values for the compressive stiffness of cartilage. The effect of these maximum possible errors in the indentation and thickness measurements on the calculated creep modulus was estimated at 4% and 2.9%.

Experiments performed on thin sheets of rubber demonstrated that correction for finite specimen thickness dramatically reduced the degree to which calculated modulus values depended on specimen thickness. There was still however a tendency for modulus values obtained for thinner specimens to be underestimated more than values obtained for thicker specimens.

Errors in the calculated modulus values arising as a consequence of the anisotropy and non-homogeneity of articular cartilage are more difficult to assess. However, consideration of available data in the literature indicated that the magnitude of such errors were insufficient to deter the use of the indentation test for a comparative study of the mechanical properties of cartilage.

6.2 Cartilage stiffness and stress

Comparisons between the mean creep modulus values and the levels of stress which occur within the knee and ankle joints during normal ambulatory activity, indicated that the stiffness of normal articular cartilage was directly related to the level of stress to which it is predominantly subjected. The more highly stressed the cartilage, the stiffer the cartilage This direct relationship was significant at the 1% level.

It was evident from the marked consistency of the stress-stiffness relationship (Table 5.9) that the level of mechanical stress was influential in determining the stiffness of normal articular cartilage. In particular, the predominant level of stress acting appears to be important. This finding is in keeping with the hypothesis of Seedhom et al (1979) regarding mechanical stress and the aetiology of osteoarthrosis.

Data regarding the stresses acting in the hip during level walking (Adams and Stevenson, 1985; Afoke et al, 1986) and the stiffness of normal articular cartilage on the femoral head (Kempson et al, 1971a) further emphasize this direct relationship between stress and cartilage stiffness. Kempson found the stiffest areas of cartilage on the femoral head to have modulus values ranging from 10 - 14 MN/M^2. These areas extended from the superior surfaces of the femoral head around both the anterior and posterior aspects, and coincided with the most highly stressed areas of the femoral head during level walking. Estimates of the typical stresses ranged from 6.8 to 10 MN/M^2. It should be noted that both the stress levels and the values for the stiffness of the articular cartilage were in excess of values obtained from areas of cartilage in either the knee or the ankle. The more lowly stressed areas of cartilage on the surfaces of the femoral head, in particular those areas surrounding the fovea, were correspondingly much softer.

On examining the stiffness variations across both the tibial and femoral condyles in the knee joint, it was interesting to note that there were no consistent medial-lateral differences in the stiffness of the cartilage. In view of the consistency found in the relationship between stress and cartilage stiffness, one might extrapolate that the predominant stresses acting on the articular surfaces in the lateral and medial compartments of the tibio-femoral joint are similar. As the contact areas in the medial compartment are larger than those in the lateral compartment (Maquet et al, 1975), it follows that a greater proportion of tibio-femoral loads are transmitted through the medial compartment. There is however little experimental data regarding what proportion of tibio-femoral

loads are transmitted through the medial and lateral compartments. Bearing in mind however, the relative position of the hip, knee and ankle joints, especially during the stance phase of normal ambulation, and that a greater number of adductor to abductor muscles have attachments at the knee, it is quite possible that loads are eccentrically transmitted across the knee joint, with the medial compartment being subjected to a larger proportion. This fact may explain the more progressive nature of medial compartment osteoarthrosis.

6.3 Stress and osteoarthrosis

In their study of the location of osteoarthrotic lesions in the patello-femoral compartment of the knee, Seedhom et al (1979) indicated that the areas most prone to osteoarthrosis were those predominantly subjected to stresses of approximately 1 MN/M^2 but less frequently to much high stresses in excess of 4 MN/M^2. Huberti and Hayes (1984) subsequently reported that the highest stresses acting in the patello-femoral joint occur at angles of flexion of 60°-90°, largely confirming the earlier observations of Seedhom and colleagues. If such patterns of stress are responsible for initiating osteoarthrosis, it would follow that other areas, also regularly exhibiting osteoarthrosis, should also be subjected to such patterns of stress.

On the tibial condyles, osteoarthrotic lesions are commonly seen in areas exposed to direct contact with the femur. The stresses acting over the tibial surfaces are to a large extent dependent on the load bearing characteristics of the menisci. Seedhom et al (1981) outlined certain circumstances in which it was possible for these

areas to be subjected to patterns of stress similar to those (Seedhom et al,1979) responsible for initiating osteoarthrosis. The fact that such patterns of stress do not necessarily occur, however, perhaps explains why the frequency with which osteoarthrosis occurs in these areas of the tibial condyles is less than that of patello-femoral osteoarthrosis.

Osteoarthrosis is also regularly reported following meniscectomy (Fairbank, 1948; Jackson, 1968). Following the removal of the menisci, reductions in the size of contact areas between the femur and tibia (Maquet et al, 1975; Fukubayashi and Kurosawa, 1980) can results in large increases in the stresses acting on the cartilage. If the cartilage is unaccustomed to the sudden increase in stress to which it is subjected following meniscectomy, it is likely to become damaged. If prior to meniscectomy, a large proportion of the load is transmitted directly between the femoral and tibial condyles, the likelihood of degenerative changes occurring in these areas of cartilage exposed by the menisci will be reduced. In such cases the uncovered cartilage will have already been regularly subjected to considerable levels of stress and therefore the increase in stress associated with the removal of the menisci will not be so dramatic.

Where prior to meniscectomy the larger proportion of the load is transmitted through the menisci rather than directly between the two opposing condyles, the likelihood of degenerative changes occurring in areas of direct articular contact following meniscectomy are increased. In such cases, relatively unstressed areas of cartilage are suddenly subject to considerably higher stresses. The cartilage is therefore likely to be unable to transmit these higher levels of stress without incurring damage.

A considerable number of osteoarthrotic lesions were also found in the medial posterior areas on the lateral tibial condyle. At full extension, most of these areas would be covered by the lateral meniscus. Such areas are typically subjected to stresses ranging from 0.63 to 2.83 MN/M^2 (Seedhom and Hargreaves, 1979). As the knee is flexed, internal rotation of the tibia and a large active posterior movement of the lateral meniscus exposes more posterior areas of the tibial condyle to direct contact with femur. The joint becomes less congruent, resulting in smaller areas of contact (Maquet et al, 1975). Although loads acting in the tibio-femoral compartment during activities where the knee is typically flexed through angles of 80° or more (such as in climbing and descending stairs and squatting or crouching) are much the same as those acting during level walking, the levels of stress are increased because of the smaller contact areas. As the frequency of such activities is low compared with level walking, the cartilage is likely to be unaccustomed to these increased stresses and consequentially, more prone to developing osteoarthrosis.

A further observation which supports the hypothesis of Seedhom et al (1977), was the absence of osteoarthrotic lesions in the ankle. During level walking, the ankle is a highly stressed joint. Jones and Metcalfe (1985) estimated stresses to be in the region of 2.0 - 5.0 MN/M^2. Unlike the knee, however, the stresses in the ankle are consistently high. The patterns of stress suspected of disposing cartilage in the knee to osteoarthrosis do not occur. This is also consistent with the fact that the incidence of osteoarthrosis in the ankle is very low.

6.4 Mechanical hypothesis of osteoarthrosis

It was suggested by Radin (1973) that stiffening of the underlying subchondral bone was the primary aetiological event in osteoarthrosis. His model of osteoarthrosis however must be regarded with some reservation. Regimes of repetitive impact loading which he demonstrated to cause stiffening of the subchondral bone, are most unlikely to occur during the normal activities of man. Although Radin (1980) went on to document associated mechanical and biochemical changes in both the underlying bone and the cartilage, their relevance to the aetiology of osteoarthrosis is somewhat diminished by the non-physiological nature of the loads used to induce them.

Another factor which has been suggested as the initial aetiological event in osteoarthrosis is the fatigue failure of the collagenous structures in articular cartilage Freeman(1972). Weightman(1976) and Weightman et al(1978) attempted to substantiate these ideas by performing tensile fatigue tests on large cartilage samples. Although a degree of age relation was found in the fatigue failure stress of the cartilage samples, the data exhibited considerable scatter which detracted from the significance of the observed age relationship.

Furthermore, if collagen fatigue failure was a significant factor in osteoarthrosis, one might also expect the onset of the disease to be age related and perhaps the stiffness of normal articular cartilage to decrease with increased age. There is however distinct evidence which is contradictory of both these suppositions.

Considerable age variation in the occurrence of osteoarthrosis in the patello-femoral compartment was observed by Seedhom et al (1979). Osteoarthrotic lesions were found in specimens in as early as their second decade whilst many older specimens exhibited extremely healthy cartilage. Likewise, there was no evidence from the present study to suggest any age related variation in the stiffness of normal articular cartilage.

The difference in the incidences of osteoarthrosis in the knee and in the ankle joints also contradicts the idea that collagen fatigue failure is the primary event in osteoarthrosis. Cartilage in the ankle joint is subjected to an identical number of stress cycles compared with cartilage in the knee. The mean level of stress to which cartilage in the ankle is subjected is also considerably higher than that in the knee. This would imply, if collagen fatigue failure was important, that the ankle would be more prone to osteoarthrosis. In reality, the ankle has a very low incidence of osteoarthrosis.

Although clinically, it is perhaps apparent that osteoarthrosis is an age related disease, such observations are of the latter stages of what is a long and complex sequence of events, rather than the initial aetiological events. This fact perhaps precludes the real extent to which osteoarthrosis is age related.

Kempson (1971b) suggested that proteoglycan depletion preceded any other change in cartilage in osteoarthrosis. In such cases, Kempson suggested that the collagen matrix became more vulnerable to failure because of increased tensile stresses in the collagen fibres especially in the superficial layers. Studies made of osteoarthrotic

cartilage (McDevitt and Muir, 1976; Byers et al, 1977) suggest, however, that an increase in the water content is the first compositional change in osteoarthrosis. Such a change reflects a disruption in the collagenous structures rather than any alteration in the proteoglycan content. Goodfellow et al (1976) in his investigations of the patello-femoral joint also came to similar conclusions regarding the significance of collagen failure in cartilage degeneration. He again suggested that collagen failure preceded any changes in the proteoglycan content. Observations of surface blisters similar to those described by Goodfellow et al(1976) have made during the present study on the surfaces of two otherwise health tibiae. Such observations suggest a disruption of the collagen fibres, particularly in the deeper layers of the cartilage layer.

Having argued the case for deep layer collagen failure as the primary event in osteoarthrosis, it seems appropriate to address the problem of establishing a criterion for its failure.Fatigue failure has already been considered and is not regarded as an important factor. As little is known about the magnitude of the physiological tensile stresses acting in the collagen network, it is difficult to assess the relevance of the fatigue results of Weightman (1976) and Weightman et al (1978).

An alternative attempt to analyse the failure of thin cartilage layers was made by Armstrong (1986). He adopted a maximum shear stress or Tresca yield criterion for the failure of the cartilage and showed that the maximum shear stresses occurred at the junction of the cartilage with the subchondral bone, beneath the point of maximum

pressure gradient. Such an analysis supports the ideas of Goodfellow (1976) regarding collagen failure in the deeper layers.

It is also interesting to note that, according to the model of Armstrong (1986), the values of the maximum shear stress are directly proportional to the thickness of the cartilage layer. Osteoarthrotic lesions found on the patellar surfaces of the femur and on the tibial condyles are generally located in areas of the greatest thickness. This may be a further factor which topographically disposes cartilage to degenerative changes.

In considering the aetiological factors important in osteoarthrosis it is insufficient to examine any one factor, such as stress, in isolation. Osteoarthrosis is a multifactorial degenerative disorder and in reality, biochemical and enzymatic changes also occur. These changes may or may not be a consequence of a preceding mechanical factor, but either way could be significant in their contribution to causing degenerative changes in articular cartilage. Possible pathways through which mechanical and biochemical changes in osteoarthrosis occur have been summarized conveniently in the form of a flow chart (Byers et al,1983). It is also worthwhile noting that the importance of any one factor will vary from individual to individual. As a consequence, the rate at which degeneration occurs and the extent to which the disease progresses will vary.

6.5 Biochemical factors and cartilage stiffness

Although intuitively, the stiffness of articular cartilage is understood to be a function of how the collagen, proteoglycan and water interact with each other, attempts to correlate specific

measurements of the biochemical constituents with the mechanical properties of articular cartilage have been largely inconclusive. One popular thesis suggests cartilage stiffness to be directly related to the proteoglycan content. Correlations performed in the present study however could not substantiate such a relationship. No significant correlation, direct or exponential, was found. The results demonstrated however certain limitations to the variation in proteoglycan content with stiffness.

It is suggested that other factors, relating to the structure and composition of the collagen and proteoglycan, rather than to the precise quantities of each constituent present, are more influential in determining the stiffness of cartilage.

The integrity of the collagen network, the size of the proteoglycan aggregates, their composition, the degree of collagen cross-linking and the degree to which collagen and proteoglycan are chemically bound are all possible factors which effect the stiffness of cartilage without necessarily affecting the proteoglycan or collagen contents of the cartilage.

Establishing the extent to which each of these factors is important in determining the stiffness of cartilage is a fundamental element to furthering our understanding of cartilage mechanics. Likewise is the understanding of the mechanisms which are involved in the maintenance of normal healthy articular cartilage. Answers to these questions will undoubtedly help in understanding the mechanical and biochemical processes involved in the aetiology and pathgenesis of osteoarthrosis.

In summary, aetiologically it appears that infrequent high stresses are important in initiating osteoarthrosis. Surveys of cartilage stiffness indicate a direct stress cartilage stiffness relationship which is consistent with this hypothesis. The initial insult resulting from these high stresses is almost certainly to the collagenous structures. Gross alterations in proteoglycan and water contents of cartilage appear to occur subsequently to such changes. It is probable that failure initially occurs in the deeper layers rather than superficially. Collagen fatigue is, however, not regarded as being the mechanism through which failure occurs. The possibility that variations in subchondral bone stiffness may dispose cartilage to osteoarthrosis cannot be over-looked. It is unlikely, however, that the stresses associated with repetitive impact loading (Radin, 1973) are aetiologically important in osteoarthrosis, as the frequency with which repetitive loading occurs is low.

6.6 Suggestions for further investigation

(a) A model of osteoarthrosis

The evidence collected in favour of the hypothesis regarding stress and cartilage stiffness is based on the statistical comparison of two relatively small data samples obtained from separate studies. An animal model involving special exercise routines, designed to dispose a joint or part of a joint to a known level of stressing, would provide an opportunity to observe directly cartilage stiffness adaptation to stress. Such a model could also be used to demonstrate the damaging effects of infrequent stresses in excess of predominant levels. An exercise routine whereby cartilage is subjected for the most part to low stresses but infrequently to higher stresses could

be used to induce osteoarthritic changes in the cartilage. Other animals models, used in similar studies of the aetio-pathogenesis of osteoarthrosis (Pond and Nuki, 1973; Radin, 1980) resulted in osteoarthrotic-like lesions, but required atypical articular alterations (Anterior cruciate ligament incision) or loading regimes (cyclic impact loading). There is some doubt, therefore, as to whether or not such models accurately mimic the aetiological events of osteoarthrosis. A model utilizing controlled stressing to generate osteoarthrosis would therefore result in a more physiologically realistic aetiology.

This type of model could also be used as the basis for further biochemical studies of osteoarthrotic cartilage aimed at understanding the important factors which contribute to the stiffness of cartilage and allow it to adapt to changes in mechanical stress. As a consequence, a fuller explanation of the biochemical factors involved in osteoarthrosis might ensue.

(b) Load distribution in the tibio-femoral compartment.

Although a great deal of experimental work aimed at investigating how the menisci distribute loads across the tibial surafces of the knee joint has been perfrmed, there remain many controversies. As it is not known what proportion of the tibio-femoral loads are transmitted through the medial and the lateral compartments, efforts to determine what loads each meniscus transmitS within their respective compartment has only limited value. In the context of the present work, futher study of the distribution of loads between the lateral and medial compartments of the tibio-femoral joint would undoubtable enable a more complete assessment of the stiffness stress relationship to be made

CONCLUSIONS

1 A testing apparatus has been developed which allows accurate measurement of both depths of indentation and the undeformed thickness of articular cartilage. Tests of the apparatus indicated significant improvements in the accuracy and repeatability of both the measurement techniques over previously employed techniques.

2 The use of modulus values, calculated using the formula of Waters (1965), for making topographical comparisons of cartilage stiffness was justified assuming a standard radius of the indenter and the size of the applied load used. Although corrected for finite thickness, the size of the calculated modulus values remained partially dependent on the specimen thickness. This however did not preclude their use in the comparison of the stiffness of articular cartilage.

3 Significant topographical variations in the compressive stiffness and the thickness of normal articular cartilage have been identified. Femoral condylar cartilage was stiffer than cartilage situated on the patella surfaces of the femur. Tibial cartilage underlying the menisci was stiffer than the cartilage left exposed by the menisci. Cartilage from the ankle joint was generally stiffer than that from the knee.

The thinest cartilage on the femur was found on the medial condyle, whilst on each of the tibial condyles, the thickest areas appeared to be those exposed by the menisci. There was

also a tendency for the lateral condyle to exhibit thicker cartilage than the medial condyle. Cartilage in the ankle was significantly thinner than that found in the knee.

4 A direct relationship has been found between the level of stress to which normal articular cartilage is subjected and its compressive stiffness. Highly stressed cartilage is stiffer than lowly stressed cartilage. This finding directly supports the hypothesis of osteoarthrosis proposed by Seedhom et al (1979).

5 No significant correlation was found between the proteoglycan content and the compressive stiffness of normal articularcartilage. This suggests that the integrity of the collagenous structure plays a more prominent role in determining the compressive stiffness of articular cartilage.

6 The initial aetiological change in osteoarthrosis appears to be to the collagenous structures rather than to the proteoglycan. The evidence suggests that high but infrequent stresses are responsible for this damage. It appears unlikely that the initial collagen failure occurs through fatigue, or as a consequence of increased stresses resulting from an increase in the stiffness of the underlying subchondral bone.

DAMAGED
TEXT
IN
ORIGINAL

APPENDIX A.

COMPUTER PROGRAMMES.

```
program channelchecker (input,output);

label 99;

type
   bits = array[1..2] of integer;
   dat  = array[1..20] of integer;
var
   prim,time,stat,flag,nbytes,nwords      : integer;
   an32ad,hscad,nochans,i                 : integer;
   data                                   : dat    ;
   an32,hsc                               : bits   ;

procedure nuo488(var ti,st,fl,nb,di,sa,pa : integer);extern;
procedure nuo488(var ti,st,fl,nb,di,sa,pa : integer);extern;
procedure tas488(var ti,st,fl,nb,di,sa,pa : integer);extern;

begin
    prim:=7;
    hscad:=0;
    an32ad:=1;
    flag:=0;

    write( no. channels      );
    readln(nochans);

    hsc[1]:=8+3;
    hsc[2]:=1;
    nbytes:=2;
    nuo488(time,stat,flag,nbytes,hsc[1],hscad,prim);

    an32[1]:=(nochans-1)*4+2;
    nbytes:=1;
    nuo488(time,stat,flag,nbytes,an32[1],an32ad,prim);

    flag:=129;
    nwords:=nochans;
99:
    tas488(time,stat,flag,nwords,data[1],an32ad,prim);

    for i := 1 to nochans do
        write(data[i]);
    writeln;
    goto 99;
end.
```

```
program indentation_data_logger (input,output) ;
const
    bell  = chr(7) ;
type
    bit = array[1..3] of integer;
    bits = array[1..10,1..2] of integer;
    units = array[0..10] of integer;
    results = array[1..8000] of integer;
    listpost = array[1..100] of integer;
var
    byte1,byte2,nochans,nomodes,trigchan     :integer;
    time,stat,flag0,flag129,nbytes,nowords   :integer;
    hscad,an32ad,prim,notests                :integer;
    i4,i5,i6,i7,i8,i9,i10                    :integer;
    clockfreq,i1,i2,i3,checkout,x1           :integer;
    filename                                 :string[12] ;
    datafile                                 :file of integer   ;
    tiletile                                 :text   ;
    adait,char1,space_bar                    :char   ;
    xsize,ysize,xpost,ypost,letter,trchar    :char   ;
    trig                                     :bit    ;
    hsc,an32                                 :bits   ;
    data                                     :results ;
    nosamps,frequency,samplesize,point       :units  ;
    timeperiod,rate                          :real   ;
    xrecord,yrecord                          :listpost ;

procedure nuc488(var ti,st,fl,nb,da,sa,pa : integer);extern;
procedure nui488(var ti,st,fl,nb,da,sa,pa : integer);extern;
procedure tas488(var ti,st,fl,nb,da,sa,pa : integer);extern;
function dosxqq(command,parameter : word): byte;extern;
function mlOrqqia : real ; i : integer) : real ; extern ;

procedure declare_variables ;
begin
    byte1 := 1;
    byte2 := 2;
    flag0 := 0;
    flag129 := 129;
    prim := 7;
    hscad := 0;
    an32ad := 1;
    trigchan := 1;
    nochans := 2;
    nomodes := 2;
    nosamps[1] := 500;
    nosamps[2] := 100;
    frequency[1] := 1000;
    frequency[2] := 10;
end;

procedure empty_buffer;
const  empty = chr(0);
var    buffer : char  ;
begin
    repeat
    buffer := chr(dosxqq(6,255));
    until buffer = empty ;
end;

procedure get_exper_code;
begin
    notests := 0;
    write(  enter experimental code letter  );
    empty_buffer;
        repeat
            repeat
            letter:=chr(dosxqq(6,255));
            until letter <> chr( 0 );
        until ( letter >= chr( a ) ) and ( letter <= chr( z ) );
    write(letter);
```

```
end;

procedure get_second_char;
begin
    write( enter (t) for tibia, (f) for femur     );
    empty_buffer;
        repeat
            repeat
            tfchar:=chr(dosxqq(6,255));
            until tfchar <> chr('0');
        until ( tfchar = chr( 't' ) ) or ( tfchar = chr('f') );
    writeln(tfchar);
end;

procedure get_grid_size;
begin
    write(  enter grid size, x coordinate     );
    empty_buffer;
        repeat
            repeat
            xsize:=chr(dosxqq(6,255));
            until xsize <> chr('0');
        until ( xsize >= chr( '1' ) ) and ( xsize <= chr('f') );
    writeln(xsize);
    write(  enter grid size, y coordinate     );
    empty_buffer;
        repeat
            repeat
            ysize:=chr(dosxqq(6,255));
            until ysize <> chr('0');
        until ( ysize >= chr( '1' ) ) and ( ysize <= chr('z') );
    writeln(ysize);
end;

function checkgridpost : integer;
begin
    checkgridpost := 0;
    for i3 := 1 to notests do
        begin
        if (xrecord[i3] = ord(xpost))
        and (yrecord[i3] = ord(ypost))
        then checkgridpost := 1;
        end;
    if result(checkgridpost) = 0
    then
       begin
       notests := notests + 1 ;
       xrecord[notests] := ord(xpost);
       yrecord[notests] := ord(ypost);
       end;
end;

procedure calculate_bytes;
begin
for i1 := 1 to nomodes do
    begin
    an32[i1,1] := (nochans - 1) * 4 + 2;
    timeperiod := 1000 / frequency[i1] ;
    rate := (timeperiod/(4 * nochans) * 1000 ) ;
    i3 := 0 ;
    clockfreq := 0 ;
       repeat
       if rate <= 250.0 then clockfreq := 8 + i3
       else
          begin
          i3 := i3 + 1 ;
          rate := rate / 10.0 ;
          end;
       until clockfreq <> 0 ;
    hsc[i1,1] := clockfreq;
    hsc[i1,2] := trunc(rate) ;
```

```
        samplesize[i] := nochans * nosamps[i] * 4 ;
        nosamps[0] := 0;
        point[1] := 0 ;
        for i := 1 to i1 do
            point[i] := point[i] + (nosamps[i-1] * nochans * 4) ;
        point[i1] := point[i] + 1 ;
        end;
end;

procedure get grid_position;
begin
    repeat
    write(  enter grid position, x coordinate     );
    empty_buffer;
      repeat
        repeat
        xpost:=chr(dosxqq(6,255));
        until xpost <> chr('0');
      until ( xpost >= chr( 1') ) and ( xpost <= xsize );
    writeln(xpost);
    write(  enter grid position, y coordinate     );
    empty_buffer;
      repeat
        repeat
        ypost:=chr(dosxqq(6,255));
        until ypost <> chr('0');
      until ( ypost >= chr( 1') ) and ( ypost <= ysize );
    writeln(ypost);
    until checkoridpost = 0;
end;

procedure assign_datafile;
begin
    filename :=  a:dummy1.ind ;
    filename[3] := letter ;
    filename[4] := tfchar ;
    filename[5] := xsize ;
    filename[6] := ysize ;
    filename[7] := xpost ;
    filename[8] := ypost ;
    assign(datafile,filename);
    rewrite(datafile);
end;

procedure assign_filefile;
begin
    filename :=  a:filinx.dum  ;
    filename[10] := letter ;
    filename[11] := tfchar ;
    filename[12] := '1'    ;
    assign(filefile,filename);
    rewrite(filefile);
end;

procedure awaiting_go_ahead ;
begin
    empty_buffer;
    write(  hit (space bar) when specimen is position correctly  );
            repeat
            space_bar := chr(dosxqq(6,255));
            until space_bar = chr(  ) ;
    writeln ;
end;

procedure awaiting_trigger ;
begin
    trig[1] := 6 * 3 ;      {1 every 1 millisec}
    trig[2] := 1     ;      {every pulse}
    trig[3] := 2     ;      {1 channel to be sampled}
    num486(time,stat,flag0,byte2,trig[1],nstad,prim);
    num486(time,stat,flag0,byte1,trig[3],an32ad,prim);
```

```
    writeln(' awaiting trigger on displacement channel no. 1 .... ');
    nowords := 1 ;
    au1486(time,stat,flag129,nowords,data[2],an32ad,prim);
    repeat
    au1486(time,stat,flag129,nowords,data[1],an32ad,prim);
    until abs(data[2] - data[1]) > 20 ;
end;

procedure sample ;
begin
    au0486(time,stat,flag0,byte1,an32[1,1],an32ad,prim);
--  for i1 := 1 to nomodes do
        begin
        au0486(time,stat,flag0,byte2,hsc[i1,1],hscad,prim) ;
        fas486(time,stat,flag129,samplesize[i1],data[point[i1]],an32ad,prim);
        end;
end;

procedure file_data;
begin
    i7 := 0 ;
    for i4 := 1 to nomodes do
        begin
            for i5 := 1 to nosamps[i4] do
            begin
                for i6 := 1 to nochans do
                begin
                i7 := i7 + 1 ;
                write(datafile,data[i7]);
                end;
                i7 := i7 + (3 * nochans) ;
            end;
         end;
    close(datafile);
end;

begin                   { main body of program }
    declare_variables;
    get_exper_code;
    get_second_char;
    get_grid_size;
    assign_filefile;
    calculate_bytes;
    repeat
        writeln;
        writeln;
        get_grid_position;
        assign_datafile;
        awaiting_go_ahead;
        awaiting_trigger;
        sample;
        write(bell);
        writeln(' writing data to disk ',filename, ' ');
        file_data;
        writeln(filefile.filename);
        writeln;
--      empty_buffer;
        write(' hit (return) for further tests ');
        repeat
            charac := chr(dosxqq(6,255));
        until (charac = chr(13)) or (charac = chr('f'));
    until charac = chr('f') ;
    writeln;
    close(filefile);
    for i8 := 1 to 23 do
        writeln;
    writeln('                indentation testing complete. ');
    for i8 := 1 to 12 do
        writeln;
end.                    { end of main program }
```

```
program indentation data logger(input,output);
const
    clear window =  2' ;
    def wind = 1  ;
    escape = chr(27) ;
    dix = escape + '5' ;
    open window = escape +  m2  +     +  8' ;
    close window = escape +  m2  + '5  +  8  ;
    disp scr =  D  ;
    work scr =  W  ;
    apost =  0  ;
    aline =  0  ;
    combination =  A  ;
    move wind =  V  ;
    carriage return = chr(13);
    cursor type =  x  ;
    clear wscr =  Z  ;
    screen dump =  ?  ;
type
    bit = array[1..3] of integer;
    bits = array[1..10,1..2] of integer;
    units = array[0..10] of integer;
    results = array[1..16000] of integer;
    listpost = array[1..100] of integer;
    odata = array[1..2000] of integer ;
var
    byte1,byte2,nochans,nomodes,trigchan     :integer;
    timeout,stat,flag0,flag129,nbytes,nowords:integer;
    hscad,an32ad,prim,notests                :integer;
    i4,i5,i6,i7,i8,i9,i10                    :integer;
    clockfreq,i1,i2,i3,checkout              :integer;
    x1,trigtime,totalsamps                   :integer;
    source,dest,comb,h,v,bone_time           :integer;
    filename,thickout                        :string[12] ;
    datafile                                 :file of integer ;
    thickfile                                :text   ;
    xsize,ysize,xpost,ypost,letter,tfchar    :char   ;
    space_bar,key                            :char   ;
    trig                                     :bit    ;
    hsc,an32                                 :bits   ;
    qdata1,qdata2,time,bigtime               :qdata  ;
    data                                     :results ;
    nosamps,frequency,samplesize,point       :units  ;
    timeperiod,rate                          :real   ;
    xrecord,yrecord                          :listpost ;

procedure nuo488(var ti,st,fl,nb,da,sa,pa : integer);extern;
procedure nui488(var ti,st,fl,nb,da,sa,pa : integer);extern;
procedure fas488(var ti,st,fl,nb,da,sa,pa : integer);extern;

function dosxqq(command,parameter : word): byte;extern;
function mlurqq(a : real ; i : integer) : real ; extern ;

procedure empty_buffer ;
const  empty = chr(0);
var    buffer : char ;
begin
    repeat
      buffer := cor(dosxqq(0,255)) ;
    until buffer = empty ;
end;

procedure declare variables ;
begin
    byte1 := 1;
    byte2 := 1;
    flag0 := 0;
    flag129 := 129;
    prim := 7;
    hscad := 0;
    an32ad := 1;
```

```
    triqchan := 1;
    nochans := 2;
    nomodes := 1;
    nosamps[1] := 1500 ;
    frequency[1] := 1000;
    v := 300 ;
    h := 100 ;
    totalsamps := 0 ;
    for i1 := 1 to nomodes do
       totalsamps := totalsamps + nosamps[i1] ;
end;

procedure get_exper_code;
begin
    notests := 0;
    write( enter experimental code letter     );
    empty_buffer ;
        repeat
            repeat
            letter:=chr(dosxqq(6,255));
            until letter <> chr('0');
        until ( letter >= chr('a') ) and ( letter <= chr('z') );
    writeln(letter);
end;

procedure get_second_char;
begin
    write( enter (t) for tibia, (f) for femur     );
    empty_buffer ;
        repeat
            repeat
            tfchar:=chr(dosxqq(6,255));
            until tfchar <> chr('0');
        until ( tfchar = chr('t') ) or ( tfchar = chr('f') );
    writeln(tfchar);
end;

procedure get_grid_size;
begin
    write( enter grid size, x coordinate     );
    empty_buffer ;
        repeat
            repeat
            xsize:=chr(dosxqq(6,255));
            until xsize <> chr('0');
        until ( xsize >= chr('1') ) and ( xsize <= chr('f') );
    writeln(xsize);
    write( enter grid size, y coordinate     );
    empty_buffer ;
        repeat
            repeat
            ysize:=chr(dosxqq(6,255));
            until ysize <> chr('0');
        until ( ysize >= chr('1') ) and ( ysize <= chr('z') );
    writeln(ysize);
end;

function checkgridpost : integer;
begin
    checkgridpost := 0;
    for i3 := 1 to notests do
        begin
        if (xrecord[i3] = ord(xpost))
        and (yrecord[i3] = ord(ypost))
        then checkgridpost := 1;
        end;
    if result(checkgridpost) = 0
    then
       begin
       notests := notests + 1 ;
       xrecord[notests] := ord(xpost);
```

```
        yrecord[notests] := ord(ypost);
        end;
end;

procedure calculate_bytes;
begin
    for ii := 1 to nomodes do
        begin
        ansiz[ii,1] := (nochans - 1) * 4 + 2;
        timeperiod := 1000 / frequency[ii] ;
        rate := (timeperiod/(4 * nochans) * 1000 ) ;
        i3 := 0 ;
        clockfreq := 0 ;
           repeat
           if rate <= 250.0 then
                 clockfreq := 8 + i3
           else
              begin
              i3 := i3 + 1 ;
              rate := rate / 10.0 ;
              end;
           until clockfreq <> 0 ;
        hsc[ii,1] := clockfreq;
        hsc[ii,2] := trunc(rate) ;
        samplesize[ii] := nochans * nosamps[ii] * 4 ;
        nosamps[0] := 0;
        point[ii] := 0 ;
        for i2 := 1 to ii do
            point[ii] := point[ii] + (nosamps[i2-1] * nochans * 4) ;
        point[ii] := point[ii] + 1 ;
        end;
end;

procedure get_grid_position;
begin
    repeat
    writeln ;
    empty_buffer;
    write( enter grid position, x coordinate     );
      repeat
        repeat
        xpost:=chr(dosxqq(6,255));
        until xpost <> chr('0');
      until ( xpost >= chr( 1 ) ) and ( xpost <= xsize );
    writeln(xpost);
    write( enter grid position, y coordinate     );
      repeat
        repeat
        ypost:=chr(dosxqq(6,255));
        until ypost <> chr( 0 );
      until ( ypost >= chr( 1 ) ) and ( ypost <= ysize );
    writeln(ypost);
    until checkgridpost = 0;
end;

procedure assign_datafile;
begin
    filename :=  a:dummy.ned ;
    filename[3] := letter ;
    filename[4] := tfchar ;
    filename[5] := xsize ;
    filename[6] := ysize ;
    filename[7] := xpost ;
    filename[8] := ypost ;
    assign(datafile,filename);
    rewrite(datafile);
end;

procedure set_thickfile ;
begin
    thickout :=  a:thickn.txt  ;
```

```
    thickout[10] := letter ;
    thickout[11] := t+char ;
end;

procedure write thickness ;
var  junky :array[1..100] of string[12] ;
     junkie :array[1..100] of integer ;
     j  : integer :
begin
    assign(thickfile,thickout);
    if notests > 1 then
       begin
       reset(thickfile);
       for j := 1 to notests-1 do
          readln(thickfile,junky[j],junkie[j]);
       rewrite(thickfile);
       for j := 1 to notests-1 do
          writeln(thickfile,junky[j],junkie[j]);
       end
    else
       rewrite(thickfile);
    writeln(thickfile,filename,bone_time);
    close(thickfile);
 end;

procedure awaiting_go_ahead ;
begin
    write(  hit <space bar> when specimen is position correctly ');
             repeat
             space_bar := chr(dosxqq(6,255));
             until space_bar = chr(  ') ;
    writeln ;
end;

procedure awaiting_trigger ;
begin
    trig[1] := 8 + 3 ;      {1 every 1 millisec}
    trig[2] := 1     ;      {every pulse}
    trig[3] := 2     ;      {1 channel to be sampled}
    nuo488(timeout,stat,flag0,byte2,trig[1],hscad.prim);
    nuo488(timeout,stat,flag0,byte1,trig[3],an32ad.prim);
    writeln(  awaiting trigger on displacement channel no. 1 .... );
    nowords := 1 ;
    nui488(timeout,stat,flag129,nowords,data[2],an32ad,prim);
      repeat
      nui488(timeout,stat,flag129,nowords,data[1],an32ad.prim);
      until abs(data[2] - data[1]) > 20 ;
end;

procedure sample ;
begin
    nuo488(timeout,stat,flag0,byte1,an32[1,1],an32ad.prim);
    for ii := 1 to nomodes do
        begin
        nuo488(timeout,stat,flag0,byte2,hsc[ii,1],hscad,prim) ;
        tas488(timeout,stat,flag129,samplesize[ii],data[point[ii]],an32ad,prim);
        end;
end;

procedure file_data;
begin
    i7 := 0 ;
    for i4 := 1 to nomodes do
        begin
            for i5 := 1 to nosamps[i4] do
            begin
                for i6 := 1 to nochans do
                begin
                i7 := i7 + 1 ;
                write(datafile,data[i7]);
                end;
```

```
                    i7 := i7 + (3 * nochans) ;
                end;
        end;
    close(datafile);
end;

function keypress : char ;
begin
    keypress := chr(dosxqq(6,255));
end;

--  procedure close_text window ;
    begin
    writeln(close_window) ;
    end;

    procedure process datafile ;
    begin
        write(' processing data..... ');
        i4:=1;
        i2:=1;
        for i1 := 1 to totalsamps do
          begin
          if i4 = 1 then
            begin
            qdata[i1]:=trunc(0.0-((1.6*((data[i2]*0.1)-150))-400)) ;
            i4 := 3 ;
            end
          else
             i4 := i4 - 1 ;
          i2:=i2+6;
          end;
        writeln(' Ok ! ');
    end;

    procedure plot_axis;
    begin
        writeln(gfx,disp_scr,1);
        writeln(gfx,work_scr,1);
        writeln(gfx,apost,100,0);
        writeln(gfx,aline,100,399);
        writeln(gfx,apost,100,390);
        writeln(gfx,aline,700,390);
    end;

    procedure bone_contact ;
    begin
        bone_time := trunc((totalsamps*0.01)/6*(h-100)) ;
    end;

    procedure time_axis;
    begin
        for i1 := 1 to totalsamps do
            time[i1] := trunc((i1*6/(totalsamps*0.01))+100);
        end;

..  procedure define_cursor_type;
    begin
        writeln(gfx,cursor_type,1);
    end;

    procedure move_cursor;
    begin
        writeln(gfx,apost,h,v);
    end;

    procedure plot_graph;
    begin
        writeln(gfx,disp_scr,1);
        writeln(gfx,work_scr,1);
        i4:=3;
```

```
    writeln(qfx,apost,time[1]:4,qdata1[1]:4);
        for i1 := 2 to totalsamps do
        begin
        if i4 = 1 then
           begin
           writeln(qfx,aline,time[i1]:4,qdata1[i1]:4);
           i4:=3;
           end
        else
           i4:=i4-1;
        end;

    writeln(qfx,apost,time[1]:4,qdata2[1]:4);
    i4:=3;
        for i1 := 2 to totalsamps do
        begin
        if i4 = 1 then
           begin
           writeln(qfx,aline,time[i1]:4,qdata2[i1]:4);
           i4:=3;
           end
        else
           i4:=i4-1;
        end;
end;

procedure combine(const comb,source,dest : integer);
begin
    writeln(qfx,work_scr,source);
    writeln(qfx,apost,0,0);
    writeln(qfx,def_wind,800,400);
    writeln(qfx,combination,comb);
    writeln(qfx,move_wind,dest);
end;

procedure clear_cross_wire;
begin
    writeln(qfx,disp_scr,0);
    writeln(qfx,work_scr,0);
    writeln(qfx,clear_wkscr);
end;

procedure open_text_window ;
begin
    writeln(open_window) ;
    clear_cross_wire;
end;

procedure plot_cross_wire;
begin
    writeln(qfx,disp_scr,0);
    writeln(qfx,work_scr,0);
    writeln(qfx,apost,h,0);
    writeln(qfx,aline,h,399);
    writeln(qfx,apost,0,v);
    writeln(qfx,aline,700,v);
end;

procedure graph_to_cross_wire;
begin
    writeln(qfx,disp_scr,0);
    combine(7,1,0);
end;

procedure clear_graph;
begin
    writeln(qfx,disp_scr,1);
    writeln(qfx,work_scr,1);
    writeln(qfx,clear_wkscr);
end;
```

```
procedure move cross wire;
begin
    writeln(gt),disp scr,0);
    writeln(gt),work scr,0);
        repeat
        key := keypress ;
        case key of
           d  : v:=v+1;
           u  : v:=v-1;
           l  : h:=h-1;
           r  : h:=h+1;
           p  : writeln(gt),screen_dump);
        otherwise     ;
        end;
        move cursor;
        if (key = carriage_return) or (key = chr(f')) then
           begin
           clear_cross_wire;
           plot_cross_wire;
           graph_to_cross_wire;
           end;
        until key = chr(f );
end;

begin                    { main body of program }
    open_text_window ;
    declare_variables;
    get_exper_code;
    get_second_char;
    get_grid_size;
    set_thickfile ;
    calculate_bytes;
    time_axis;
    repeat
    get_grid_position;
    assign_datafile1;
    awaiting_go_ahead;
    awaiting_trigger;
    sample;
    writeln(chr(7));
    file_data;
    process_datafile;
    close_text_window;
    define_cursor_type;
    clear_graph;
    plot_axis;
    plot_graph ;
    plot_cross_wire;
    graph_to_cross_wire;
    move_cross_wire;
    done_contact;
    write_thickness;
    open_text_window ;
    writeln;
        writeln;
        write(' hit (space bar) for further tests ');
    until chr(dos(qqil,0)) = chr('f');
    writeln;
    clear_cross_wire;
    for i8 := 1 to 23 do
        writeln;
    writeln(                    needling of cartilage complete.');
    for i6 := 1 to 12 do                .
        writeln;
end.                     { end of main program }
```

```
program calculate_indentation (input,output);
type
   numbers = array [1..600] of integer ;
var
   range,average,dispcal,ic              : integer ;
   start_of_data,i1,i2,i3,two_seconds    : integer ;
   data,data1                            : numbers ;
   datafile,filefile,sumfile             : string[12] ;
   datain                                : file of integer ;
   run_tot,depth                         : real ;
   filein,summary                        : text ;

function dosxqq(command,parameter : word ) : byte ; extern ;

procedure get_cal ;
begin
    write(' enter displacement calibration figure, units per mm... ');
    readln(dispcal);
end;

procedure initial_contact;
begin
    range := 8 ;
    start_of_data := 0 ;
    i1 := 0 ;
    i2 := 0 ;
    repeat
         i1 := i1 + 1 ;
         for i3 := i1 to (i1+range-1) do
           begin
           if data[(i3+1)] > data[i3] then
                  i2 := i2 + 1 ;
           end;
         if i2 = range then
           start_of_data := i1
         else i2 := 0 ;
    until (i1 = 500) or (i2 = range) ;
end;

procedure two_secs_reading ;
begin
    i1 := 0 ;
    repeat
         i1 := i1 + 1 ;
         if start_of_data < (50+i1) then
             begin
             two_seconds := 514 + i1 ;
             i1 := 0 ;
             end;
     until i1 = 0 ;
end;

procedure read_datafile;
begin
    for i1 := 1 to 600 do
        read(datain,data[i1],data1[i1]);
end;

procedure offset_initial_contact;
begin
    if start_of_data <> 1 then
    begin
    run_tot := 0.0 ;
    average := 0 ;
    for i1 := 1 to (start_of_data-1) do
        run_tot := run_tot + data[i1] ;
    average := trunc((run_tot/(start_of_data - 1))) ;
    if average > data[start_of_data] then
        start_of_data := start_of_data +1 ;
    end;
end;
```

```
procedure set sumfile;
begin
    sumfile :=  a:summary.xxx  ;
    for io := 10 to 11 do
        sumfile[io] := tilefile[io] ;
    assign(summary,sumfile);
    rewrite(summary);
end;

begin  { main body of prog }
    write(' enter file index identifiers.. a:filinx. ');
    tilefile :=  a:filinx.xxx  ;
    for ii := 10 to 12 do
     tilefile[ii] := chr(dosxqq(1,0)) ;
writeln;
set sumfile;
get_cal;
assign(filein,tilefile);
reset(filein);
while not eof(filein) do
   begin
   readln(filein,datafile);
   assign(datain,datafile);
   reset(datain) ;
   read_datafile;
   close(datain);
   initial_contact;
   offset_initial_contact;
   two_secs_reading;
   depth := (data1[start_of_data] - data1[two_seconds])/dispcal ;
   writeln(summary,datafile,data1[start_of_data]:6,data1[two_seconds]:6,depth:8:4);
   end;
close(summary);
    close(filein);
end.
```

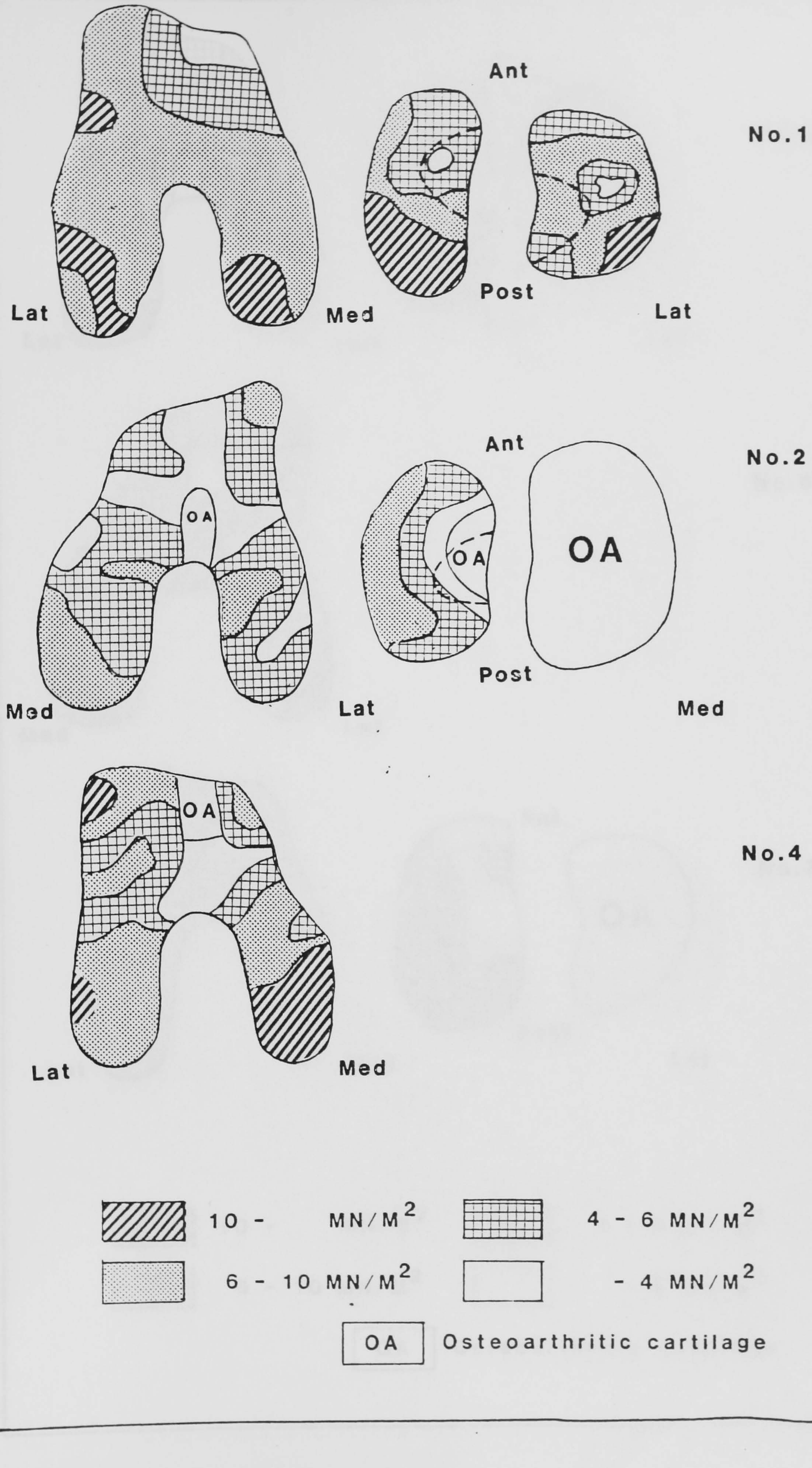
```
program calculate thickness (input,output);
type
   numbers = array [1..5000] of integer ;
   names = array[1..100] of string[12];
   ints = array[1..100] of integer ;
   reels = array[1..100] of real ;
var
   range,average,flag,junk,done contact          : integer ;
   start_of_data,i1,i2,i3,two_seconds,i5,i6,i4   : integer ;
   ndispcal,idispcal                             : integer ;
   data,data1                                    : numbers ;
   datafile,thickfile,sumfile,result             : string[12] ;
   datain                                        : file of integer ;
   run_tot,thick,creep2,ratio                    : real ;
   thickin,summary,final                         : text ;
   of                                            : names ;
   twos,ic                                       : ints ;
   ans                                           : reels ;

function dos(go(command,parameter : word ) : byte : extern ;

procedure get_cal ;
begin
    write( enter NEEDLE displacement calibration figure, units per mm... );
    readln(ndispcal);
    writeln;
    write( enter INDENTATION displacement calibration figure, units per mm... );
    readln(idispcal);
end;

procedure initial_contact;
begin
    range := 8 ;
    start_of_data := 0 ;
    i1 := 0 ;
    i2 := 0 ;
    repeat
        flag := 0;
        i1 := i1 + 1 ;
        for i3 := i1 to (i1+range-1) do
          begin
          if data[(i3+1)] > data[i3] then
                  begin
                  i2 := i2 + 1 ;
                  flag := 0 ;
                  end
          else if (data[(i3+1)] = data[i3]) and (flag = 0) then
                  begin
                  i2 := i2 + 1 ;
                  flag := 1 ;
                  end;
          end;
        if i2 = range then
              start_of_data := i1
            else i2 := 0 ;
      until (i1 = 1000) or (i2 = range) ;
    end;

    procedure set_thickfile ;
    begin
        assign(thickin,thickfile);
        reset(thickin);
    end;

    procedure read_datafile;
    begin
        j4 := 0 ;
        while not eof(datain) do
           begin
           j4 := j4 + 1 ;
           read(datain,data[j4],data1[j4]);
```

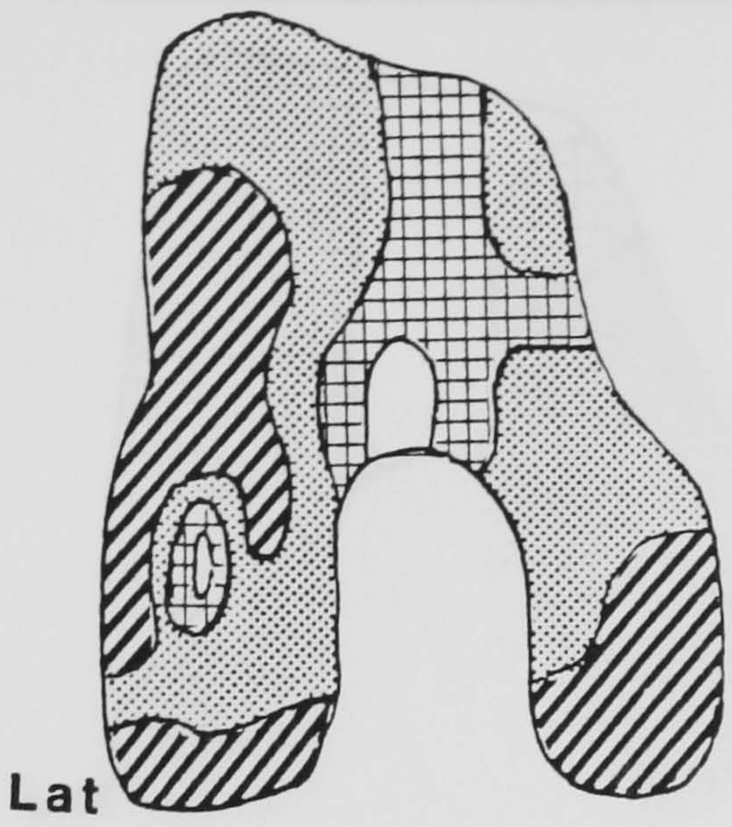
```
        end;
end;

procedure set sumfile;
begin
    sumfile :=  a:summary.xxx  ;
    for ii := 10 to 11 do
        sumfile[ii] := tricktile[ii] ;
    assign(summary,sumfile);
    reset(summary);
end;

procedure calculate creep;
const
    r         = 1.5                 ;   { millimeters }
    p         = 10.93e15            ;   { newtons }
var
    top,bottom,bracket,quote,constant    : real ;
begin
    thick    := (data[start of data] - data[bone contact])/ndispcal ;
    constant := (9*p) / (16*sqrt(r)) ;
    top      := (0.6 - 0.42) * thick ;
    bottom   := sqrt((2 * r * ind[19])-(ind[19] * ind[19])) ;
    quote    := top/bottom ;
    bracket  := (1-exp(quote)) / ind[19] ;
    creep2   := ( sqrt(bracket*bracket*bracket) ) * constant ;
end;

procedure read sumfile ;
begin
    19 := 0 ;
    while not eof(summary) do
      begin
      19 := 19 + 1 ;
      readln(summary,df[19],ic[19],twos[19],ind[19]);
      end;
    close(summary);
end;

procedure spot ;
var
  dummyfile                    : string[12] ;
  jj                           : integer ;
begin
    19 := 0 ;
    dummyfile :=  a:xxxxxx.ind  ;
    for jj := 3 to 8 do
        dummyfile[jj] := datafile[jj] ;
    repeat
       19 := 19 + 1 ;
    until df[19] = dummyfile ;
end;

procedure write final result ;
begin
    writeln(final,d[19],ic[19]:9,twos[19]:9,ind[19]:8:4,
            start of data:6,bone contact:8,     ,thick:8:4,     ,
            creep2:8:4,ratio:6:2);
end;

procedure calculate ratio;
const rr = 1.5 ;
var   a  : real ;
begin
    a := sqrt ((2*rr*ind[19])-(ind[19]*ind[19])) ;
    ratio := thick / a ;
end;

begin { main body of program}
    writeln enter file index identifiers.. a:thickn. );
    indexfile :=  a:thickn.xxx  ;
```

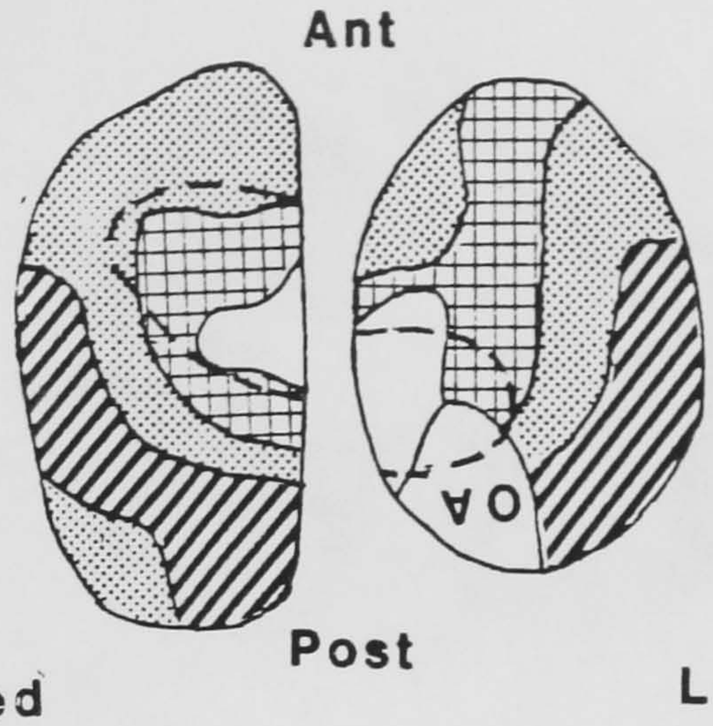
```
    result :=  a:result.xxx ;
    for ii := 10 to 12 do
        begin
        thicktile[ii] := chr(ord(xx[ii,0])) ;
        result[ii] := thicktile[ii];
        end;
    result[12] :=  r  ;
    writeln;
    get car;
    assign(final,result);
    rewrite(final);
--  writeln(final,        INDENTATION displacement calibration :=  ,idispcal,
                  digits per mm );
    writeln(final,          NEEDLE displacement calibration :=  ,ndispcal,
                digits per mm );
    writeln(final);
    writeln(final,  Filename    surface  two sec depth of  time of  time of cartilage  creep  thickness :
    writeln(final,              position position indent   pin/sur pin/bone thickness modulus   / mm    :
    writeln(final, ------------------------------------------------------------------------------------ );
    set subtile;
    read subtile ;
    set thicktile;
    while not eof(thickin) do
       begin
       readln(thickin,datafile.bone contact);
       writeln(datafile);
       assign(datain,datafile);
       reset(datain) ;
       read datafile;
       close(datain);
       initial_contact;
       spot ;
       calculate_creep;
          calculate_ratio;
          write_final_result ;
          end;
       close(thickin);
       close(final);
end.
```

APPENDIX B.

TOPOGRAPHICAL MAPS OF CARTILAGE STIFFNESS.

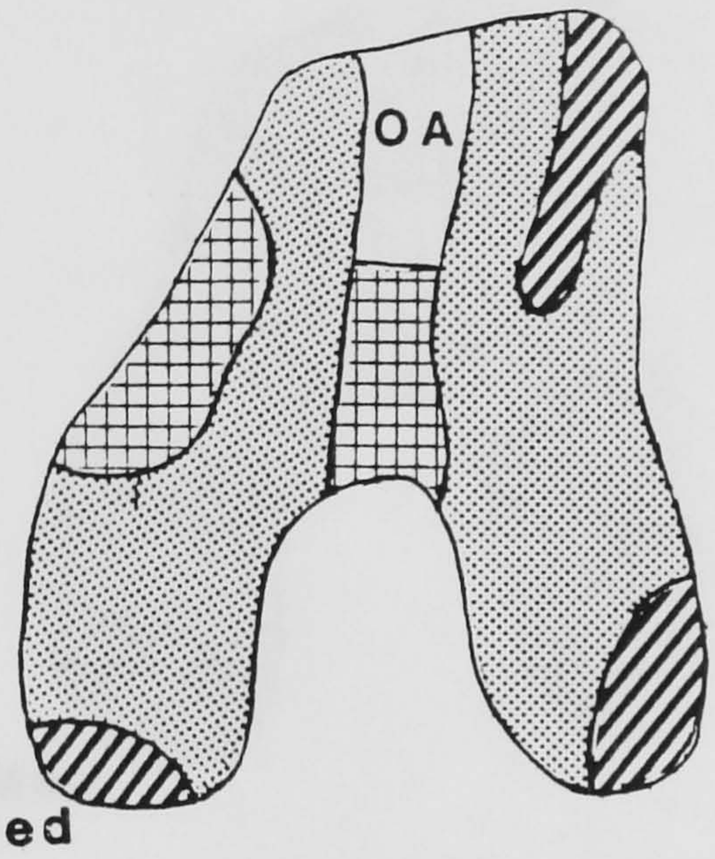

Ant
Post
Lat
Med
No.1
OA
Med
Lat
Ant
Post
OA
OA
No.2
OA
Lat
Med
No.4
10 - MN/M2
4 - 6 MN/M2
6 - 10 MN/M2
- 4 MN/M2
OA
Osteoarthritic cartilage





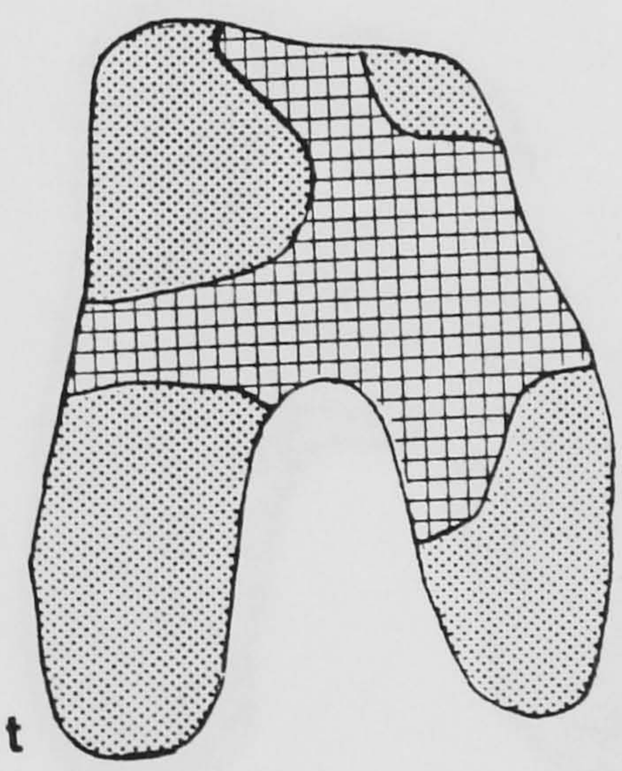
No.5

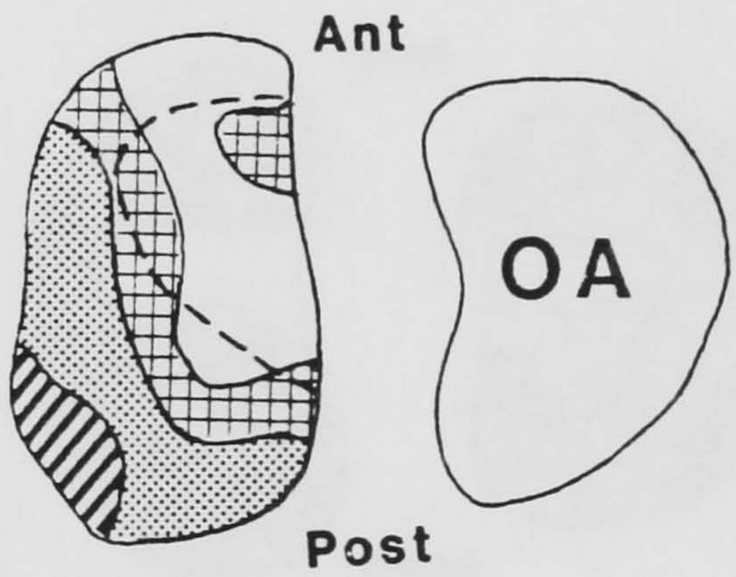
Lat



Lat

No.6





No.7

Lat

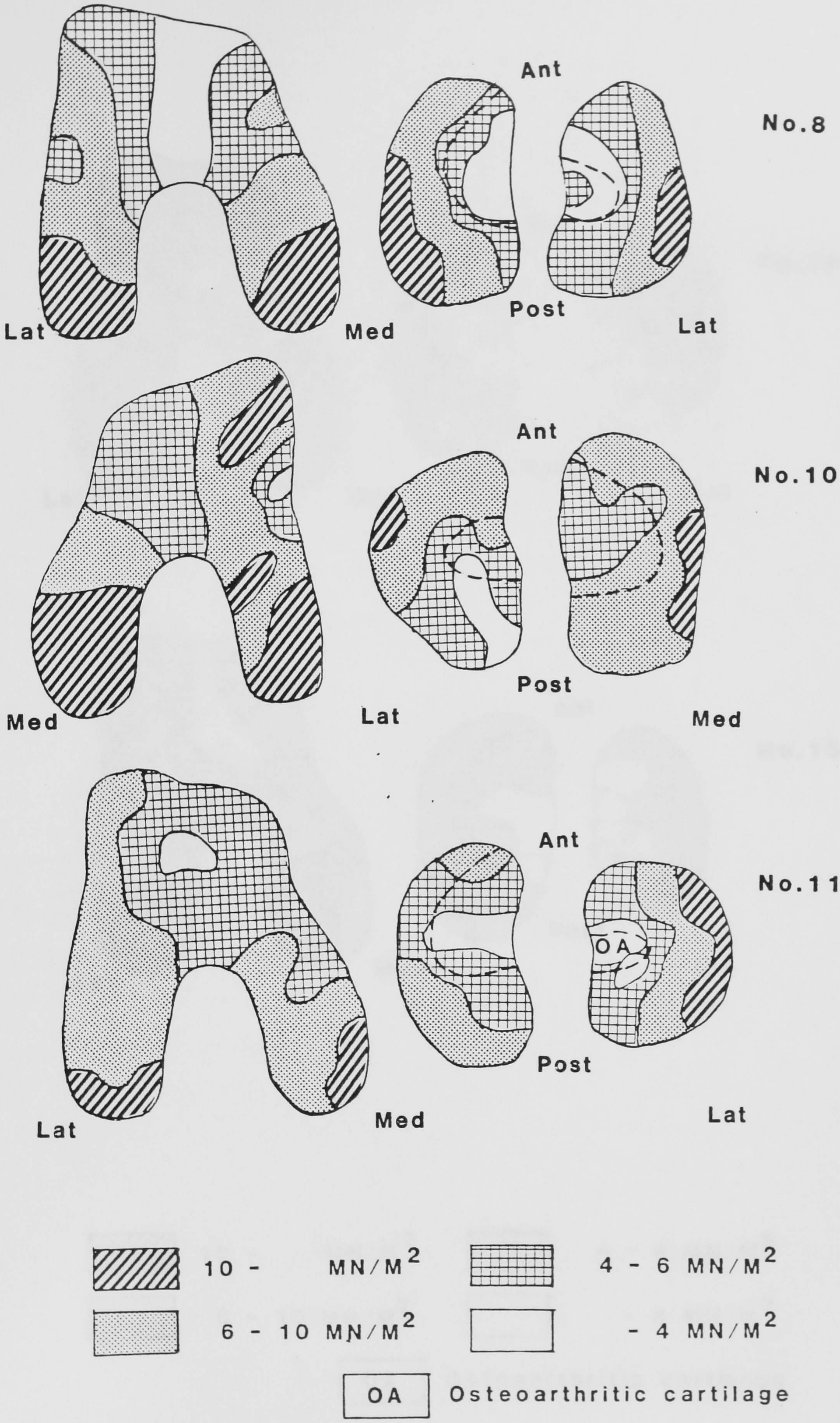
10 - MN/M^2

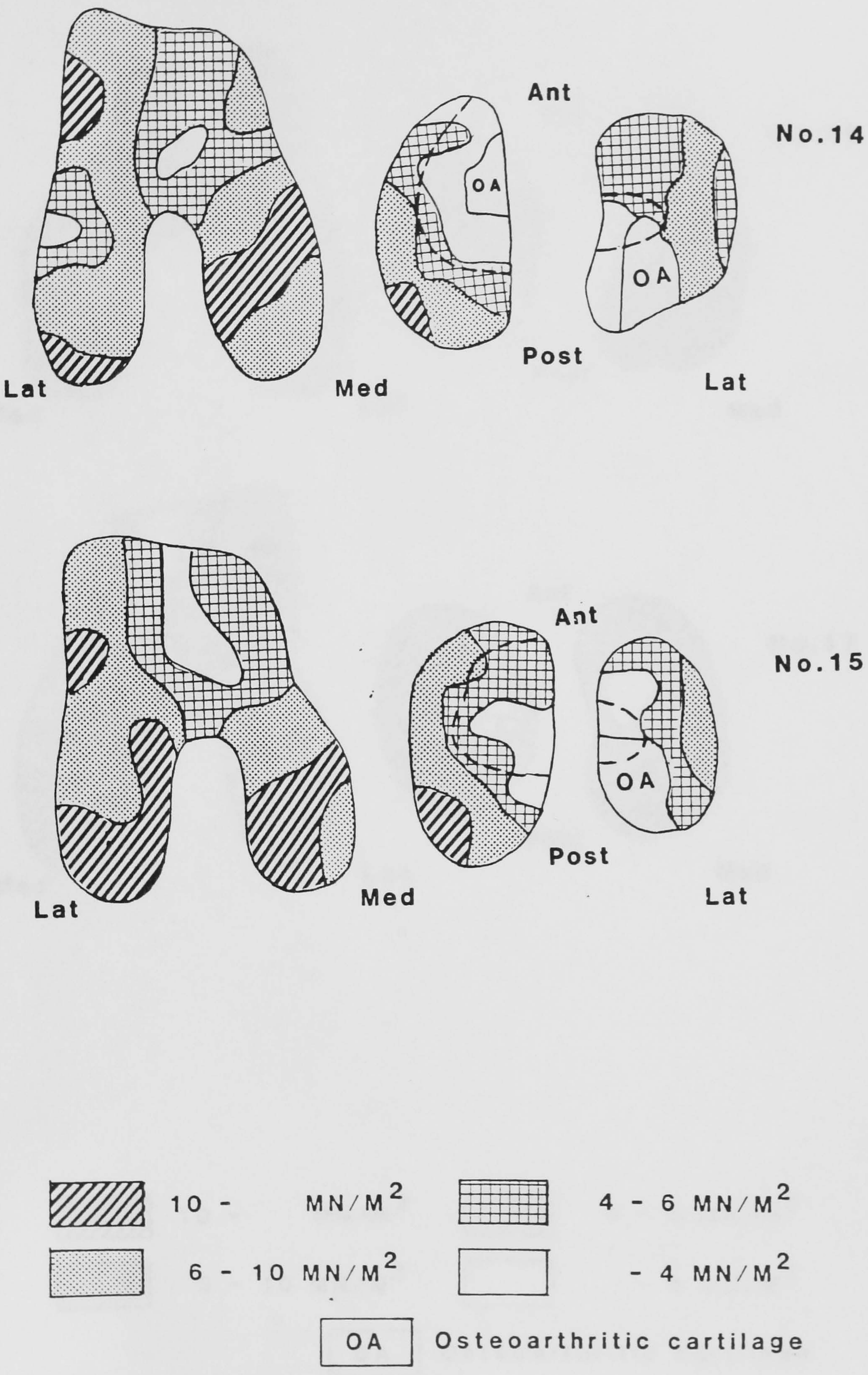
4 - 6 MN/M^2

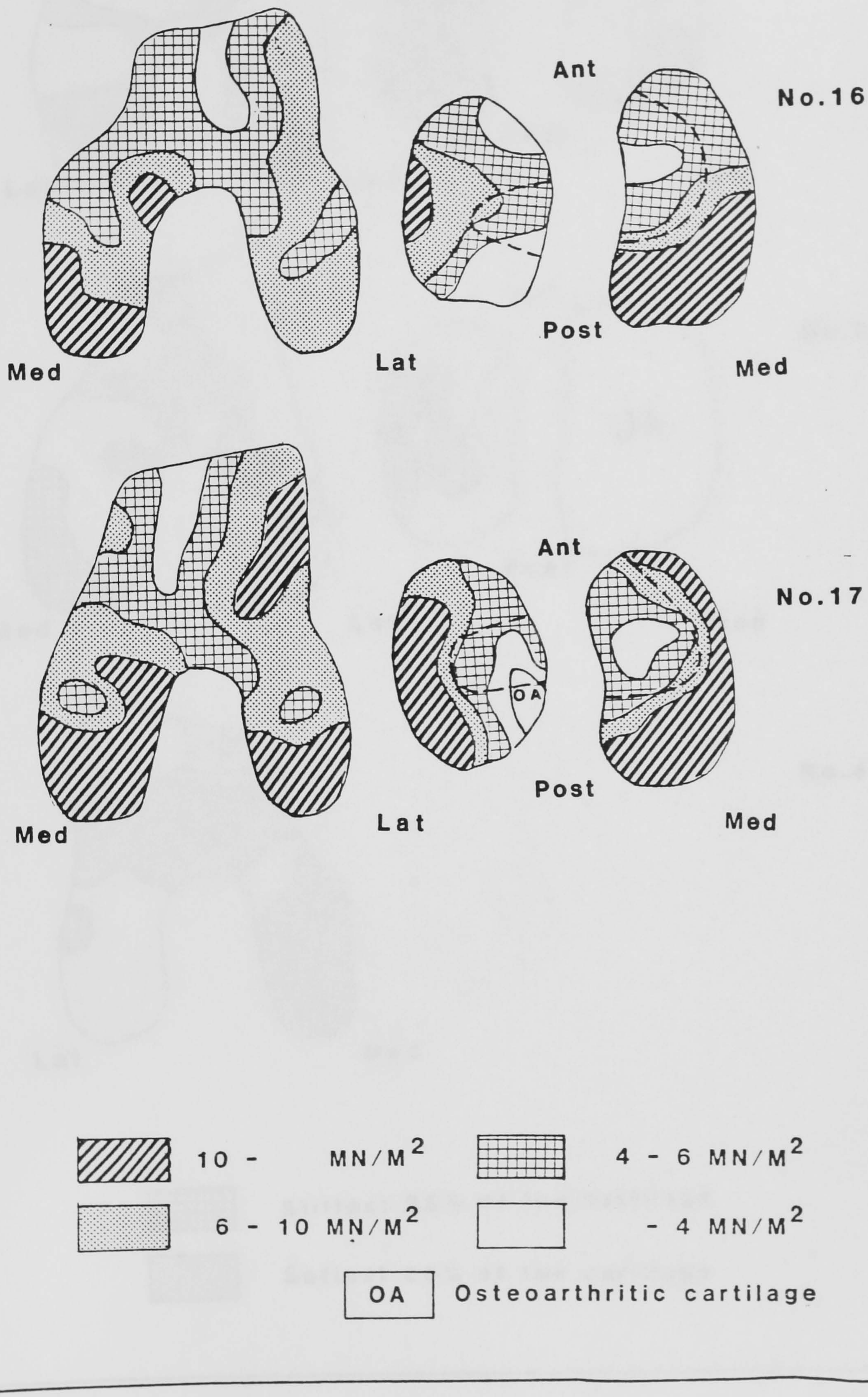
6 - 10 MN/M^2

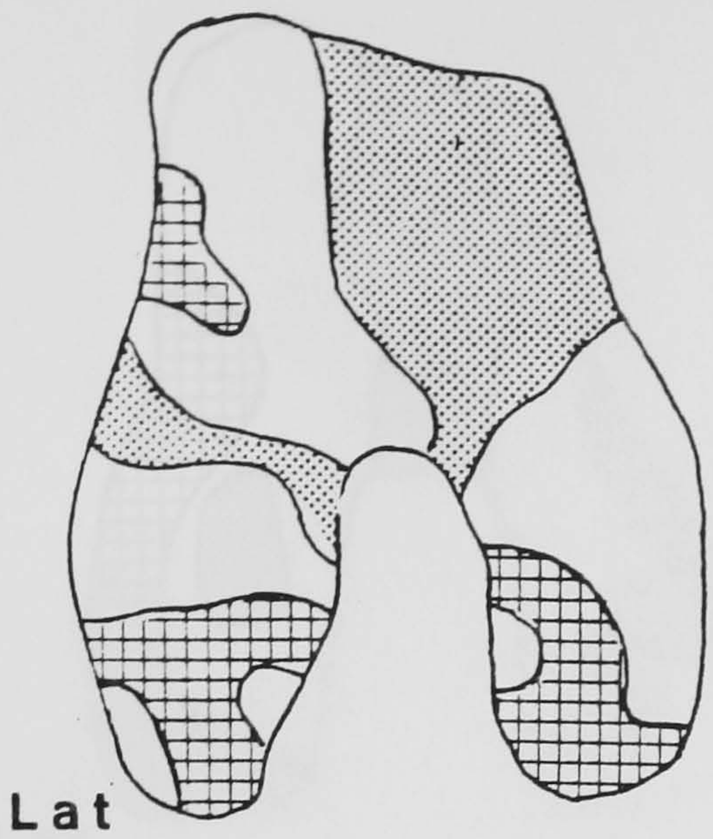
- 4 MN/M^2

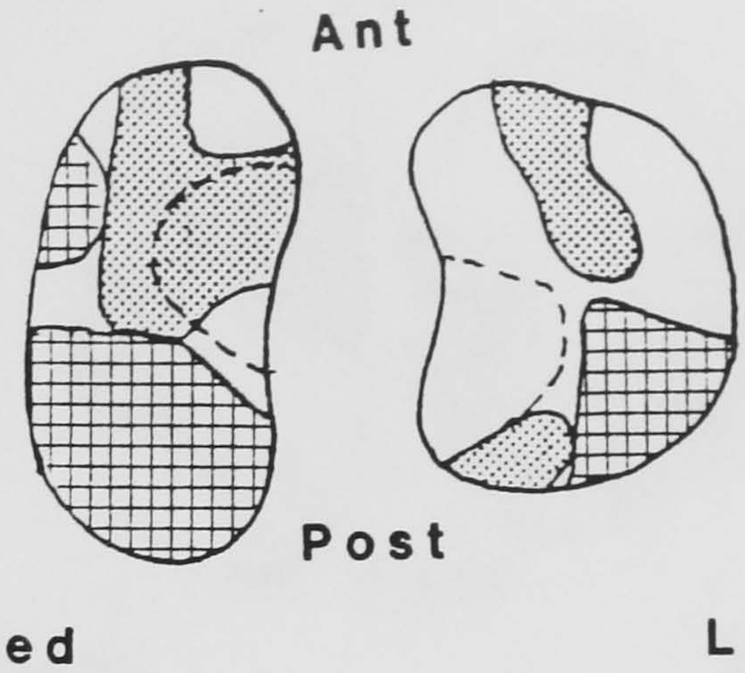
OA Osteoarthritic cartilage


Lat
Med
Ant
Post
Lat
No.8
Med
Ant
Post
Lat
Med
No.10
Lat
Med
Ant
Post
OA
Lat
No.11
10 - MN/M2
4 - 6 MN/M2
6 - 10 MN/M2
- 4 MN/M2
OA
Osteoarthritic cartilage

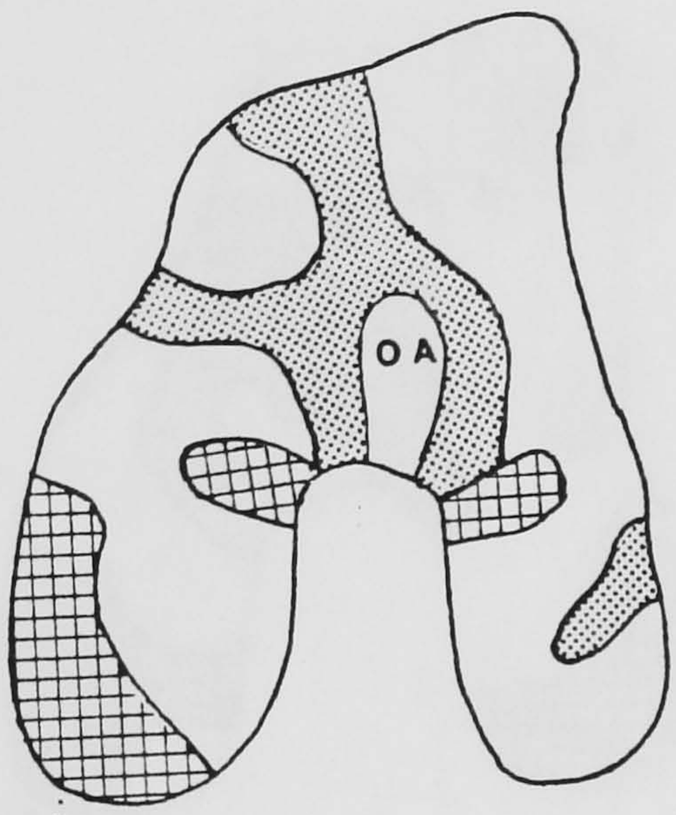

Ant
OA
Post
Lat
Med
Lat
No.14
Ant
OA
Post
Lat
Med
Lat
No.15
10 - MN/M2
4 - 6 MN/M2
6 - 10 MN/M2
- 4 MN/M2
OA
Osteoarthritic cartilage

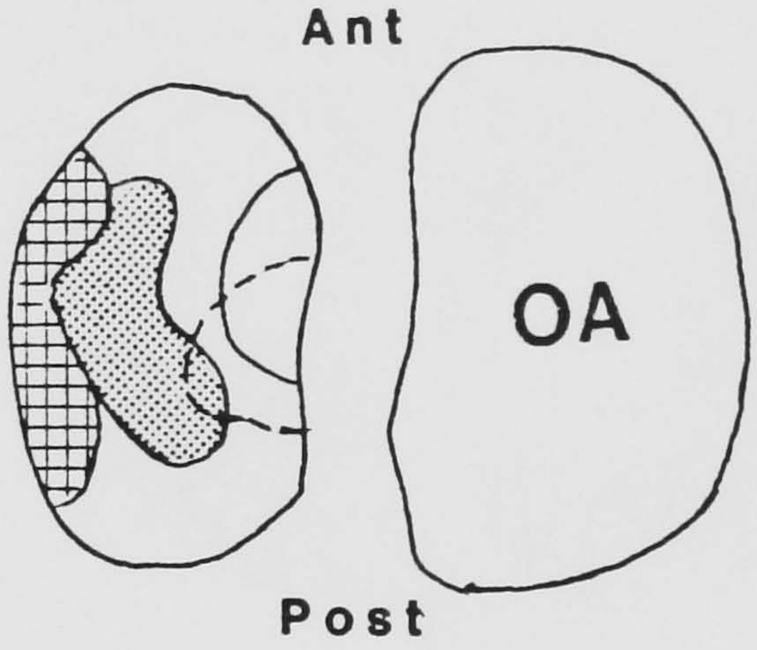

Ant
No.16
Med
Lat
Post
Med
Ant
No.17
OA
Med
Lat
Post
Med
10 - MN/M2
4 - 6 MN/M2
6 - 10 MN/M2
- 4 MN/M2
OA
Osteoarthritic cartilage



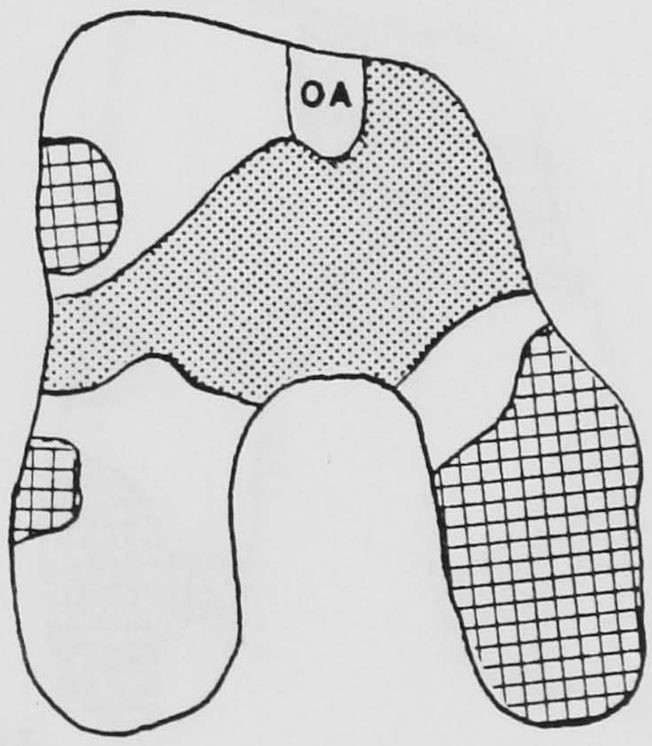


No.1





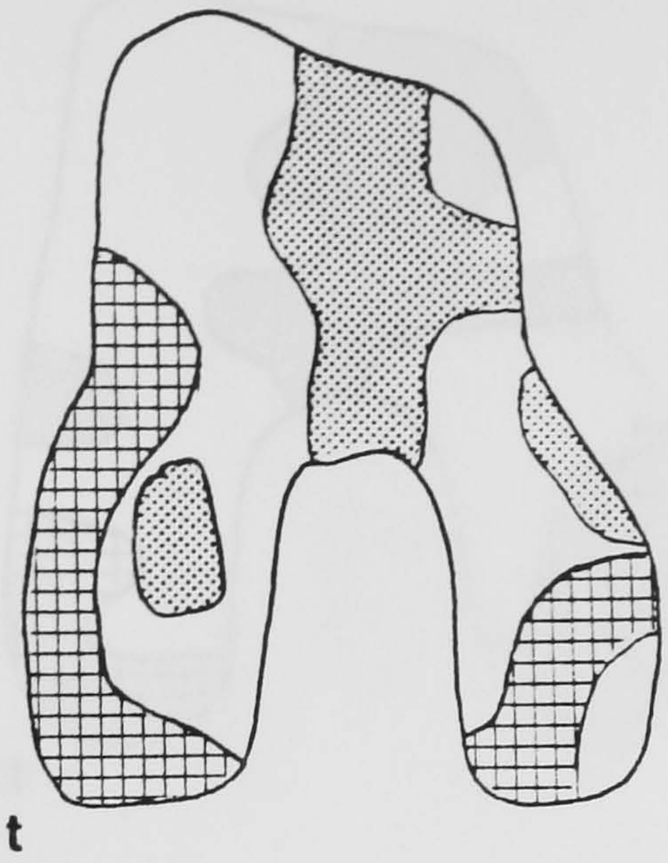
No.2

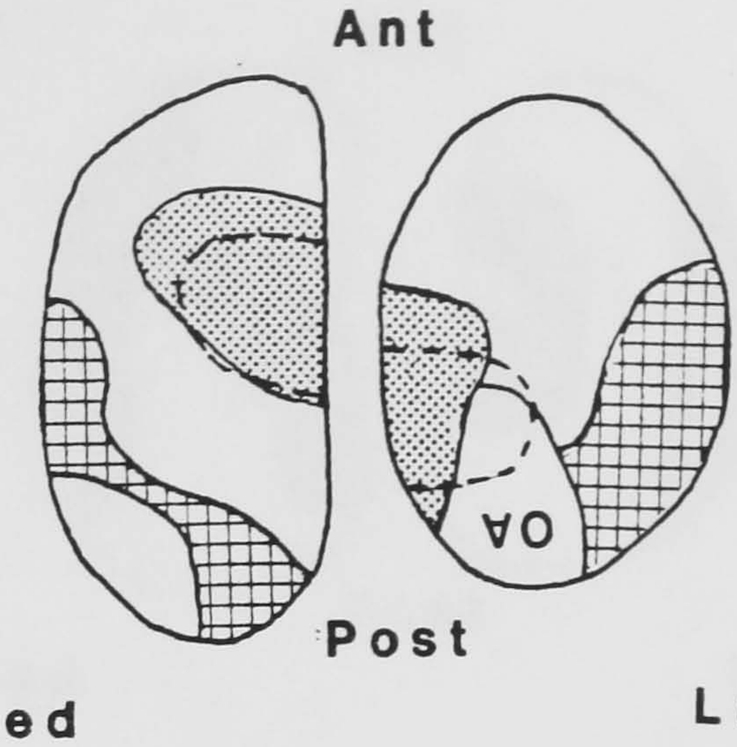


No.4

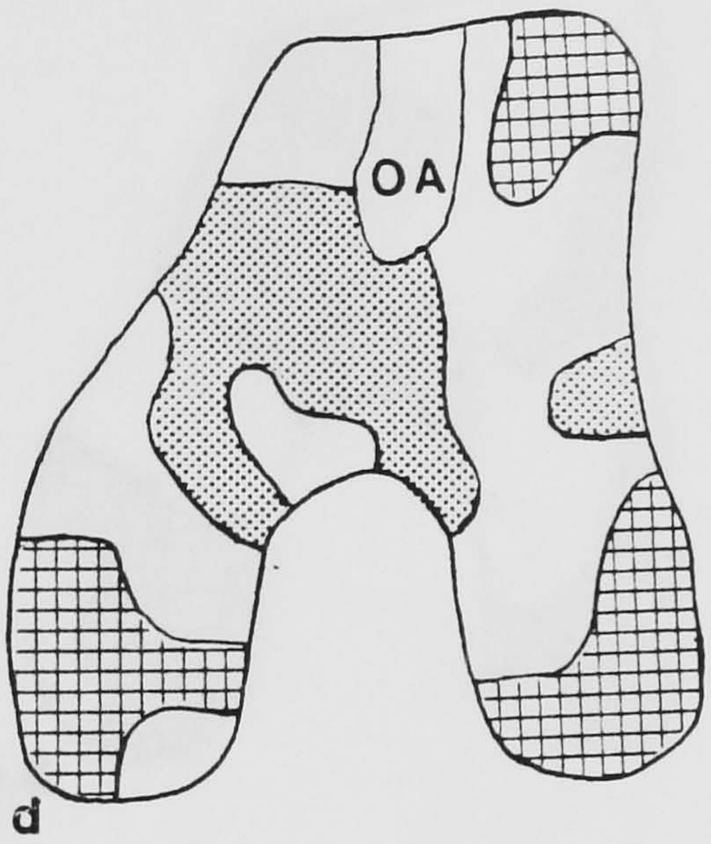
Stiffest 25% of the cartilage

Softest 25% of the cartilage

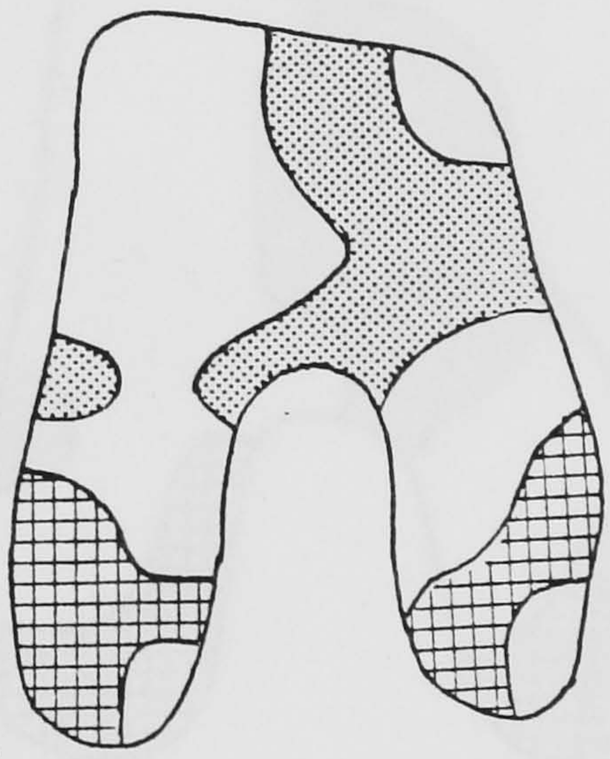


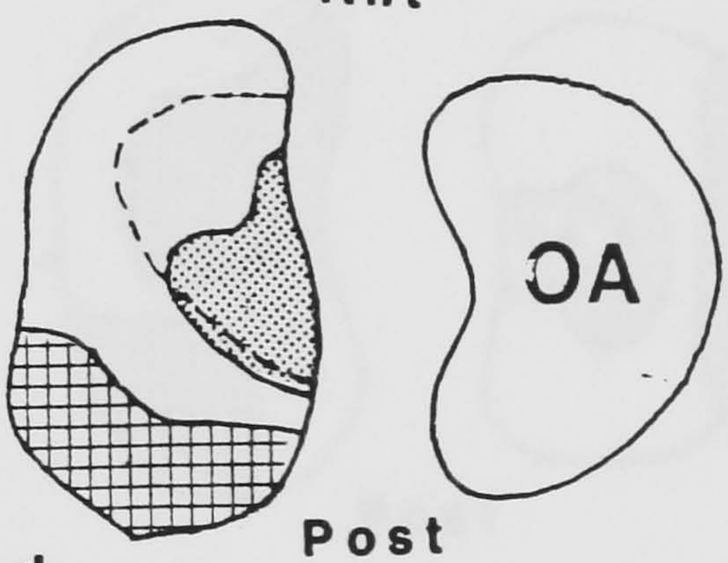


No.5



No.6

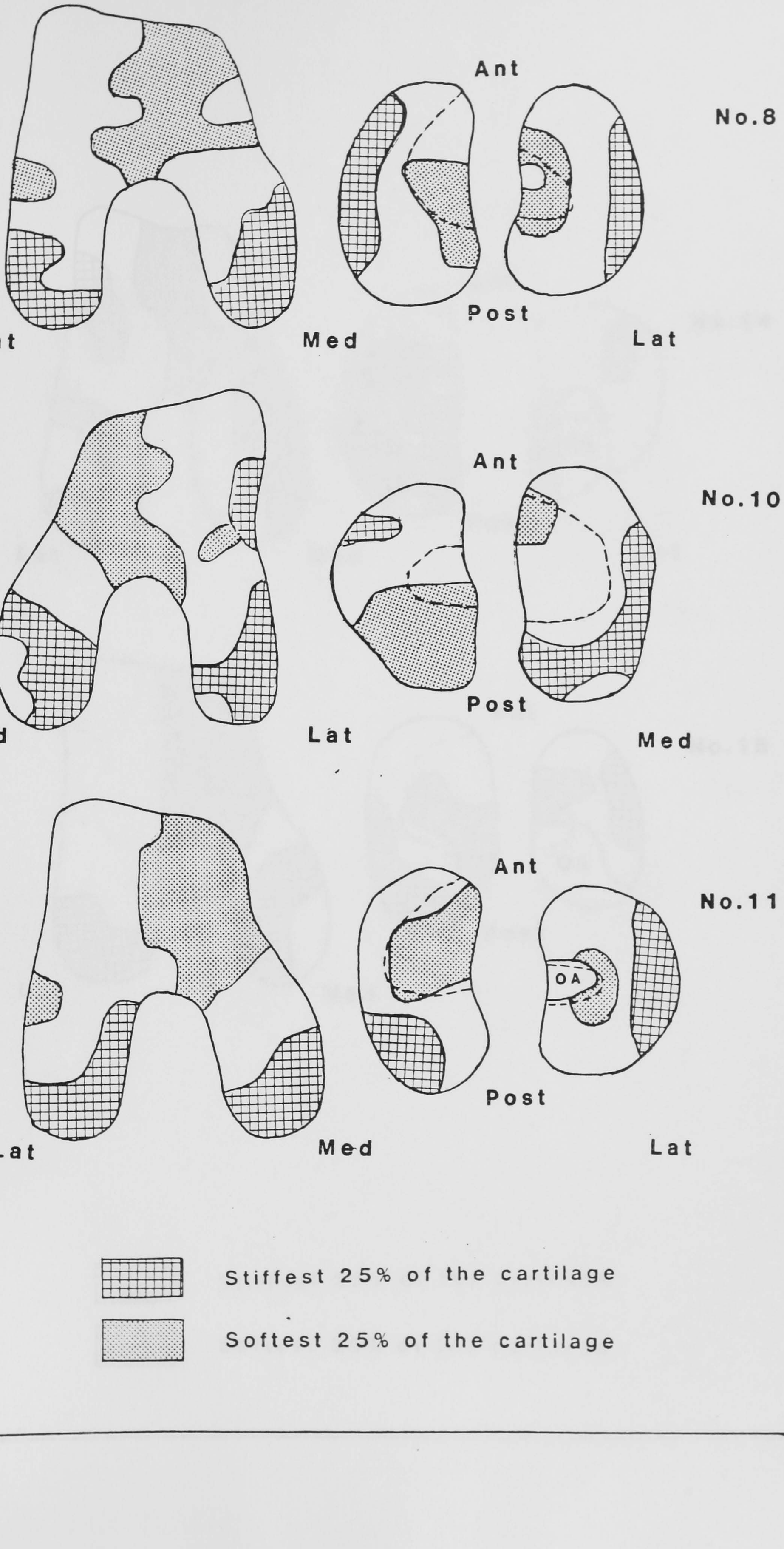


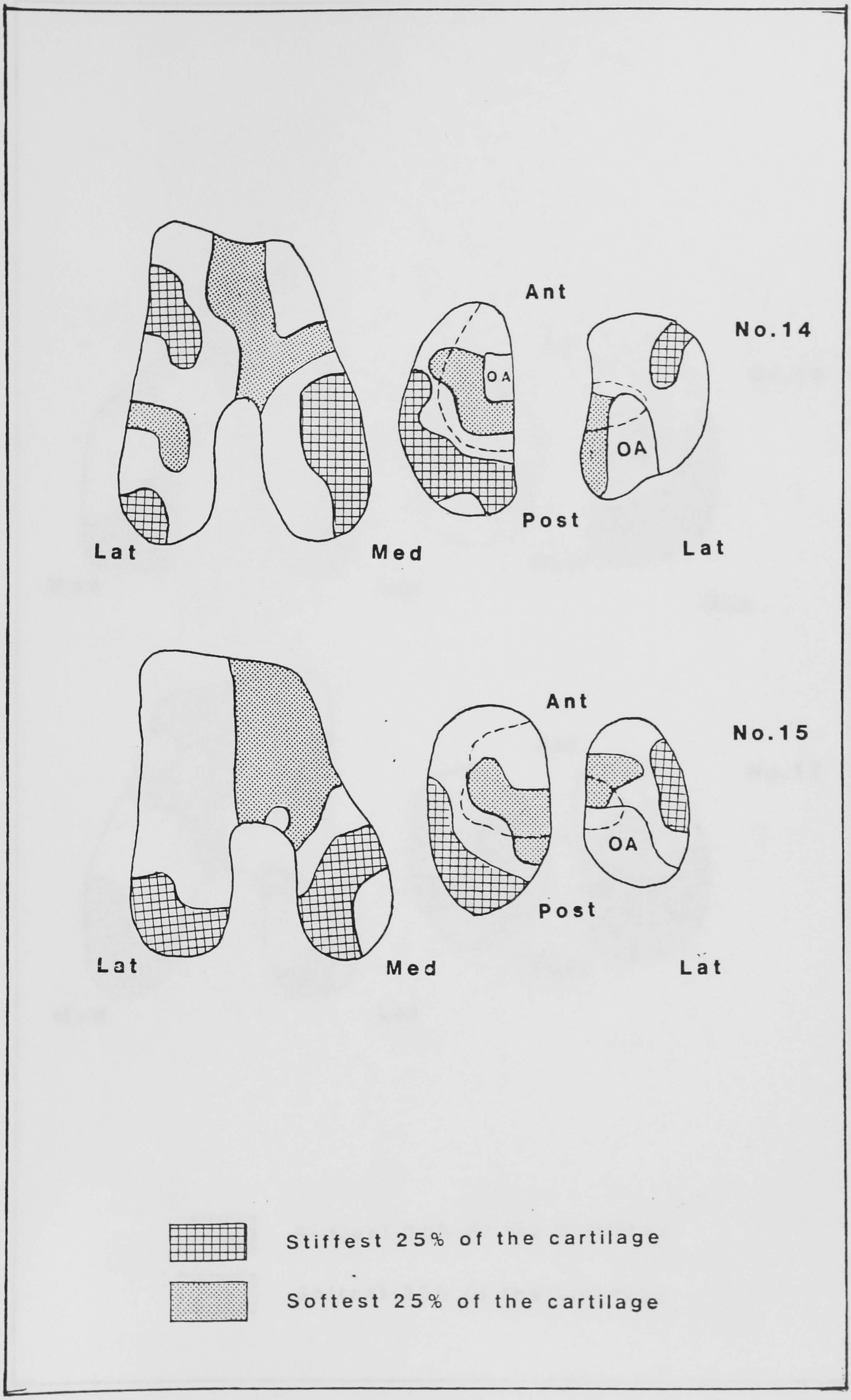


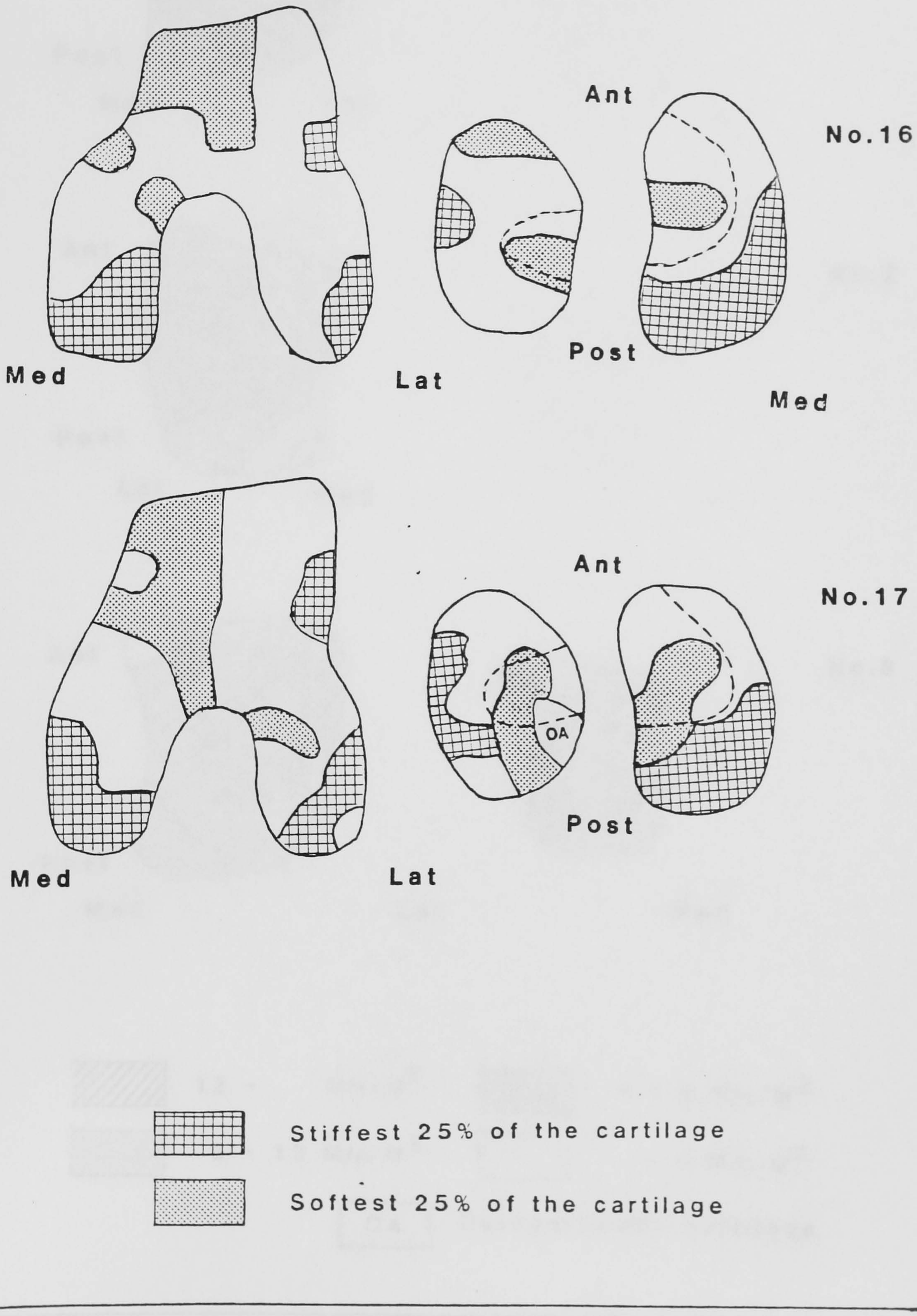
No.7

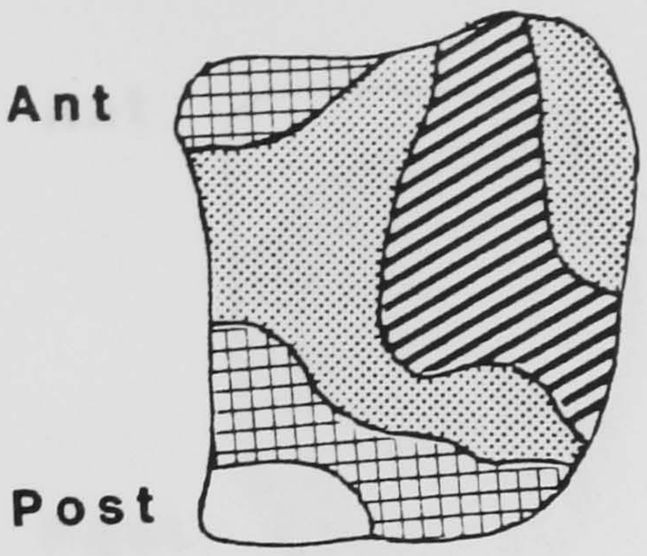
Stiffest 25% of the cartilage

Softest 25% of the cartilage

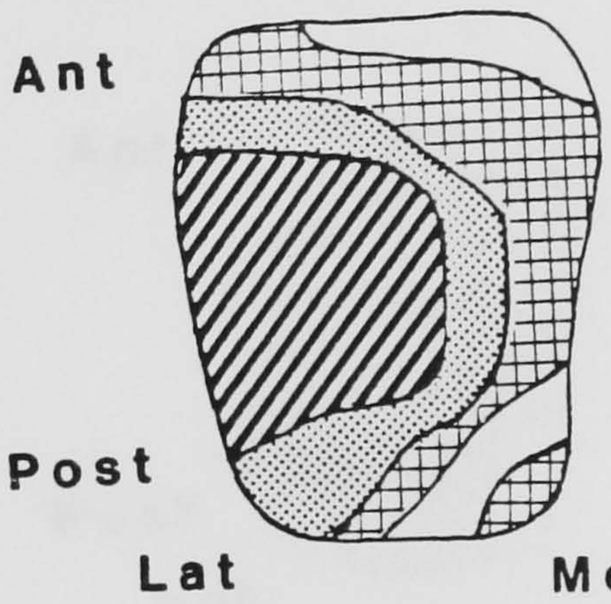

Ant
No.8
Lat
Med
Post
Lat
Ant
No.10
Med
Lat
Post
Med
Ant
No.11
OA
Lat
Med
Post
Lat
Stiffest 25% of the cartilage
Softest 25% of the cartilage


Ant
OA
No.14
Post
Lat
Med
Lat
Ant
OA
No.15
Post
Lat
Med
Lat
Stiffest 25% of the cartilage
Softest 25% of the cartilage

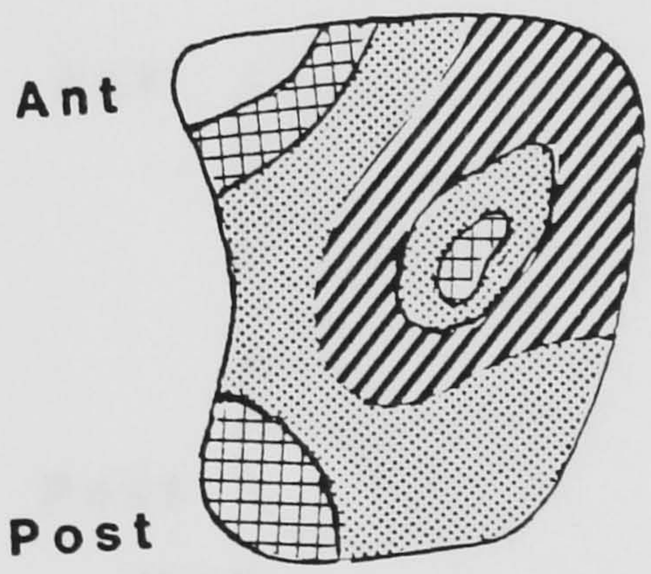

Ant
No.16
Post
Med
Lat
Med
Ant
No.17
OA
Post
Med
Lat
Stiffest 25% of the cartilage
Softest 25% of the cartilage

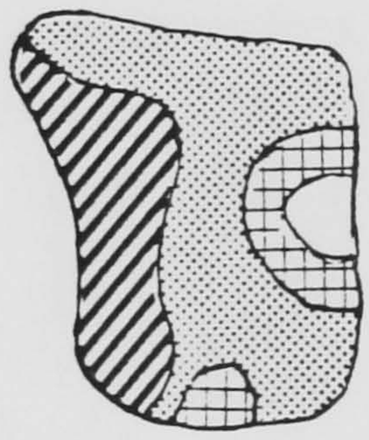


No.1



No.2





No.3

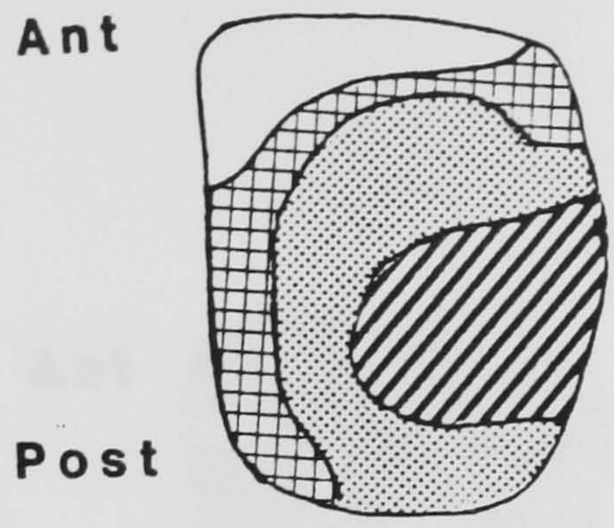
12 - MN/M^2

6 - 9 MN/M^2

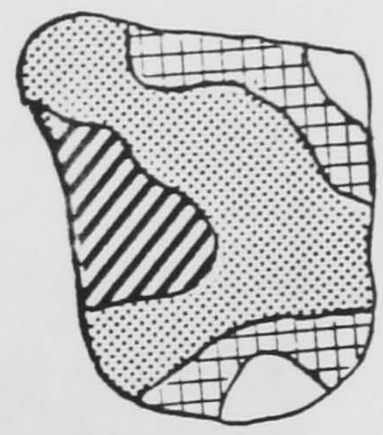
9 - 12 MN/M^2

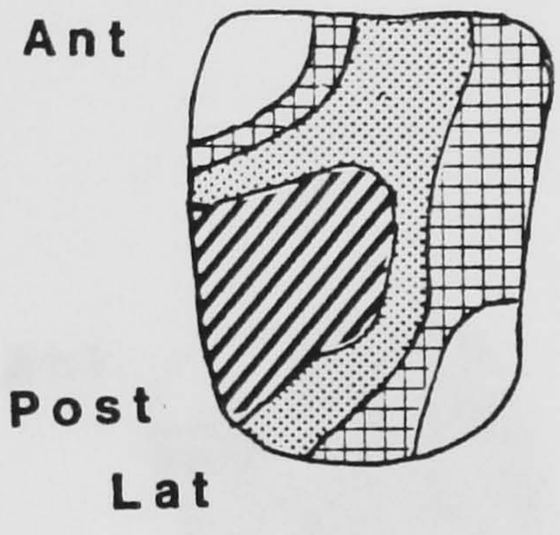
- 6 MN/M^2

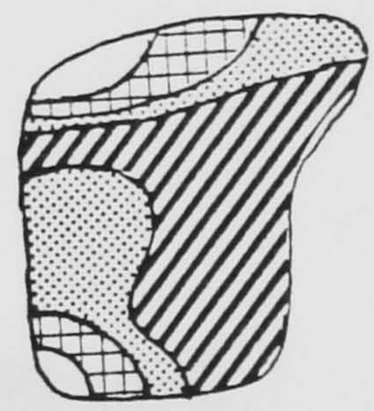
OA Osteoarthritic cartilage



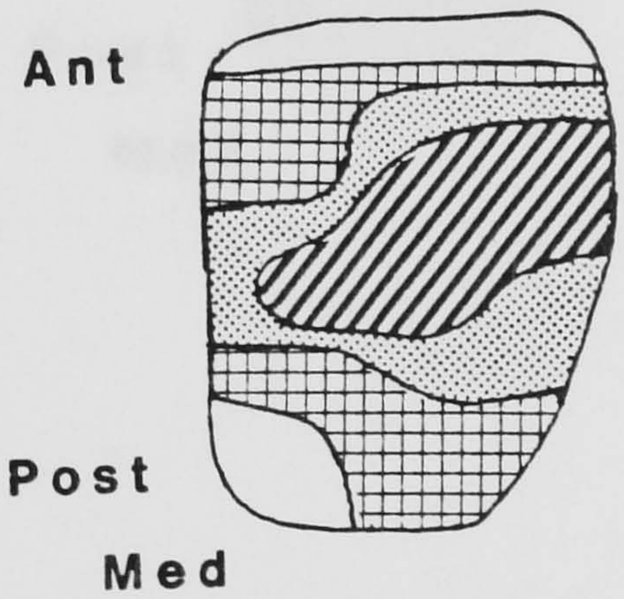
No.4

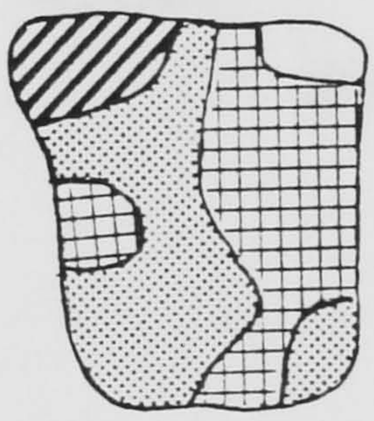






No.5





No.6

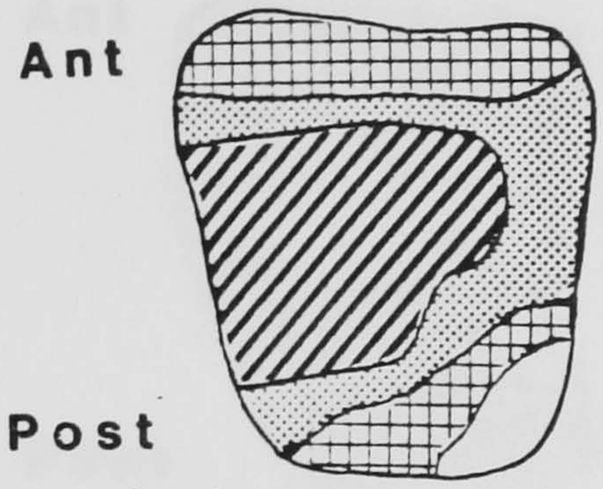
12 - MN/M^2

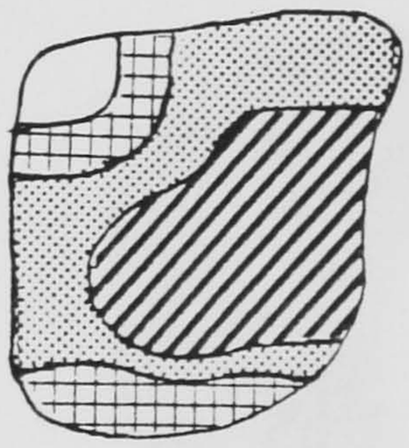
9 - 12 MN/M^2

6 - 9 MN/M^2

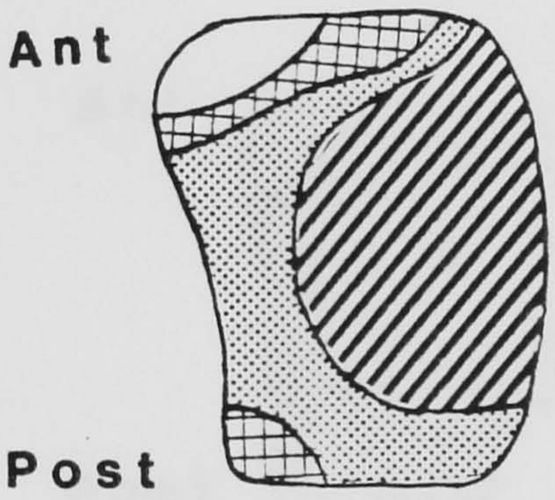
- 6 MN/M^2

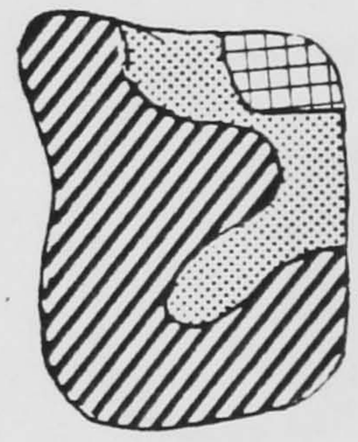
OA Osteoarthritic cartilage





No.7





No.8

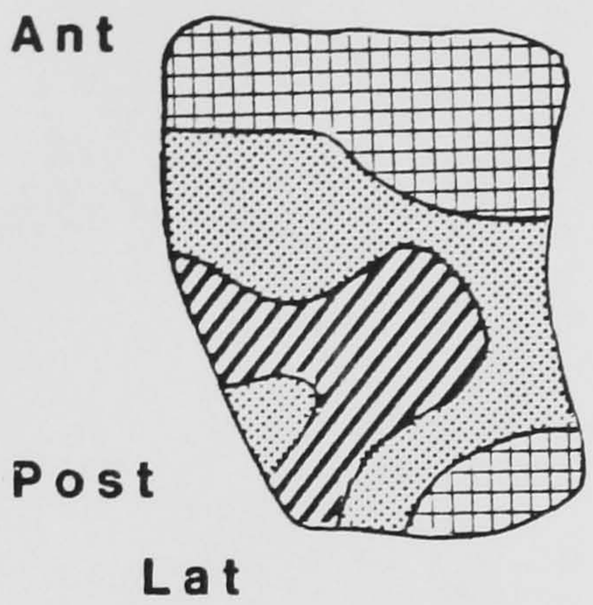
12 - MN/M^2

9 - 12 MN/M^2

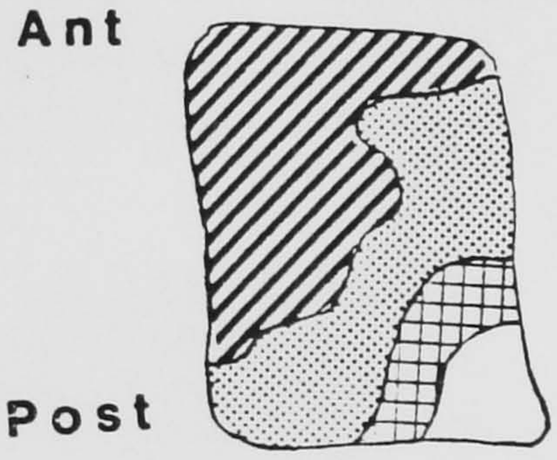
6 - 9 MN/M^2

- 6 MN/M^2

OA Osteoarthritic cartilage



No.9



No.10

12 - MN/M^2

9 - 12 MN/M^2

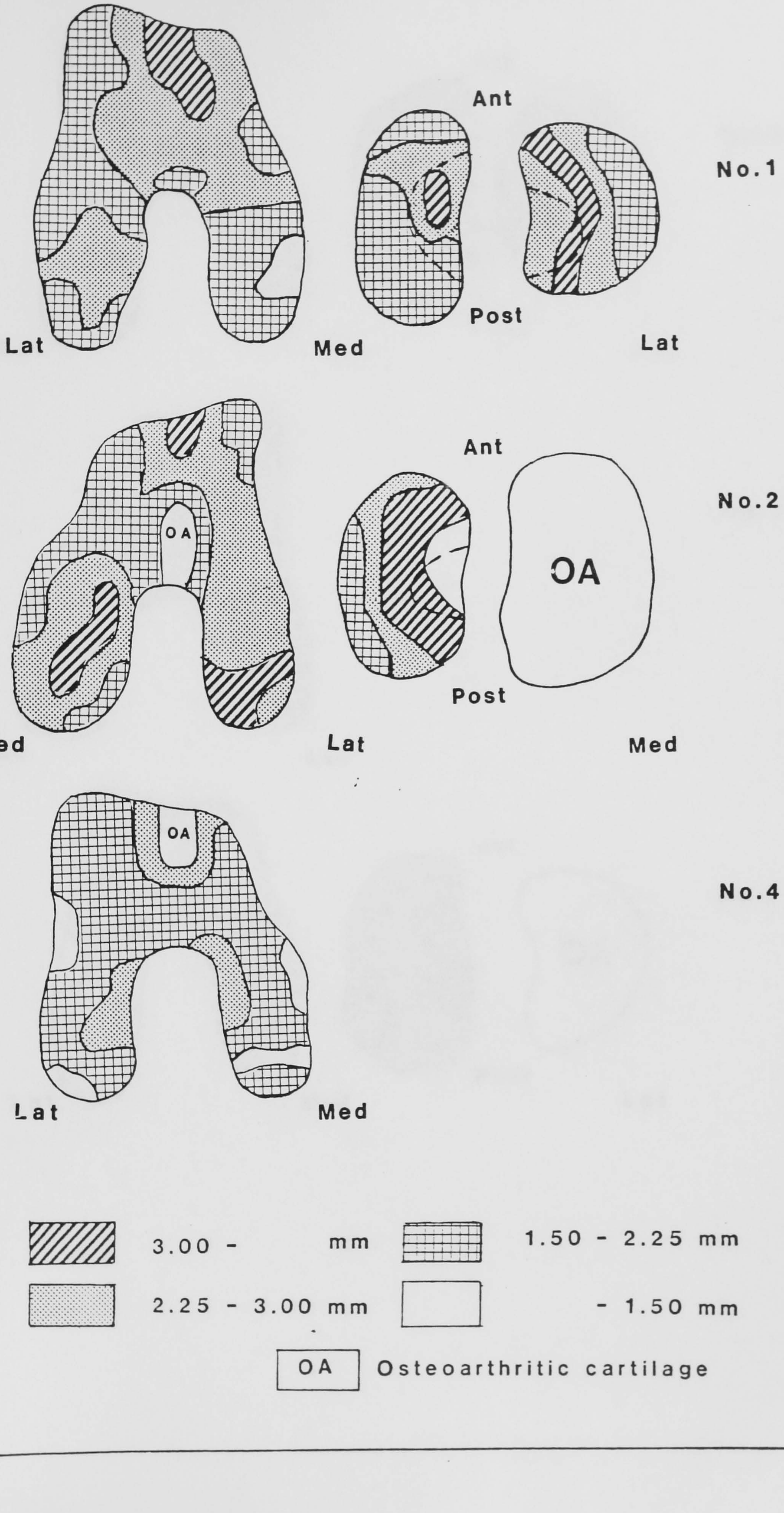
6 - 9 MN/M^2

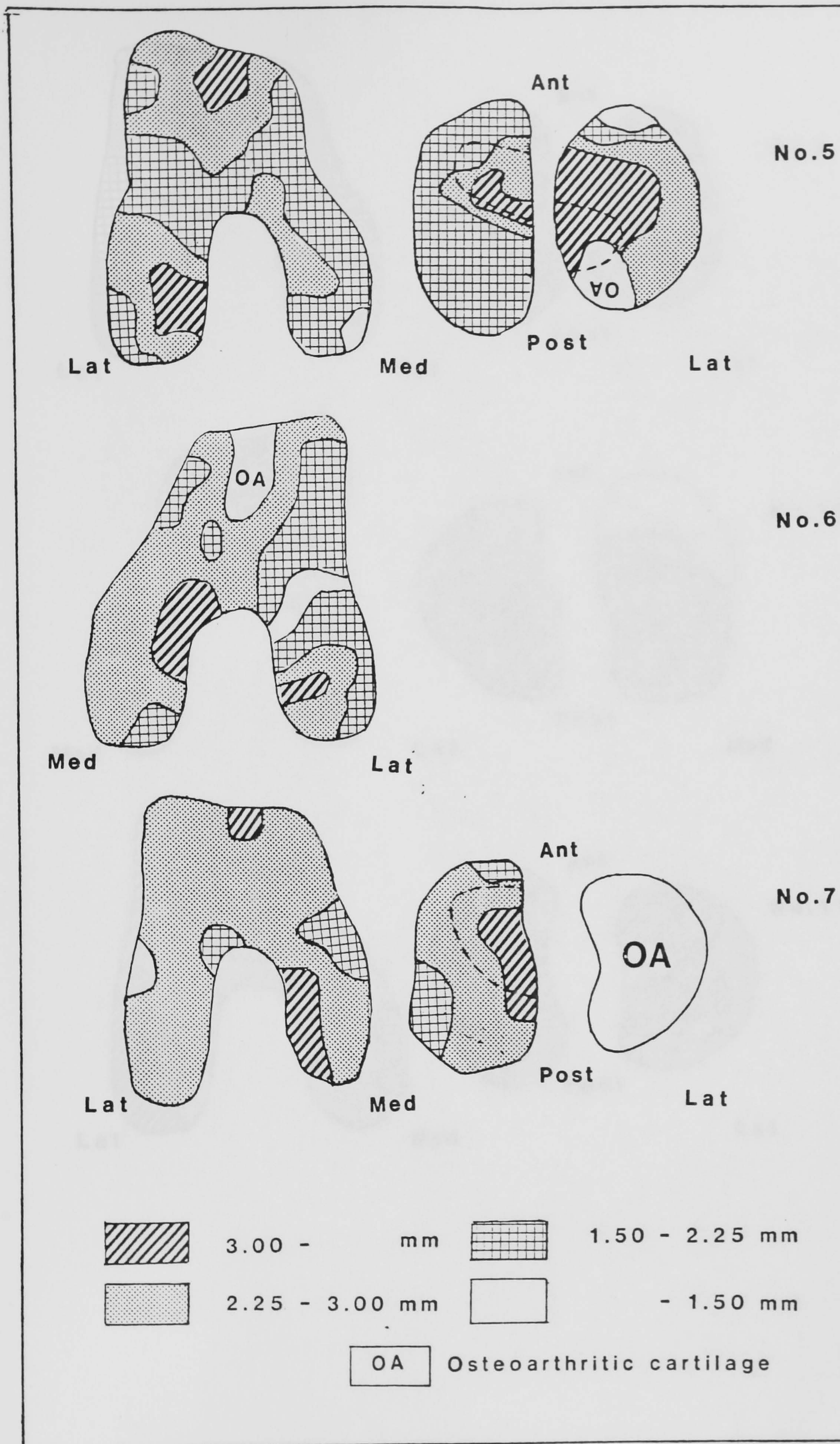
- 6 MN/M^2

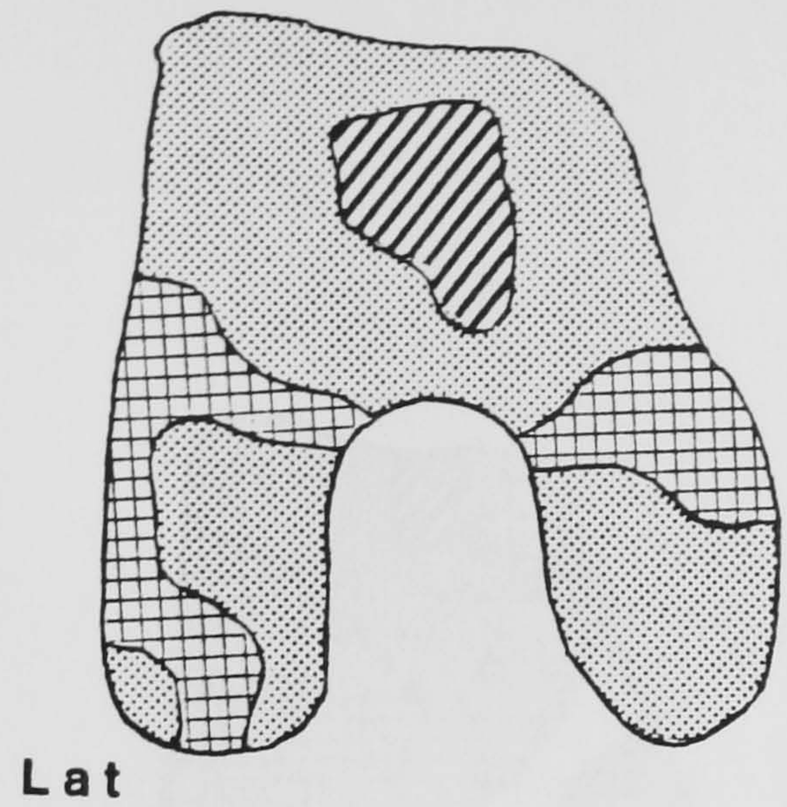
OA Osteoarthritic cartilage

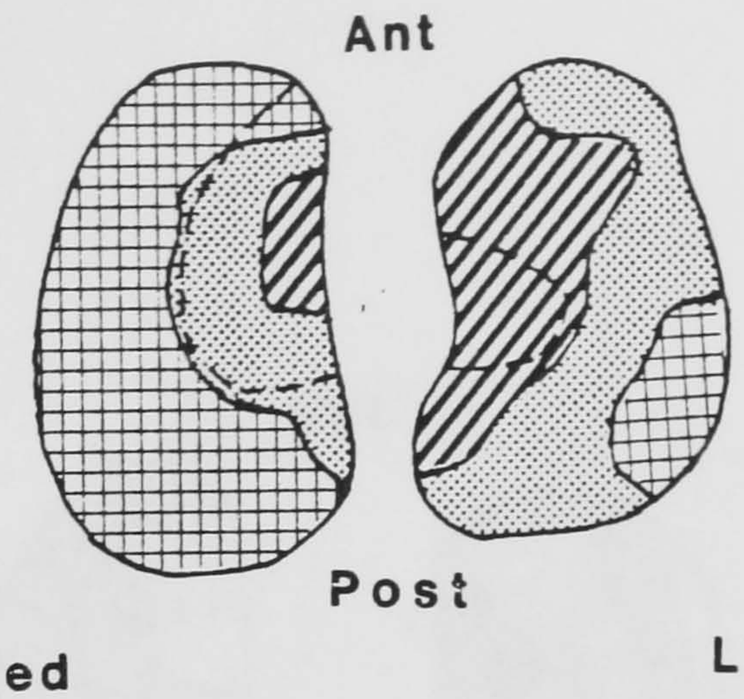
APPENDIX C.

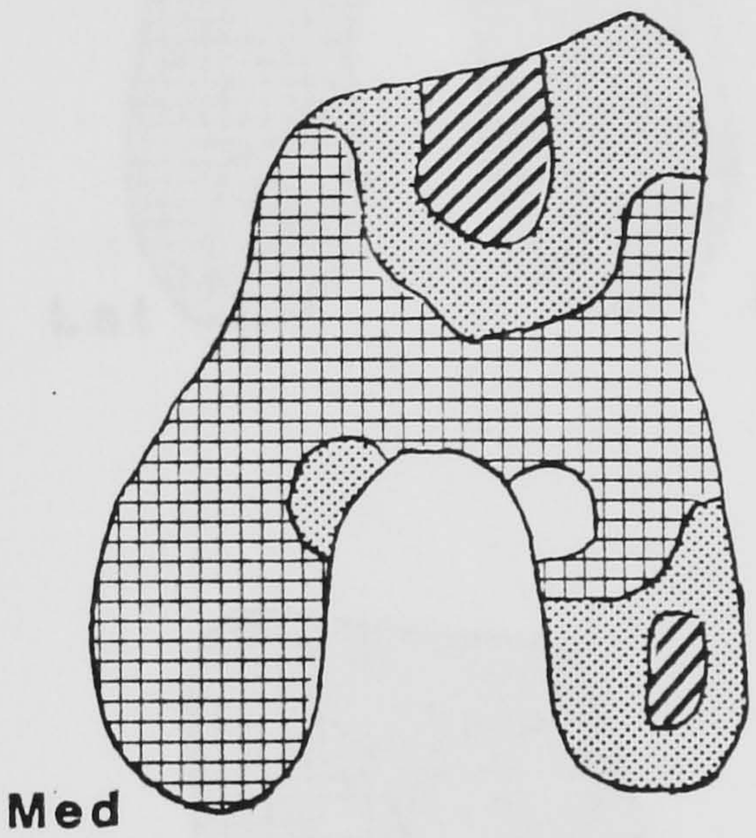
TOPOGRAPHICAL MAPS OF CARTILAGE THICKNESS.

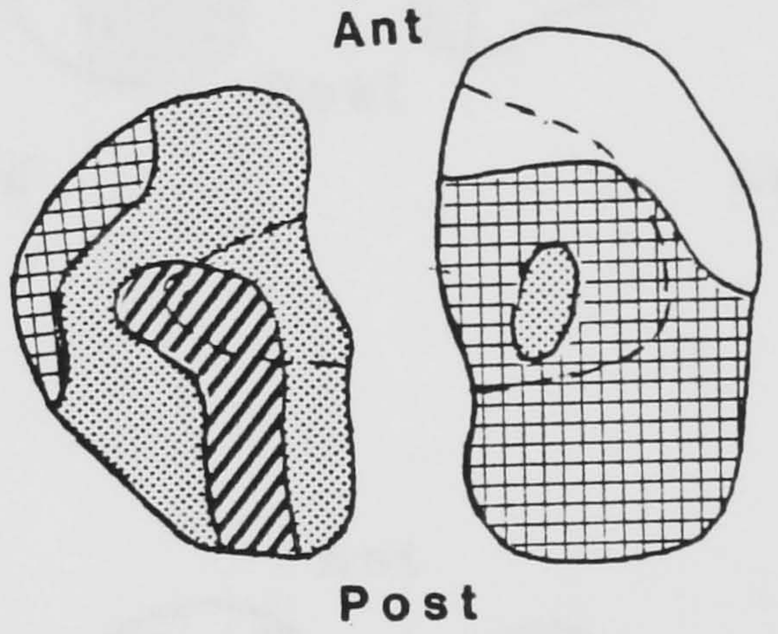

Ant
Post
Lat
Med
Lat
No.1
OA
Med
Ant
Post
Lat
Med
No.2
OA
Lat
Med
No.4
3.00 - mm
1.50 - 2.25 mm
2.25 - 3.00 mm
- 1.50 mm
OA
Osteoarthritic cartilage

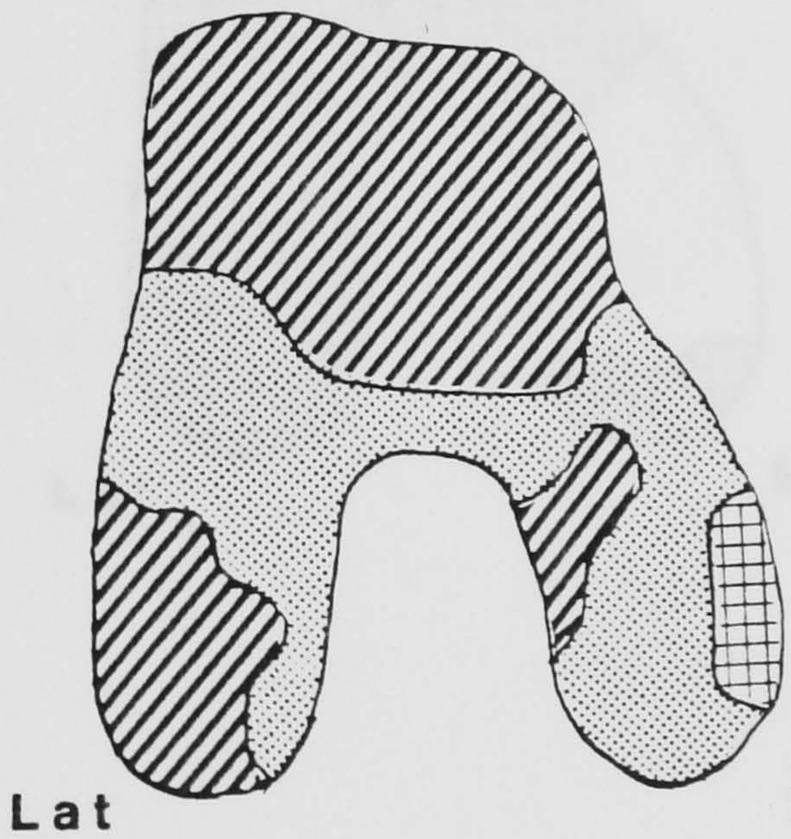

Ant
No.5
OA
Post
Lat
Med
OA
No.6
Med
Lat
Ant
No.7
OA
Post
Lat
Lat
Med
3.00 - mm
1.50 - 2.25 mm
2.25 - 3.00 mm
- 1.50 mm
OA
Osteoarthritic cartilage

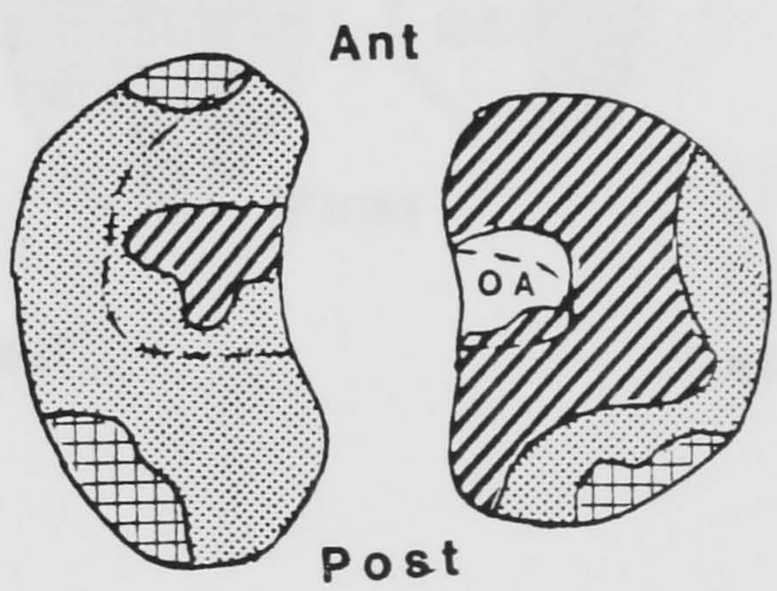












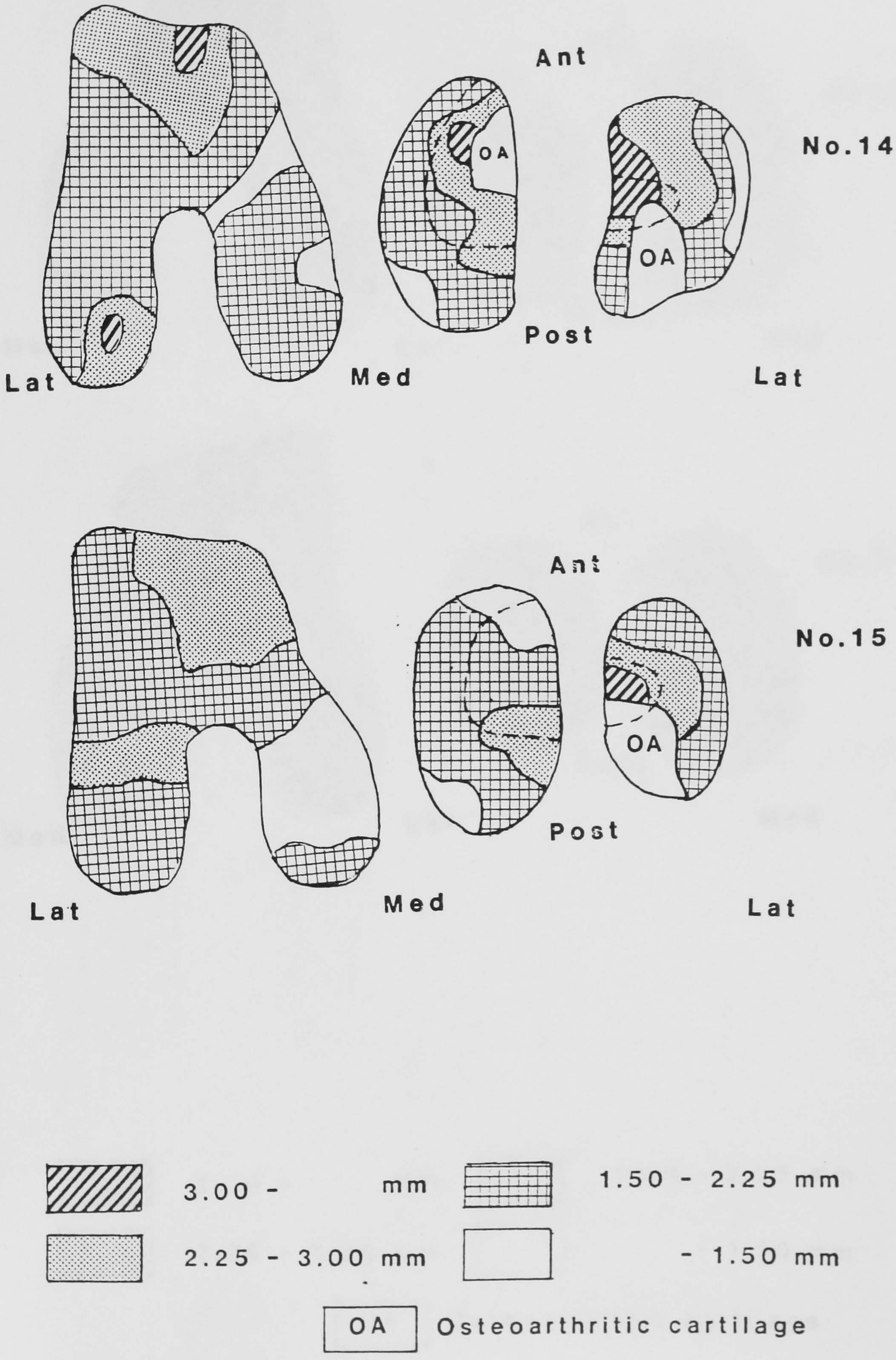
3.00 - mm

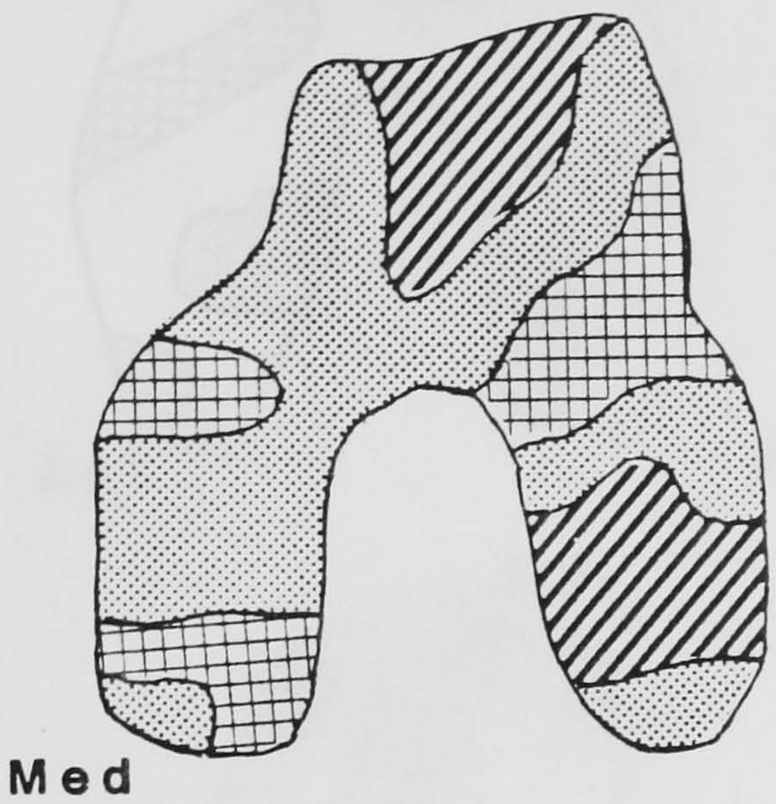
1.50 - 2.25 mm

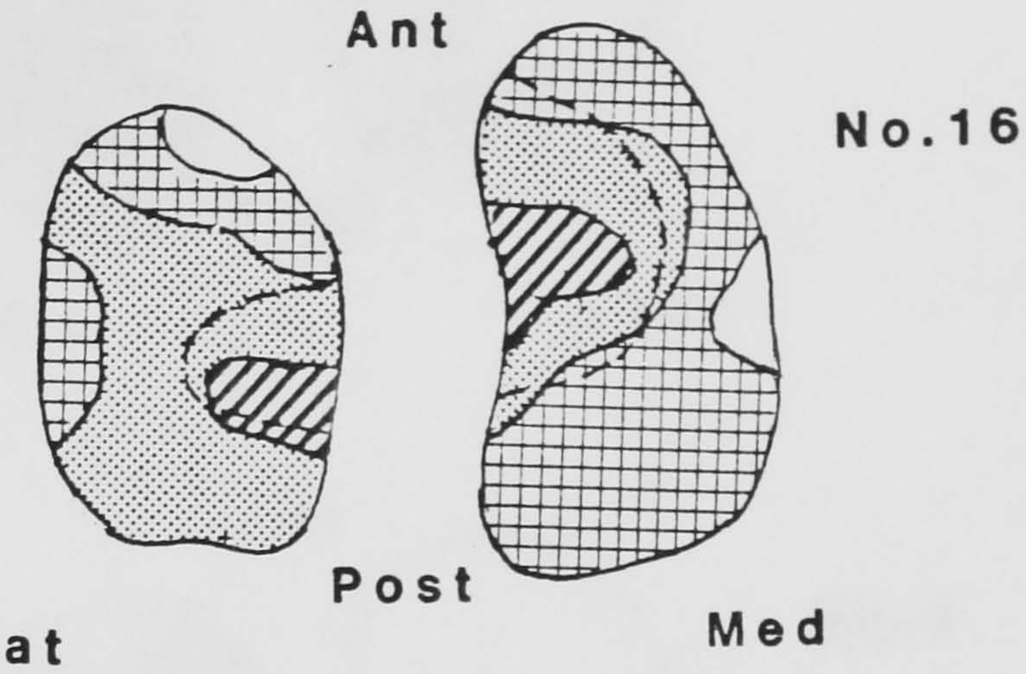
2.25 - 3.00 mm

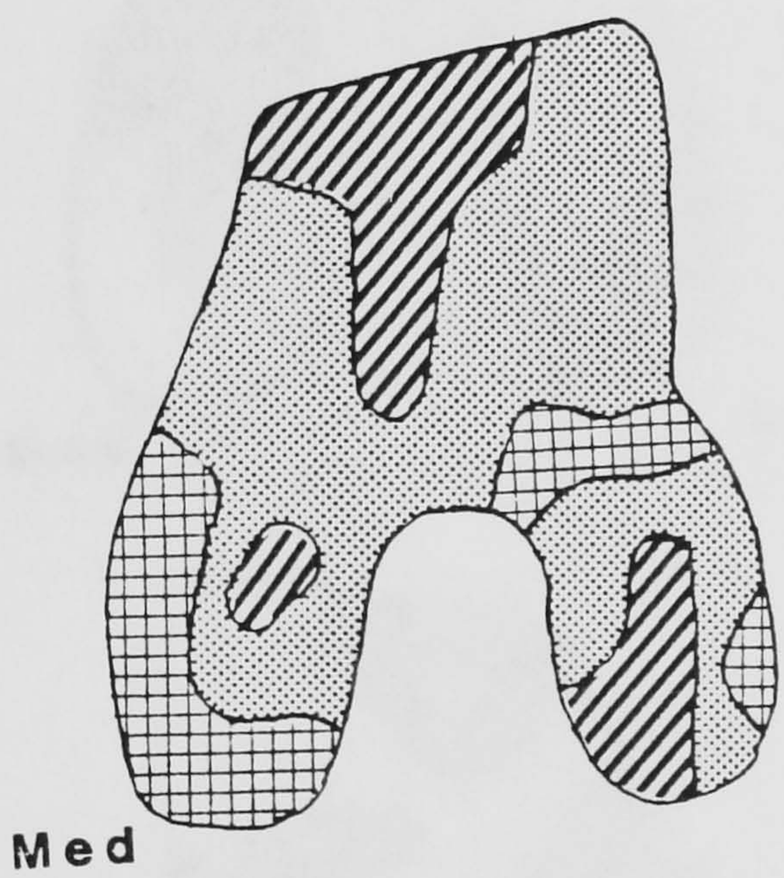
- 1.50 mm

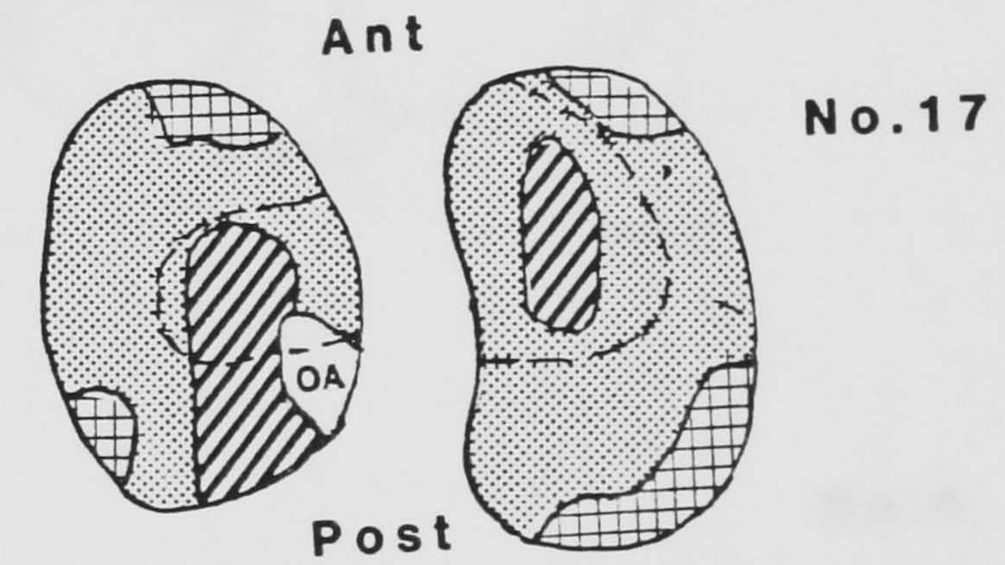
OA Osteoarthritic cartilage


Ant
OA
Post
Lat
Med
Lat
No.14
Ant
OA
Post
Lat
Med
Lat
No.15
3.00 - mm
1.50 - 2.25 mm
2.25 - 3.00 mm
- 1.50 mm
OA
Osteoarthritic cartilage









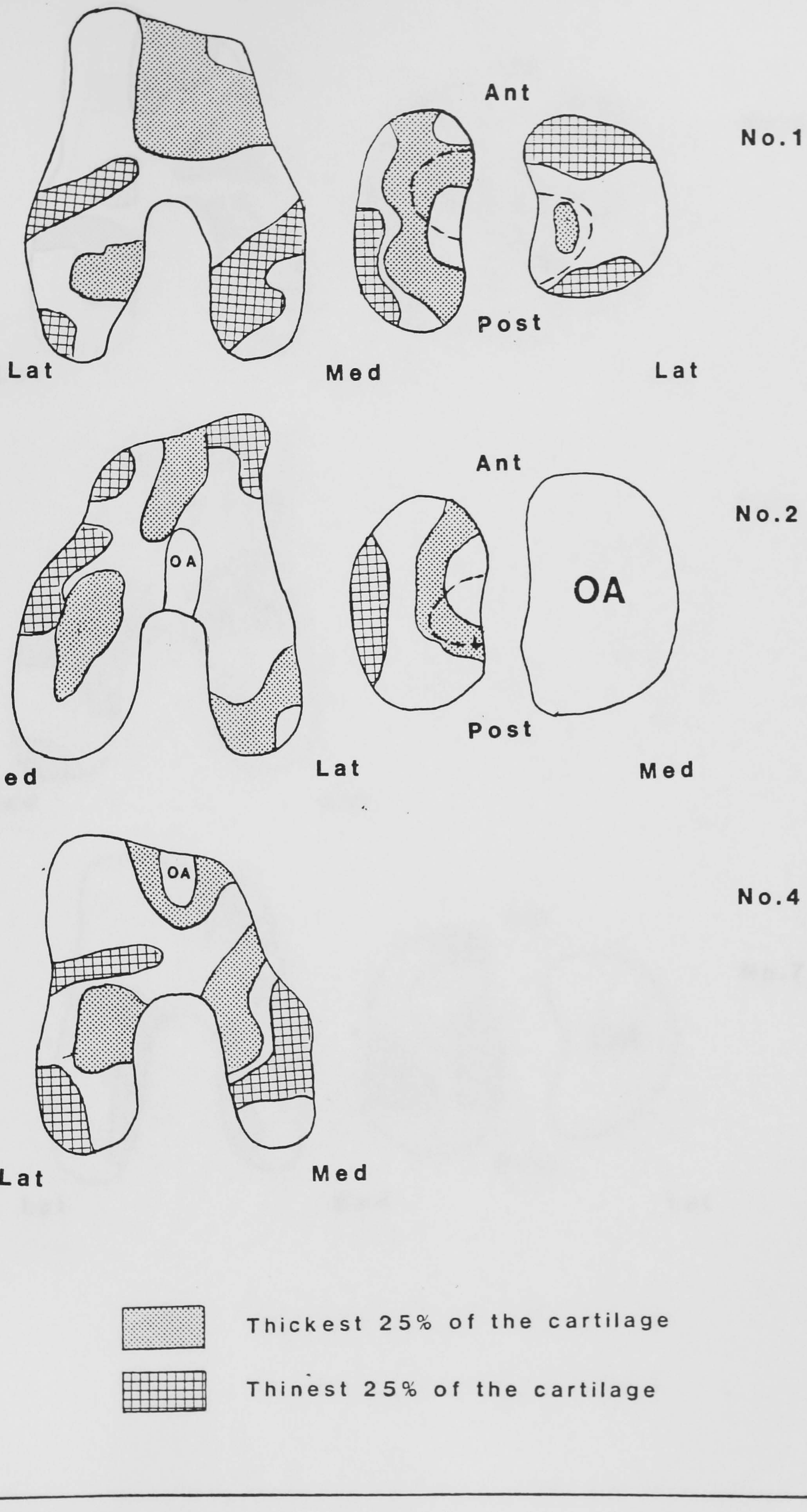
3.00 - mm

2.25 - 3.00 mm

1.50 - 2.25 mm

- 1.50 mm

OA Osteoarthritic cartilage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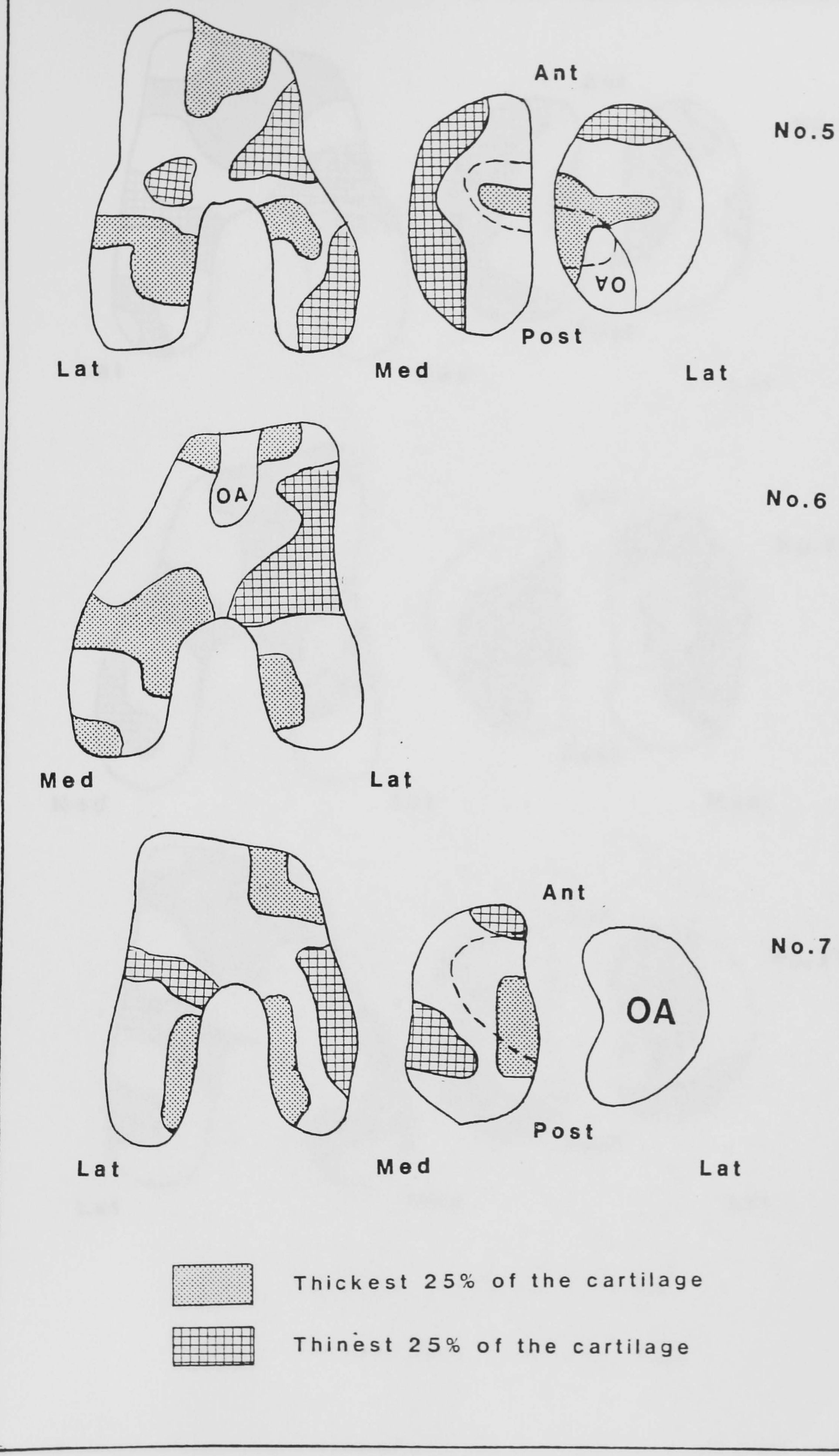
No.4
Thickest 25% of the cartilage
Thinest 25% of the cartilage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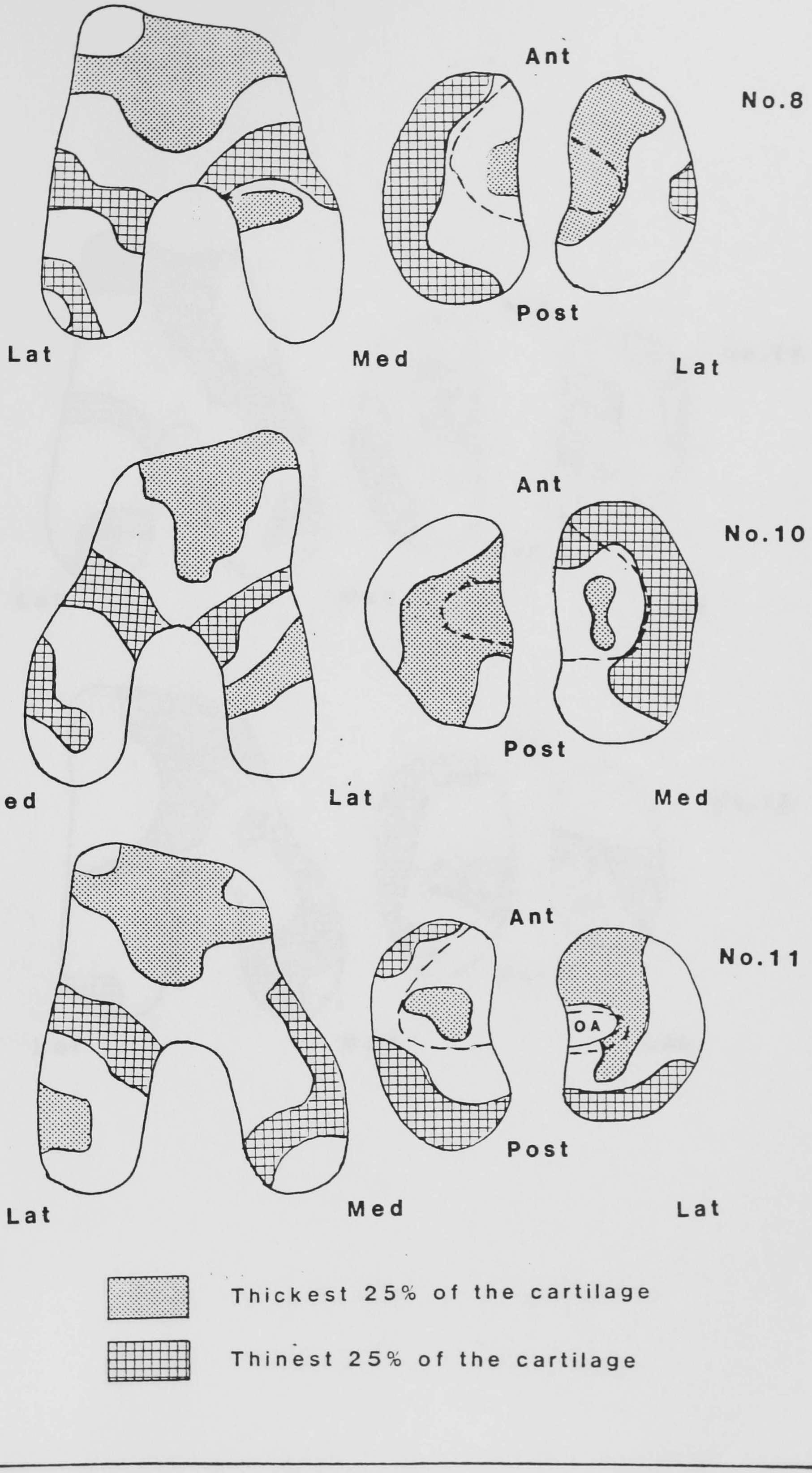
Thickest 25% of the cartilage
Thinest 25% of the cartilage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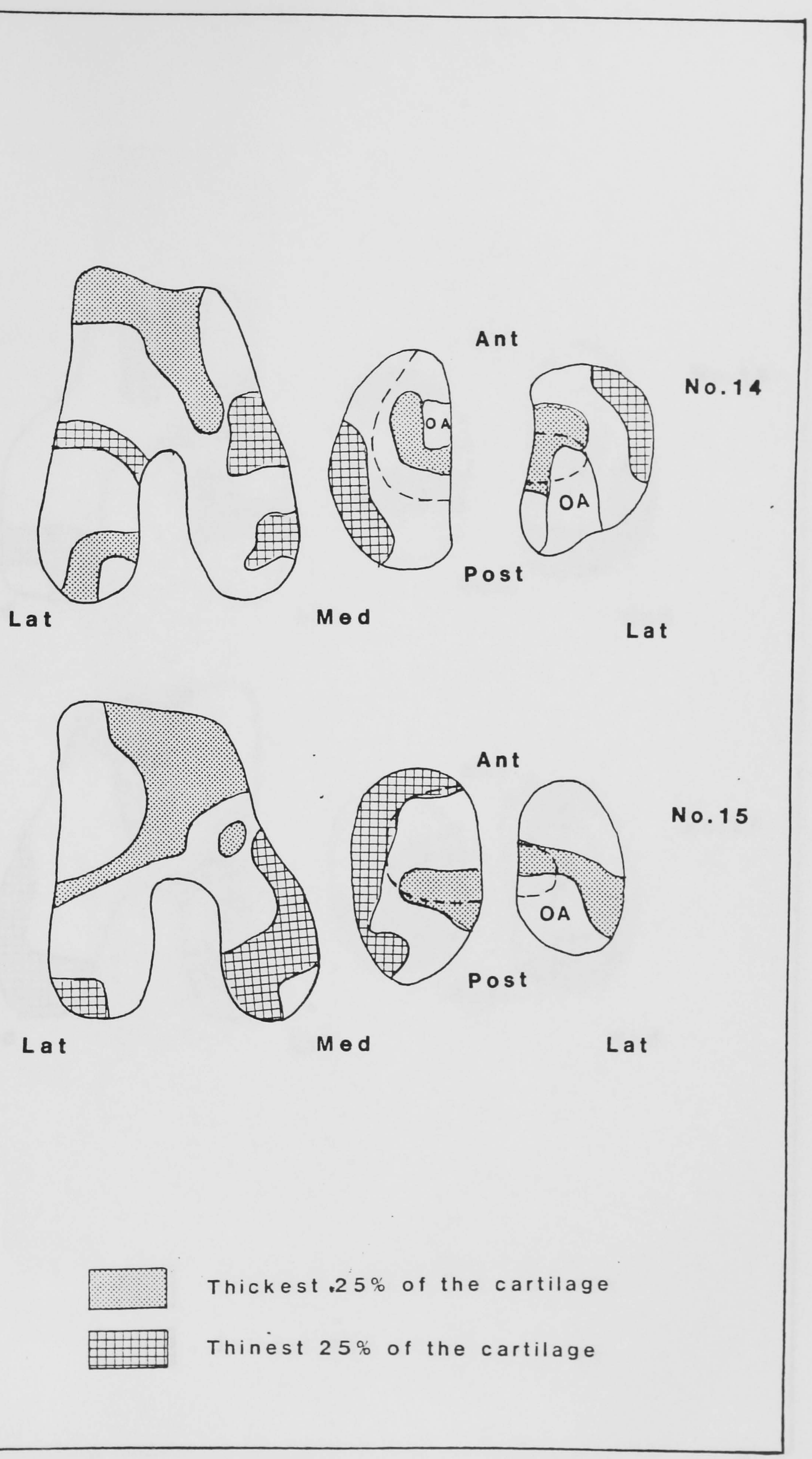
Thickest 25% of the cartilage
Thinest 25% of the cartilage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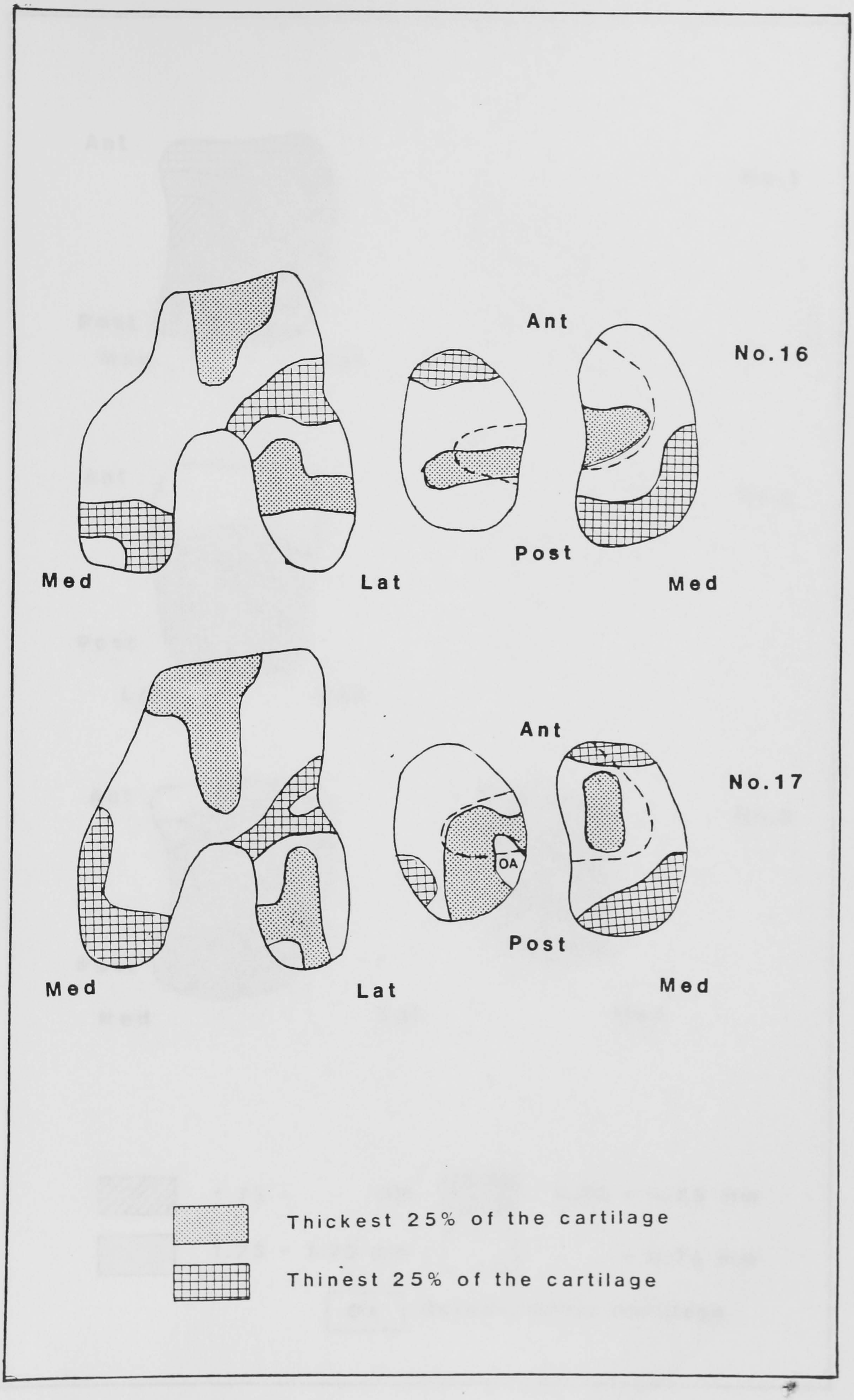
Thinest 25% of the cartilage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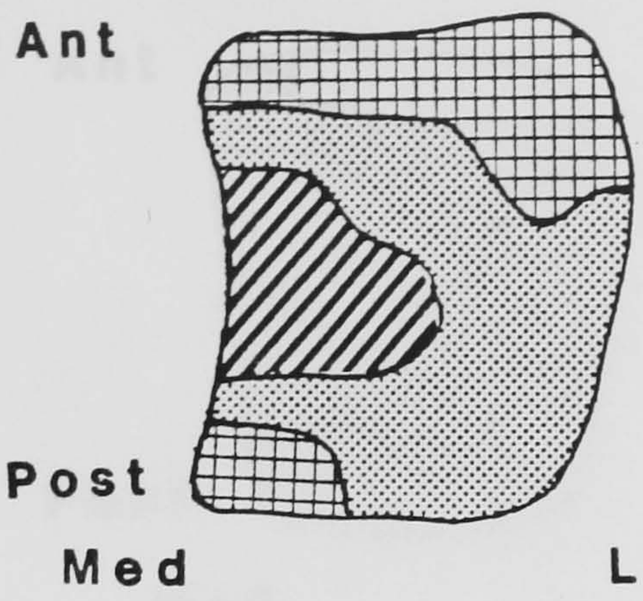
Thickest 25% of the cartilage
Thinest 25% of the cartilage



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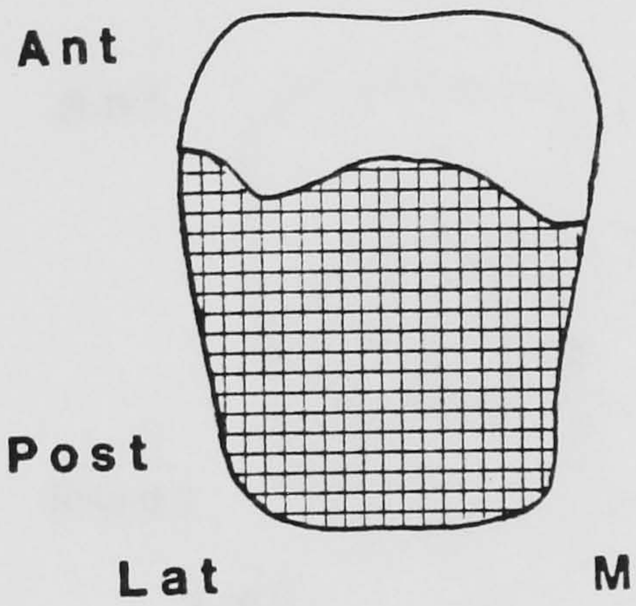

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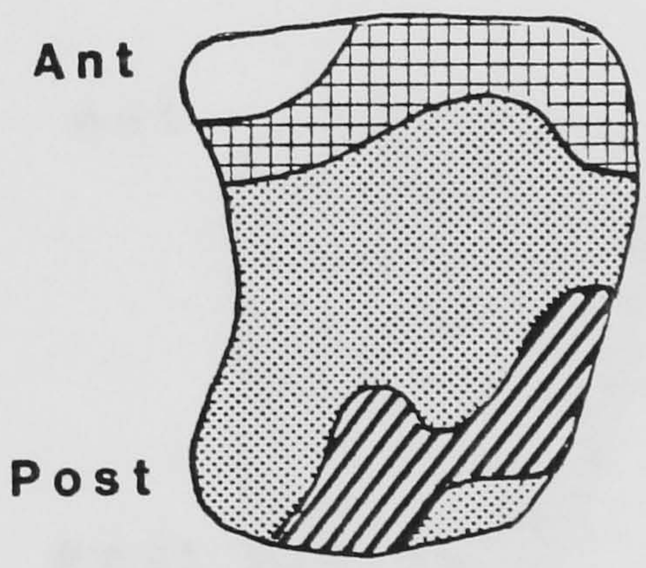
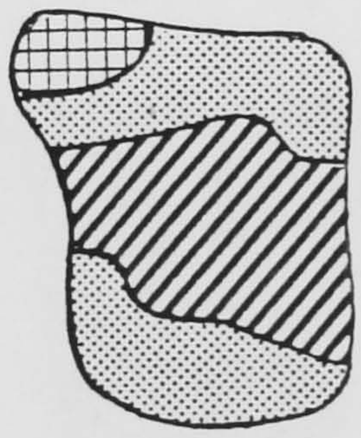
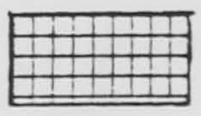
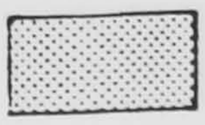




No.3


1.75 - mm


0.75 - 1.25 mm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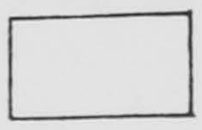
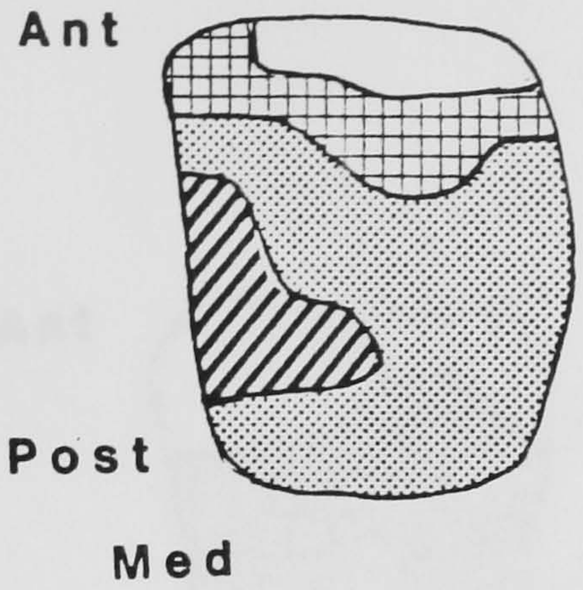
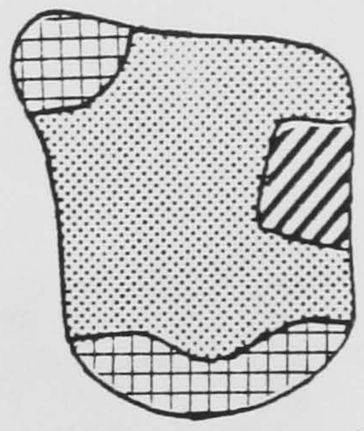

- 0.75 mm

OA Osteoarthritic cartilage





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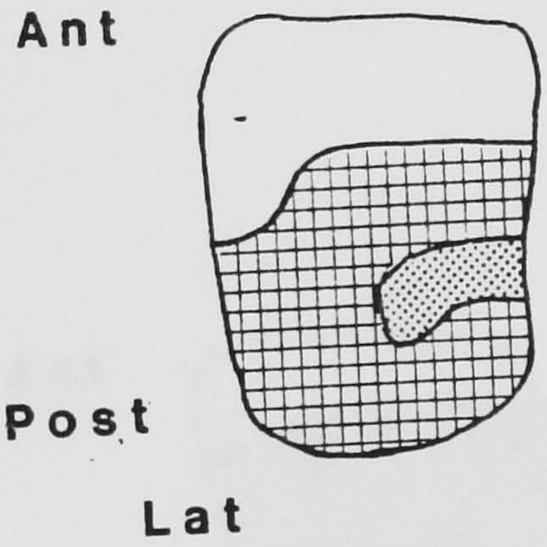

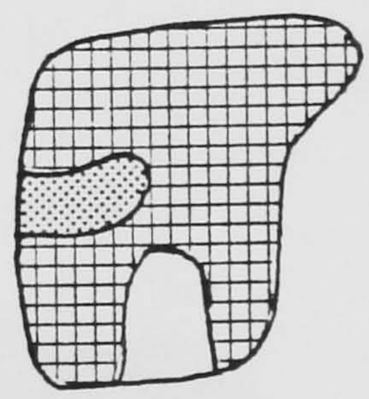

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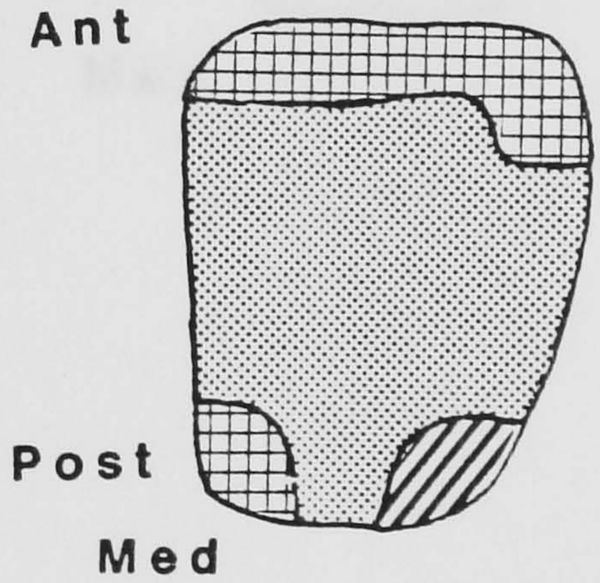
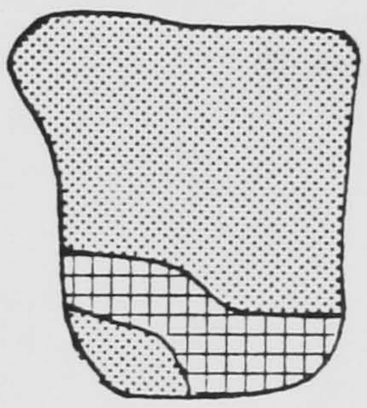




No.6

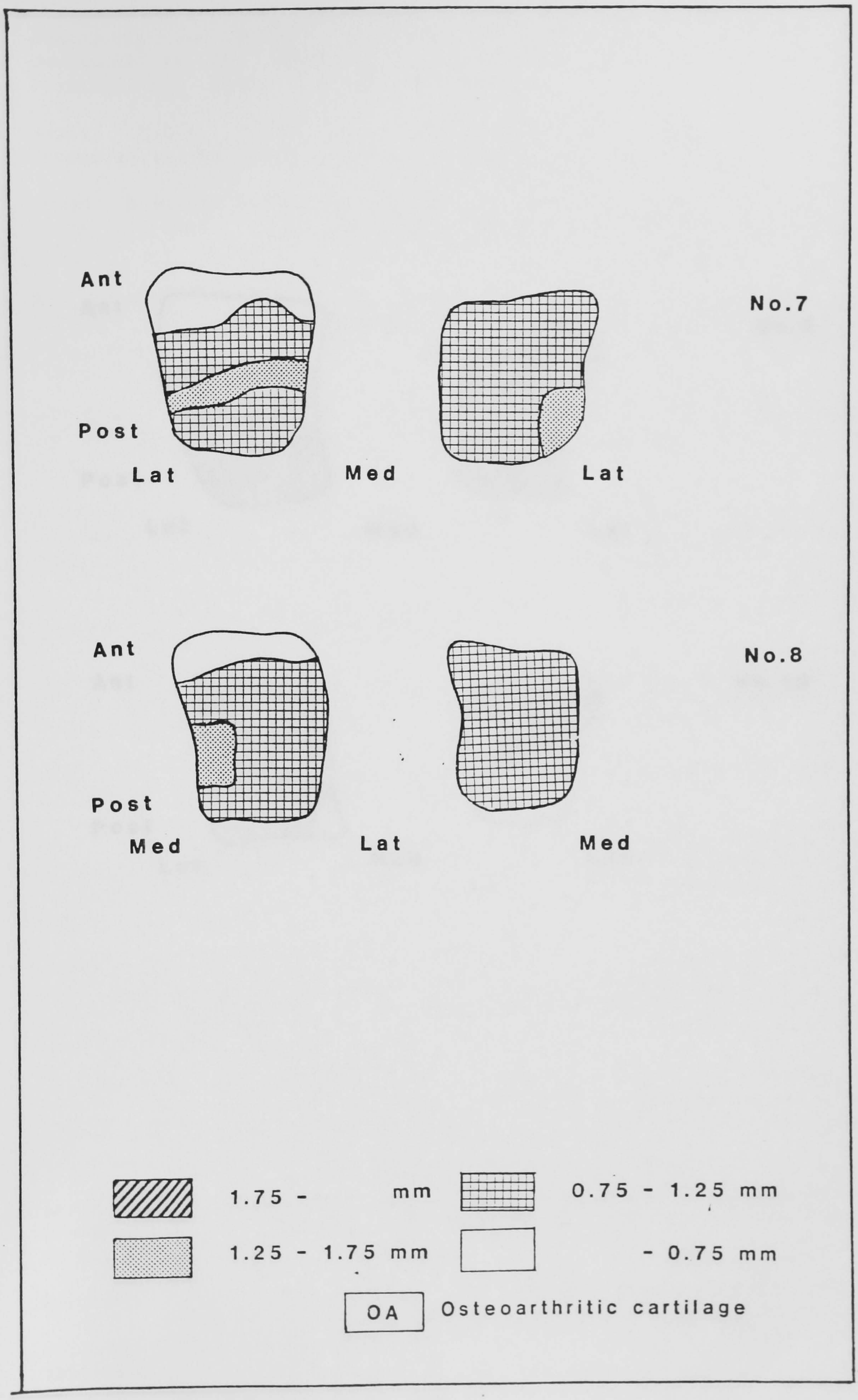
1.75 - mm

0.75 - 1.25 mm

1.25 - 1.75 mm

- 0.75 mm

OA Osteoarthritic cartilage


Ant
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No.7
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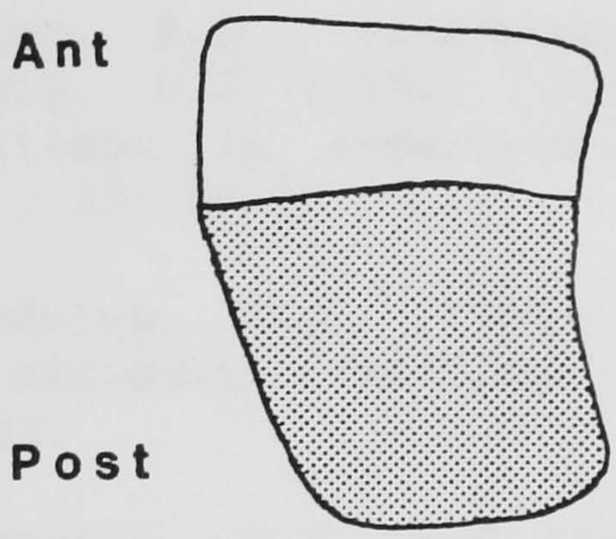
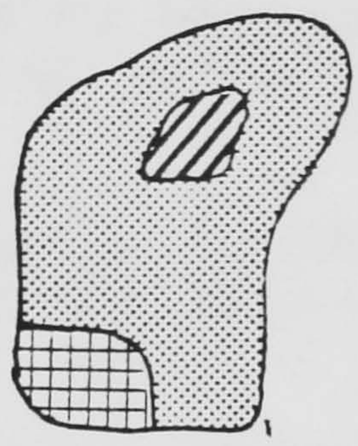
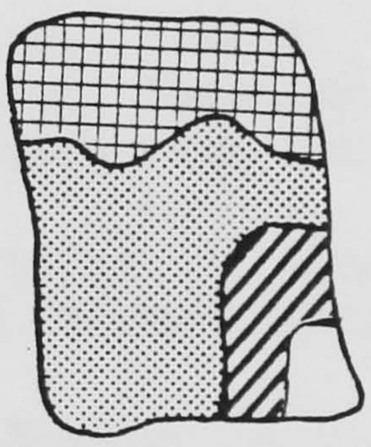
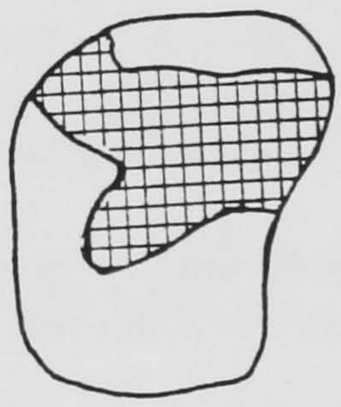
No.8
1.75 - mm
0.75 - 1.25 mm
1.25 - 1.75 mm
- 0.75 mm
OA
Osteoarthritic cartilage





No.9





No.10

1.75 - mm

1.25 - 1.75 mm

0.75 - 1.25 mm

- 0.75 mm

OA Osteoarthritic cartilage

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ACKNOWLEDGEMENTS

There are many people, too numerous to mention, whom I would like to thank for their help and assistance in writing this thesis. In particular however, I would like to extend my thanks to Professor Verna Wright for allowing me to work at the Rheumatology and Rehabilitation Research Unit, Dr Bahaa Seedhom for his invaluable supervision and encouragement, Messrs Pullan and Whitham for their inimitable technical expertise and Mesdames Sharp and Dibb for their haste in typing this thesis.

I am also grateful to Professor Helen Muir and Dr Stephen Carney of the Kennedy Institute of Rheumatology, London for their advice and assistance in the biochemical analysis

My thanks are also due to the Science and Engineering Research Council and the Emmandjay trust, for the financial support which has enabled this project to continue to its present conclusion. I am also indebted to the Arthritis and Rheumatism Council who generously support the work of the Rheumatology and Rehabilitation Research Unit.