Mechanical properties of articular cartilage: variation with depth

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MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE - VARIATION WITH DEPTH

by

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Abstract

The response of human, adult articular cartilage to compressive loading has been investigated in this study.

Full thickness specimens of patellar cartilage were subjected to compressive loading either in a Scanning Electron Microscope (SEM) or in one of the two conventional testing machines.

Testing in the SEM meant that video recordings could be made of the deformation which occurred during loading, and subsequent analysis of these video recordings enabled the determination of the variation in the mechanical properties through the thickness of the cartilage.

Testing of individual slices of cartilage and repeated testing of specimens reduced in thickness by the removal of successive slices of cartilage were performed in the conventional testing machines. The results of these tests also give an indication as to the variation in mechanical properties with depth.

Results indicate that the compliance of normal patellar cartilage is highest in the superficial zone, decreases to a minimum in the mid-zone and increases again slightly in the deep zone.

The full thickness tests were used to determine the effect (if any) of age, site of specimen, storage procedure and variations in water and uronic acid content on the response of the tissue.

These results show that the compliance of articular cartilage is highly dependent on the age of the tissue, increasing with age, and that specimens stored at - 20^{o}C show, on average, a higher value of compliance than specimens stored at + 4^{o}C.

No systematic variation in compliance of specimen with site was observed and there was no correlation between either water or uronic acid content and compliance.

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

The human body is made up of a great variety of tissues, each performing specific functions and each, in its healthy state, well suited to the performance of these functions.

One of the main groups of tissues of interest to bioengineers are the load bearing or structural tissues of the body. An understanding of the loading to which these tissues are subjected, how the healthy tissues withstand and transmit the loads and why changes in the tissue make it unable to perform its required function, may help in the diagnosis and treatment of many diseases.

Articular cartilage is a tissue which has been studied by many bioengineers. It is a very important structural tissue of the body which, in its healthy state, enables the articulating joints of the body to function properly. For many years it has been postulated that the cause of arthritis is degeneration of the articular cartilage in the affected joint. However, despite the amount of time and effort which has been applied to the problem the mechanisms by which articular cartilage becomes disrupted and, therefore, unable to perform its normal functions, are not fully understood.

Articular cartilage is a thin layer of connective tissue, approximately 2 - 4 mm thick in the large human joints such as the knee and hip, which covers the articulating ends of bones in synovial joints.

The main functions of articular cartilage appear to be to enable the articulating bones to transmit high loads whilst maintaining low contact stresses and to move over each other with very little frictional resistance.

Although articular cartilage can successfully perform these functions for the entire life-span of an individual, disruption of its structure and consequently failure of function is a common occurrence among the population.

Disruption of articular cartilage, known as fibrillation, is characterised by splitting and fraying of the tissue and, on naked eye examination, fibrillated cartilage has a 'matt' appearance in comparison with its normal 'glossy' appearance.

Although it is thought that fibrillation may occur as an age related change of no clinical significance, it also seems likely that it represents the initial stages of osteoarthrosis.

Osteoarthrosis is a non-inflammatory disorder of articulating joints where disruption and thinning of the cartilage, often leading to exposure of underlying bone, is accompanied by formation of new bone at the articulating surface. The result of these changes is often severe pain and disablement of the affected joint which in most cases can only be relieved by replacement of the joint by a prothesis.

The incidence of osteoarthrosis and its relationship to age was investigated by Heine (1926). In one thousand routine post-mortems he found that in the knee the incidence was approximately 3% at 20 years, 45% at 40 years, 80% at 60 years and 95% at 80 years.

The social significance of such widespread occurrences of articular cartilage disruption is highlighted by the fact that in the U.K. arthritis and rheumatism together rank fourth as the cause of days lost from work because of sickness, seventh in general practioner consultations, eighth in out-patient referrals and eleventh in days spent in hospital (Freeman and Swanson 1975).

One of the fundamental starting points in determining how and why osteoarthrosis develops must be a complete understanding of the mechanical properties of articular cartilage. Only when this information is available, combined with information about the loading and possible biochemical changes due to enzyme activity, which can occur in articular cartilage in vivo, can we hope to develop therapeutic methods of either preventing or curing osteoarthrosis.

In this investigation the mechanical properties of both normal and osteoarthrotic human articular cartilage was studied. In particular the variation in properties throughout the thickness of the cartilage has been studied, by direct observation in a Scanning Electron Microscope (SEM) and by indirect measurement in two mechanical testing machines.

Although there have been many previous investigations into the mechanical properties of articular cartilage, none has yielded

detailed information as to the variation in properties with depth during compressive loading. It is felt that this information will be useful not only in predicting the stresses and strains which occur in articular cartilage *in vivo* but also possibly in determining the aetiology of osteoarthrosis.

The first technique entailed dynamic testing of articular cartilage in an SEM and visually recording the deformation which occurred, using video recording equipment. Subsequent analysis of the video recording, combined with measurements of overall deformation and loading, enabled correlation between local deformation and gross loading and deformation.

An SEM was used rather than a conventional light microscope because of the greater depth of field available and the ease with which recordings can be made. It was felt that these advantages would outweigh any disadvantages due to the tissue having to be tested *in vacuo*. However, the technique could only be developed once the feasibility of observing 'wet' articular cartilage in the SEM had been established. Part of this thesis is therefore concerned with microscopic procedures which have previously been used and with the development of new procedures necessary for this work.

The two other techniques entailed testing specimens in either a Dartec or Instron testing machine. When using the Dartec, thin layers of cartilage were removed from the specimen and the remaining cartilage was re-tested. The contribution of each layer to the overall deformation was then determined.

The Instron test machine became available quite late during the research and in this machine individual slices of cartilage could be tested.

In addition to the mechanical tests performed, measurements were made of the biochemical content of the tissue (water and uronic acid). The relationship between these values and the mechanical properties of the cartilage was investigated. Further investigation as to the effect of age, site of specimen and storage procedures were also made.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

One of the most common types of connective tissue found in the human body is cartilage. It is found at the ribs, spinal column, external ear, nose larynx and tracheal rings and covering the bone ends of all articulating joints.

The tissues which are known by the general name of cartilage can be subdivided into three major groups, classified by their appearance in the light microscope

i) Elastic where a large number of elastic fibrils have been demonstrated e.g. external ear cartilage.

ii) Fibrous where there is an abundance of large fibrils e.g. semi-lunar cartilage of the knee joint.

iii) Hyaline where the cartilage has a glossy homogeneous appearance e.g. cartilage covering the ends of all articulating bones.

Articular cartilage is an example of hyaline cartilage. Because of the important role articular cartilage plays in maintaining the normal function of articulating joints many workers have been interested in it and have studied a wide range of aspects of its composition, structure and properties.

Consequently there is a vast amount of literature on the subject not all of which is directly relevant to this thesis. The following pages are therefore a brief review of the investigations, and conclusions which have been drawn from these investigations, in those areas which are relevant.

A more extensive review covering all aspects of articular cartilage can be found in a recent book on the subject (Freeman 1979).

2.2 Composition of Articular Cartilage

2.2.1 Introduction

Any investigation into the mechanical properties of a human tissue must take into account the chemical and physical changes which can occur in the tissue due to both aging and diseases or disorders of the tissue. An understanding of the biochemical composition and the interactions which exist between the components of both the normal and degenerative tissue is therefore essential.

Articular cartilage is composed of three non-aqueous components; cells, protein fibres and a non-fibrous hydrophillic filler substance. The relative amounts of these three components, and the structure of the fibre network, varies with depth from the articular surface. Cells are most numerous near the articular surface, the proportion of the filler substance increases with depth and the proportion of protein is highest in the surface layer.

Based on the variation in constituents which can be observed under light microscopy, articular cartilage has been classified into four zones parallel to the articular surface (Collins 1949, McCall 1969).

Zone 1 Superficial - adjacent to the joint cavity: fibres are tangential to the surface, cells are discoidal with their long axis parallel to the surface.

Zone 2 Intermediate- fibres form an interlocking meshwork. Cells are spheroidal and equally spaced.

Zone 3 Deep - fibres form a tighter meshwork predominantly normal to the surface. Spheroidal cells are arranged in columnar groups of four to eight.

Zone 4 Calcified - adjacent to the subchondral bone, matrix is heavily impregnated with crystals of calcium salts.

Although this classification is useful as a general description of the zonal variations found in articular cartilage, more detailed analysis of the composition and structure of articular cartilage is also required. Investigations using histological, biochemical and electron microscopy techniques have revealed much about the detailed structure and composition of articular cartilage.

2.2.2 Cellular Component

The cells present in articular cartilage are called chondrocytes and they manufacture and maintain the extracellular matrix. Although the number of chondrocytes per unit volume of cartilage is small, only 1 - 10% of the volume (Hamerman & Schubert 1962), there is considerable metabollic activity which is associated with the synthesis and degradation, or turnover, of the non-fibrous filler or ground substance. Metabollic studies using radioactive substrates have shown that the cells synthesise and assemble the components of the ground substance intracellularly. The macromolecules are then extruded into the surrounding matrix. (Mankin 1974).

The turnover rate of the ground substance is slow compared to other human tissues. The half life of femoral condyle cartilage has been shown to be approximately 300 days and that of femoral head cartilage to be approximately 800 days (Maroudas 1975). The chondrocytes apparently only synthesise ground substance and there seems to be no turnover of collagen in normal human articular cartilage (Libby et al 1964).

2.2.3 Ground Substance

The ground substance of articular cartilage is the 'filler' material which surrounds the fibrous component of the matrix. It has been shown (Gersh & Catchpole 1960) that the ground substance contains a great variety of chemical constituents, its main component however is proteinpolysaccharide or proteoglycan macromolecules. These macromolecules are composed of a central core to which glycosaminoglycans, carbohydrate chains containing acidic groups, are attached. The proteoglycan macromolecules are thought to be attached at intervals along a linear cartilage hyaluronate molecule (Hardingham & Muir 1972, Rosenberg et al 1975).

The length of the hyaluronate molecule backbone of these aggregates varies from 400 to 4000 nm with proteoglycan subunits arising laterally at intervals of 20 - 30 nm. The length of the proteoglycan subunits varies from 100 to 400 nm. This is shown diagramatically in Fig. 2.1.

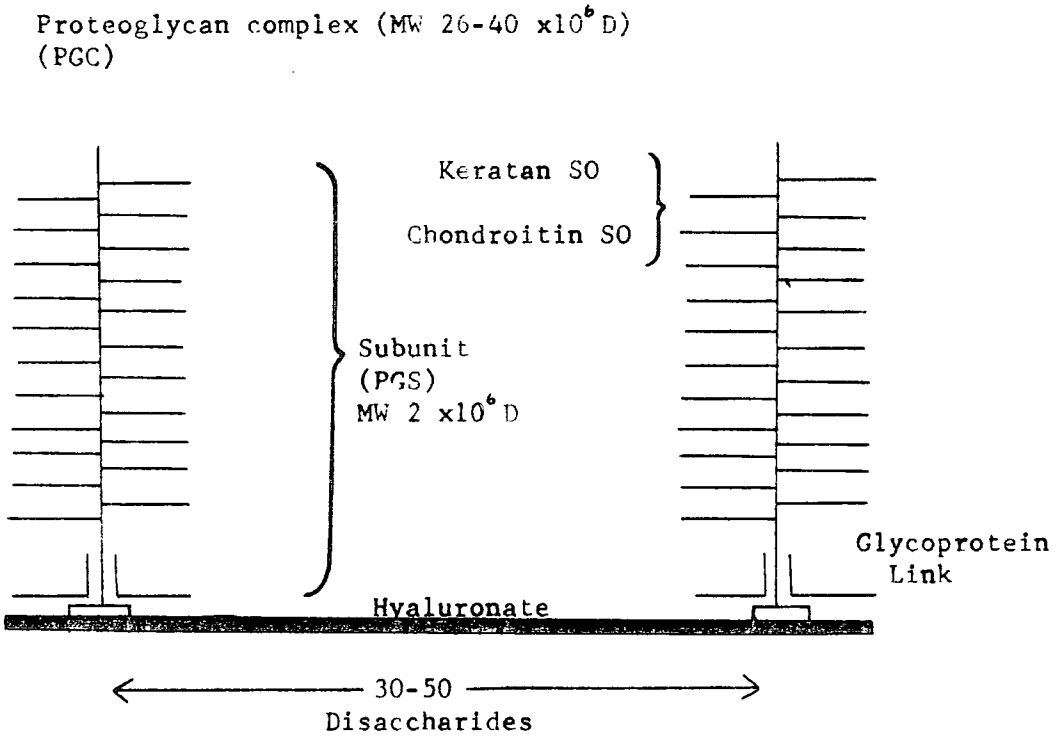


Fig2.1 Diagramatic representation of proteoglycan aggregates.

In human articular cartilage two types of glycosaminoglycans have been identified, chondroitin sulphate and keratan sulphate (Seno et al 1965, Tsiganos and Muir 1967, Heinegard and Grodell 1967, Hoffman et al 1967).

The relative proportion of these two glycosaminoglycans which are present in the macromolecule varies from almost entirely chondroitin sulphate to equal amounts of both (Muir and Jacobs 1967, Pedrini 1969, Tsiganos et al 1971).

It is thought that the chondroitin sulphate is linked to the serine residues of the protein core by a bridge of neutral sugars (Roden and Armand 1966, Lindahl and Roden 1966, Roden and Smith 1966, Helting and Roden 1968) and that keratan sulphate is linked by the terminal sugar galactosamine and the theorine residues of the protein core (Tsiganos and Muir 1967).

The variation in proteoglycan concentration with distance from the surface has been investigated using histological, biochemical and physio-chemical analysis techniques. Glycosaminoglycans have characteristic ion-binding properties and can therefore be demonstrated histologically by staining with cationic dyes. Differentiation between the two glycosaminoglycans is also possible by using the 'critical electrolytic concentration' (CEC) principle (Scott and Darling 1965).

Alcian blue is the most commonly used cationic dye, with various concentrations of magnesium chloride added to vary the electrolytic concentration.

Staining with Alcian blue at low CEC indicates both keratan sulphate and chondroitin sulphate and is most intense in the deep and intermediate zones of articular cartilage, decreasing in intensity towards the surface (Stockwell and Scott 1965). Staining with Alcian blue at high CEC indicates only keratan sulphate and is much less intense than with low CEC. It is again most intense in zone 3 and decreases towards the surface.

More quantitative measures of the glycosaminoglycan concentration can be obtained using biochemical analysis. The most common procedure is to estimate the uronic acid concentration using the Bitter and Muir procedure (1962) and the hexosamine content using Elson and Morgan (1933) reaction.

The chondroitin sulphate concentration can then be estimated by multiplying the uronic acid content, expressed as a percentage of the wet or dry weight, by 513 which is the molecular weight of the disacchoride. The keratan sulphate concentration is estimated by subtracting the uronic acid from the hexosamine content and multiplying by 464.

Using these procedures it has been shown that there is considerable variation in composition of articular cartilage from joint to joint and with site on each joint (Muir et al 1970, Kempson et al 1973). At each particular site there seems to be little variation in chondroitin sulphate concentration with depth, the average value for femoral head cartilage being out 0.055 mM/g wet weight, but there is a significant increase in keratan sulphate with depth. There is also an increase in the concentration of keratan sulphate with age (Venn 1978).

An alternative procedure for the estimation of glycosaminoglycans has been developed by Maroudas (1968). This method measures the concentration of negatively charged fixed groups in the wet tissue and is useful in that it is a non-destructive technique and can be applied to amounts of tissue as small as 1 mg wet weight. Fixed charge density measurements correlate extremely well with traditional chemical analysis (Venn and Maroudas 1977) and again indicate that the total glycosaminoglycan content increases with both depth from the surface and with age of the specimen.

2.2.4 Fibrous Component

The collagen fibres of articular cartilage are formed by the aggregation of rod shaped tropocollagen molecules each of about 300 nm length. Tropocollagen is made up of three α_1 II polypeptide chains wound together to form a helix (Miller et al 1971). Inherent in the structure of the tropocollagen molecule is the ability to form cross-links between molecules. This property of tropocollagen results in the formation of a highly cross-linked fibrous network which can be chemically disrupted only by certain digestive enzymes and which, except during growth and repair, is metabolically inert in adult human tissue.

In human articular cartilage collagen accounts for about 50% of the dry weight (20 - 30% wet weight) of the tissue (Bollet et al 1963, Anderson et al 1964). As with the ground substance there is considerable variation in collagen content with depth from the joint surface and it has been shown (Muir et al 1970) that in zone 1, the superficial layer, almost all of the dry weight is accounted for by collagen.

In addition to the variation in collagen content, there is also variation in both the size and structural arrangement of the fibres with depth.

Using Scanning Electron Microscopy it has been shown (Weiss et al 1968, Minns and Steven 1976) that in the superficial layer the fibres are approximately 0.03μm in width, packed closely together and orientated parallel to the surface of the cartilage. In the intermediate and deep zones the fibres are generally broader than in the superficial layer, varying from 0.1 - 1μm, and are both more randomly orientated and widely spaced. The predominant direction of the fibres is radial to the articular surface.

2.2.5 Interaction of Ground Substance and Collagen Fibres

The interaction of the ground substance and collagen fibres of articular cartilage can be considered from two points of view.

Firstly it seems likely that there is a chemical interaction of some kind between the proteoglycans and the collagen, although the exact nature of this interaction is not known (Muir 1979).

Secondly there is a physical interaction between the ground substance and the fibres, the nature of which obviously determines the overall response of articular cartilage to the loads it is subjected to.

Proteoglycans are hydrophylic colloids which swell in the presence of water to form a gel and in normal articular cartilage approximately 75% of the wet weight is due to water (Miles and Eichelberger 1964, Linn and Sokoloff 1965). Although some of this water is intracellular almost all of it is in the extracellular matrix.

In an unloaded state the pressure due to the swelling of the proteoglycans is balanced by tensile elastic forces in the cross-linked collagen network (Maroudas 1979).

When a load is applied to the articular cartilage the equilibrium of forces is disturbed and, if the load is maintained, water is driven out of the loaded region by the pressure gradient. As the water is expelled from the loaded region a difference in osmotic pressure develops between the loaded and unloaded regions. The rate at which water is expelled will therefore decrease with time until equilibrium is again reached. In this state the increased fluid pressure in the matrix will balance the applied load and the tensile stresses in the collagen.

Load carriage by articular cartilage is therefore dependent on both the proteoglycans, which retain the water within the matrix and regulate its flow, and on the collagen fibres which resist tensile forces within the matrix and confine the proteoglycans. Disruption of either of these components of articular cartilage will affect the load bearing properties of the tissue. In addition to altering the mechanical properties of the tissue disruption or removal of one component will facilitate easier disruption or removal of the other. If the ground substance is removed the increased strains which develop in the fibres when the tissue is loaded could result in mechanical failure of the fibres.

Conversely if the collagen network is disrupted there would no longer be a confining force on the proteoglycans and ground substance depletion could occur.

2.2.6 Ageing, Fibrillation and Osteoarthrosis

Human articular cartilage frequently develops areas of fibrillation or degenerative cartilage. Fibrillation has been defined as a state in which the articular cartilage surface no longer appears structurally intact when examined by the light microscope (Freeman and Meachim 1979).

Although the incidence of fibrillation of articular cartilage increases with age it does not necessarily accompany ageing and therefore when considering age related changes in the composition

of articular cartilage a distinction must be made between normal or intact cartilage and cartilage exhibiting any degree of degeneration.

The following changes in biochemistry have been observed in normal or histologically intact articular cartilage; there is no significant variation during ageing in the cell density of either full thickness femoral condylar or humeral head cartilage (Stockwell and Meachim 1979). It has been shown however that although the full thickness cell density remains constant there is in fact a decrease in the cellularity of the superficial layer combined with an increase in cellularity of the deeper layers (Stockwell 1967).

The ground substance content of articular cartilage, as measured by fixed charge density increases slightly with age (Venn 1977).

There is no significant change in the collagen content of articular cartilage with age (Maroudas 1979).

Recent studies as to the water content of articular cartilage indicate that there is a steady decrease in water content with age (Venn 1977).

As already mentioned, although fibrillation does not always develop as articular cartilage ages, the likelihood of fibrillated areas being present in a joint is higher the older the person, rising to 95% incidence in the knee joint at 80 years (Heine 1926). It has been shown that two types of fibrillation can occur (Byers et al 1970). The most common form occurs in non-pressure bearing areas of articular joints and is termed non-progressive fibrillation, as it does not lead to osteoarthrosis. In pressure bearing areas of the joint fibrillation can also occur and in these cases the lesions are progressive, leading to osteoarthrosis of the joint. It is quite likely that the aetiology of these two types of disruption of articular cartilage is different and as the progressive lesions are the most clinically significant it is this type of fibrillation that has been studied most extensively.

Although fibrillation can be easily identified by the disruption of the articular surface, it has been shown that changes in the chemical composition of the tissue precede the development of fibrillated areas. When considering osteoarthrosis it is these chemical changes which therefore give the most accurate measure of the degree of degeneration which is present.

By experimentally inducing osteoarthrosis in dogs it has been shown that the initial change in osteoarthrosis is an increase in the water content of the tissue (McDevitt and Muir 1974, 75, 76, McDevitt et al 1977).

This increase in water content continues as degeneration progresses and is accompanied by a decrease in the glycosaminoglycan content of the tissue, both on a dry weight and wet weight basis. The collagen content on a dry weight basis does not appear to change although due to the increase in water content there is a decrease on a wet weight basis (Venn and Maroudas 1977).

2.3 Biomechanics of Human, Synovial Joints

2.3.1 Introduction

There are two possible approaches to the study of the mechanical properties of articular cartilage. The first is to consider the tissue purely as an engineering material and to investigate the properties of that material under various loading conditions, such as long duration compressive loading and tensile loading. The second is to consider the physiological role of the tissue and the loading condition it experiences *in vivo*, and to investigate the response of the tissue to these loading conditions *in vitro*. The first approach has been adopted by many workers and the results of these investigations are discussed in 2.4. Although this type of test procedure yields interesting information as to the properties of articular cartilage and may ultimately lead to a complete description of its mechanical properties, the relevance of the results at present, to the normal function of synovial joints and the development of joint disease is not always immediately obvious.

The second approach has therefore been adopted during this research and, to establish the test conditions, a knowledge of the loading of articular cartilage *in vivo* is necessary.

2.3.2 In Vivo Loading of Articular Cartilage

It is not ethically possible to directly measure the forces transmitted by synovial joints in normal patients. The forces transmitted by a prosthesis can be measured by incorporating force transducers in the prothesis (Rydell 1966, Carlsan *et al* 1974). However these results are not in general relevant to normal subjects.

Non-invasive techniques which enable the calculation of the forces acting have been developed (Paul 1967, 1976, Morrison 1968, 1970). By measuring limb position, ground foot force and either measuring or assuming which muscle groups are acting during various activities, it is possible to estimate the magnitude and line of action of the resultant forces. If there are significant accelerations

of the limbs during the activity then the corresponding inertia forces and movements must also be included in the calculations.

Obviously the assumptions which must be made about body segment masses, muscle forces and lines of action and the corces exerted by ligaments lead to likely errors in the calculated forces. Taking into account the assumptions made, the calculated values of joint force are likely to have errors of up to 20% (Swanson 1980).

However, as the variation in forces in individuals of a large population is likely to be as high as 20% the forces calculated by the method above give a useful estimate of the magnitude of the forces acting at the joints.

Calculations based on the above assumptions indicate that during normal walking, at approximately one cycle per second, the maximum joint force at the hip is approximately 5 times the body weight and at the knee approximately 3 times the body weight.

Taking an average body weight of 70Kg this would indicate maximum forces during walking of about 3.4 KN at the hip and 2 KN at the knee.

2.3.3. Contact Areas and Stresses

In addition to calculating the forces that act at the joint it is also necessary to measure or calculate the contact area at the joint to enable the stresses which exist *in vivo* to be determined.

Various techniques have been used to measure these contact areas, the most common of which are dyeing, casting and radiographic procedures. Using a dyeing technique the contact areas at the hip have been estimated as varying from 3.83 in^2 to 4.38 in^2 during the normal walking cycle which indicates peak stresses of 326 lb/in^2 (0.22 MN/m^2) (Greenwald and O'Connor 1971).

Using the same technique the contact area in the patello/femoral joint has been estimated as ranging from 170 - 320 mm^2 at 150^o flexion and from 340 - 440 mm^2 at 60^o flexion (Matthews *et al* 1977).

These contact areas indicate a maximum stress of 2.5 NM/m^2 in level walking.

Casting techniques indicate contact areas in the patello/femoral joint of between 1.48 and 5.46 cm^2 with average stresses of 0.6 - 2.42MN $/m^2$ (Seedhom and Tsubuku 1977), in the tidio/femoral joint of about 12 cm^2 with average stresses of 12 Kg/cm^2 (0.12 MN/m^2) (Walker and Erkman 1975) and in the hip of about 4.5 cm^2 (Dowson et al 1967). Using radiographic methods the contact area of the tibio/femoral joint has been estimated as between 4.24 and 11.84 cm^2 (Kettelkamp and Jacobs 1972) and between 18.22 and 21.95 cm^2 (Maquet et al 1975).

One of the main problems with all the techniques used, and a possible explanation of the variations in results obtained, is that the joint has to be held under compressive load for a longer time than during normal walking, to enable either the casting material to set, the injection of a suitable dye or the X-Ray to be taken. It is likely therefore that the contact areas measured by these techniques are larger than those which occur in vivo, due to the time dependent deformation of articular cartilage, and that the stresses in vivo are higher than those calculated. These results can, however, be taken to give an indication of the stresses which occur and can be used as a basis for the design of test equipment to enable testing of articular cartilage within the physiological range of stresses.

2.4 Mechanical Properties

2.4.1 Introduction

During the lifetime of an individual the articular cartilage of all the joints is subjected to many millions of compressive load cycles. At the load bearing joints such as the ankle, knee and hip, in addition to the compressive loads exerted by the surrounding muscles, which occur at all joints, the articular cartilage also transmits the load due to body weight. It has been estimated that at these load bearing joints the articular cartilage is subject to, on average, 10^6 load cycles per year. When considering the mechanical properties of articular cartilage it is therefore the response of the cartilage to compressive loads which has been most extensively studied.

The most commonly used test procedure has been to apply a static load to a small area of cartilage and to measure, and try to characterise, the subsequent deformation with time. Test procedures which more closely mimic the loading which articular cartilage is subjected to *in vivo* such as sinusoidal compressive loading have also been used.

In addition to investigating the properties of articular cartilage during compressive loading, some investigations into the tensile properties of articular cartilage have been carried out. These include studies into the variation in tensile strength with depth from the articular surface and into the tensile fatigue properties of articular cartilage.

Whichever test mode is used it must always be remembered that articular cartilage is a living tissue in the body and any test performed *in vitro* can only be used as an indication of what the mechanical peoperties of articular cartilage might be *in vivo*. In assessing the relationship between the response of articular cartilage during *in vitro* tests and its response *in vivo* two main differences in conditions must be considered.

Firstly the tissue being studied has been removed from the body and secondly the load may not be applied as under physiological conditions.

The first difference can be considered negligible if the tissue is tested soon after removal from the body, due to the avascular nature of articular cartilage and its very low turnover rate under normal conditions. If the tissue cannot be tested immediately, however, the storage procedures used must be taken into account, as it is possible that these will affect the mechanical properties of the tissue.

There is considerable disagreement in the literature as to whether or not the usual method of storage of tissue, frozen at - 20°C, affects the mechanical properties of the tissue.

Several investigators claim that storage has no effect on the mechanical properties, providing the tissue is only frozen and thawed once (Maroudas 1968, Kempson et al 1971a). Other workers, however, have measured difference in properties between fresh and stored frozen tissue (Viidik and Lewin 1966, Hori and Mockros 1976).

The difference in loading conditions must be considered carefully, particularly if the test mode used is designed to be as close to physiological loading as possible.

During compression tests the use of small indentors will induce a stress field which is not representative of the physiological stresses in cartilage, particularly in the surface layers.

Alternatively, if the load is applied over the whole surface area of the specimen, then spreading of the specimen will occur if it is unconfined.

With either of these arrangements, therefore, although the load being applied may be within the physiological range, the response of the tissue is not necessarily the same as the response in vivo.

The tests may be used however, as an indication of the physiological response of articular cartilage and are also extremely useful as a comparative test between specimens.

2.4.2 The Mechanical Properties of Articular Cartilage in Compression

Indentation tests of unconfined specimens have been the most common method of studying the mechanical properties of articular cartilage. The general procedure in these tests is to apply a constant load to

an area of cartilage and to measure the subsequent deformation.

The main features of deformation-time response of articular cartilage are an 'instantaneous' deformation followed by time dependent deformation, known as the creep phase.

The variation in mechanical properties of articular cartilage with relation to histological appearance was first investigated by Hirsch (1944) using indentation tests on human patellar cartilage. He observed that the magnitude of deformation varied with site and that at fibrillated areas the deformation was greater than at non-fibrillated areas. Hirsch performed his experiments in air and a feature of the results he obtained was incomplete recovery of the cartilage after removal of the load. This incomplete recovery was shown (Elmore et al 1963) to be an artefact due to testing in air. They performed indentation tests on human and animal patellae immersed in Hanks balanced salt solution at enutral pH and found similar 'instantaneous' deformation followed by creep deformation. On removal of the load however there was an 'instantaneous' recovery followed by time dependent recovery back to the original dimensions. They concluded that the incomplete recovery previously reported was due to the specimens drying out.

Sokoloff (1966) found a similar deformation-time response to those of earlier workers and noted that after 1 hour of loading the deformation had reached an equilibrium value. The creep phase accounted for about $\frac{2}{3}$ of the total deformation. He also noted that deformation of fibrillated cartilage was much greater than normal cartilage. Kempson (1970) and Kempson et al (1971a,b) performed indentation tests using two different cylindrical indentors, one being plane ended and the other hemispherically ended. The applied stress was approximately 3 MN/m^2 in each case. The deformation-time response showed the same main characteristics as had been observed earlier and they found that for times greater than 2 seconds, i.e. after the 'instantaneous' deformation, the curves were related by the equation

$$y = kt^n \qquad \text{(i)}$$

where y = deformation

t = time

and k and n are constants.

Kempson found that for visually normal cartilage the values of the constants were

$$0.044 \lesssim k \lesssim 0.066$$

$$0.15 \lesssim n \lesssim 0.21$$

For fibrillated cartilage the values of n were lower and the values of k were larger than for normal cartilage.

Repo et al (1974) found that their indentation responses could not be described by equation (i) and instead suggested that a log-normal relationship exists.

$$\delta(t) = \delta_i - \delta_e \int_{\ln\tau}^{\infty} \exp\left[-\left(\frac{\ln\tau - \mu}{\sigma}\right)^2 \right] d \ln\tau \quad \text{(ii)}$$

Repo et al found that their creep data could be described by equation (ii) for the time period 5 seconds to 1 hour.

Mulholland (1974) found that his creep data were not log-normally distributed for times greater than 7.5 minutes.

Because of the apparent difficulty in mathematically describing the complete time-deformation response of articular cartilage, the compressive stiffness at a particular time after the application of load has been used to compare the mechanical properties of one specimen with another.

McCutchen (1962) calculated the Young's modulus of bovine 'leg joint' cartilage by applying a series of increasing loads. The deformation was measured 30 minutes after each load was applied, which was approximately the equilibrium deformation. He calculated the Youngs Modulus on the first 0.28mm of deformation and on the deformation 'immediately after application of load' and obtained the values

$$E = 0.58 \text{ MN/m}^2 \text{ at } 0.28 \text{ mm deflection}$$

$$E = 11.1 \text{ MN/m}^2 \text{ instantaneous.}$$

The stress level used was about 0.2 MN/m^2 which is low compared to physiological stresses.

Sokoloff (1966) used a plane ended cylindrical indentor and calculated Young's modulus for 'Instantaneous' deformation using the equation

$$E = \frac{F}{2.67\ PR}$$

where F = applied force

P = depth of 'Instantaneous' deformation

R = radius of indentor

This gave $E = 2.28 \text{ MN/m}^2$

Hayes et al (1972) and Hori and Mockros (1976) considered only the 'instantaneous' deformation of articular cartilage. By modelling the cartilage as an infinite elastic layer bonded to a rigid half space they found reasonable agreement between predicted and experimentally determined values of shear and bulk moduli of the cartilage.

The shear modulus for both healthy and diseased cartilage was found to vary over the range $4 - 35 \times 10^5$ N/m^2 and the bulk modulus over the range $9 - 170 \times 10^6$ N/m^2.

Kempson et al (1971) used the Young's modulus calculated 2 seconds after loading to investigate the variation in stiffness over the femoral head. The equations they used to calculate this Young's modulus, called the 2 second creep modulus, were originally formulated by Waters (1965) for indentation tests on thin sheets of rubber.

For the hemispherically ended indentor the equation used was

$$E = \frac{K^{\frac{1}{4}} P \phi(t/a)}{d^{\frac{1}{4}} R^{\frac{1}{2}}} \qquad \text{(iii)}$$

where
- P = applied load
- d = depth of deformation
- R = radius of indentor
- t = thickness of cartilage
- a = radius of contact area
- K = constant

ϕ (t/a) is an empirical correction factor which Waters calculated for rubber.

Kempson et al (1971) found their results were systematic and reproducible , to a certain extent, between specimens.

They found the creep modulus varied from $E \simeq 1.9$ MN/m^2 to $E \simeq 14.0$ MN/m^2. The stiffest cartilage was found in the load bearing areas, a band of cartilage which extends from the anterior aspect of the femoral head over the superior surface to the posterior aspect, and the softest in the perifoveal region which is the area where early fibrillation of articular cartilage occurs (Byers et al 1970).

Using the same technique Kempson et al (1970) found that there was a statistically significant increase in creep modulus with increased glycosaminoglycan content and that removal of glycosaminoglycan by incubation with enzymes reduces the stiffness of articular cartilage (Kempson 1979).

Simon (1971) found that repeated tests on the same area of cartilage using different radius indentors gave a greater variation in values of E, as calculated by equation (iii) when the correction factor was included and concluded that the use of this equation to calculate Young's modulus could not be applied to cartilage.

In addition to indentation tests and the various different methods of analysis of results already described, there have been several investigations using different compression tests.

Compression tests of intact femoral heads (Armstrong et al 1979) indicate that the method of loading greatly influences the apparent results. Armstrong et al have shown that although indentation tests of the intact femoral head indicate no relationship between stiffness of cartilage and age, loading of the femoral head by its natural acetabulum indicated a marked increase in deformation with increasing age. It is suggested that the increase in deformation is due to an increase in fluid flux under load, with age. Higginson et al (1976) have shown that if specimens are compressed in a confined state, as they are under physiological conditions, the 'instantaneous' deformation is much less than for unconfined specimens. They suggest that by confining the specimen the instantaneous deformation is restricted to the order of the bulk modulus effect. Non-confined cyclic compressive loading of articular cartilage has indicated that at normal walking frequencies the response of the tissue is essentially elastic although non-linear (Johnson et al 1977). In the case of confined cyclic compression the response of bovine articular cartilage has been shown to be almost linear-elastic (Higginson & Snaith 1979)

2.4.3 Tensile Properties of Articular Cartilage

Recently there have been several studies into the mechanical properties of articular cartilage in tension. The use of this test mode has been justified on several grounds. Weightman (1976) suggests that information as to the tensile properties of articular cartilage is relevant to the understanding of osteoarthrosis, as tensile failure of the collagen fibres during compressive loading

of the whole tissue is a possible cause of the development of fibrillation. Woo et al (1976, 1979) used tensile testing both because interpretation of the results is easier than with compression tests, and because the data are required if the constituitive equations of articular cartilage are to be developed.

In all the investigations dumb-bell shaped specimens, either of full thickness cartilage or of slices parallel to the articular surface, were used. Kempson et al (1968) showed that cartilage was both stiffer and stronger when loaded in tension parallel to the predominate fibre orientation of the surface layer than when loaded perpendicular to it. They also showed that both the tensile stiffness and strength of articular cartilage decreases with depth from the articular surface. Kempson (1973) reports that there is a decrease in tensile strength and stiffness with increasing age.

Woo et al (1976, 1979) measured values of stiffness similar to those of Kempson; however, their study indicates higher ultimate stress levels in the mid zone than in the surface and deep zones.

Chemical analysis has shown that cartilage stiffness increases with increasing collagen content of the tissue but is independent of glycosaminoglycan content (Kempson et al 1972)

2.4.4 Fatigue of Articular Cartilage

As articular cartilage is loaded in a cyclic manner many millions of times during the normal lifetime of an individual, it has been suggested that fatigue of the cartilage may be a cause of failure of the tissue (Kempson 1973, Freeman 1975).

Repeated cyclic compression has been shown to cause splitting and fraying of the articular surface similar to that seen in fibrillation (Weightman et al 1973, Gore 1977).

Tensile fatigue tests indicate that articular cartilage is fatigue-prone and that there is a decrease in fatigue resistance with increasing age (Weightman 1975, 1976).

CHAPTER 3

SCANNING ELECTRON MICROSCOPY

3.1 Introduction

The use of Scanning Electron Microscopy (SEM) in the study of articular cartilage structure and surface contours was mentioned briefly in Chapter 2. As the use of SEM is central to the research described in this thesis a more detailed review of the use of the SEM in this field, and the preparation techniques which have been used, will now be given. Equally important to this thesis is the observation of untreated tissue in the SEM and previous work in this field is also reviewed.

Based on the results of research in these two areas of electron microscopy, techniques which would possibly enable the observation of dynamic testing of articular cartilage in the SEM have been developed. These techniques are also described in this chapter.

3.2 Traditional Methods of Studying Articular Cartilage in SEM

The SEM has been used extensively in the study of articular cartilage (McCall 1968, Walker et al 1969, Clark 1971,72, Redler et al 1973, Mow et al 1974, Cameron et al 1976, Minns and Steven 1976). In all these studies the specimens were treated prior to observation in the SEM to remove the water present in the tissue. The most common technique used to dehydrate the tissue is to place it in increasing strengths of acetone followed by removal of the acetone either by vacuum or by Critical Point Drying.

There is reasonable agreement in these studies about the structure of the collagen fibre network within the matrix of articular cartilage and this observed structure is shown diagramatically in Fig. 3.1.

In the surface layer the collagen fibres are closely packed, with very little intervening ground substance, and are orientated parallel to the articular surface. In the mid-zone the fibres are randomly orientated and are much more widely spaced than in the surface layer, and in the deep-zone the fibres are again widely spaced but are orientated predominantly normal to the articular surface.

The results of studies of the surface contours of articular cartilage show considerable variation from worker to worker and it

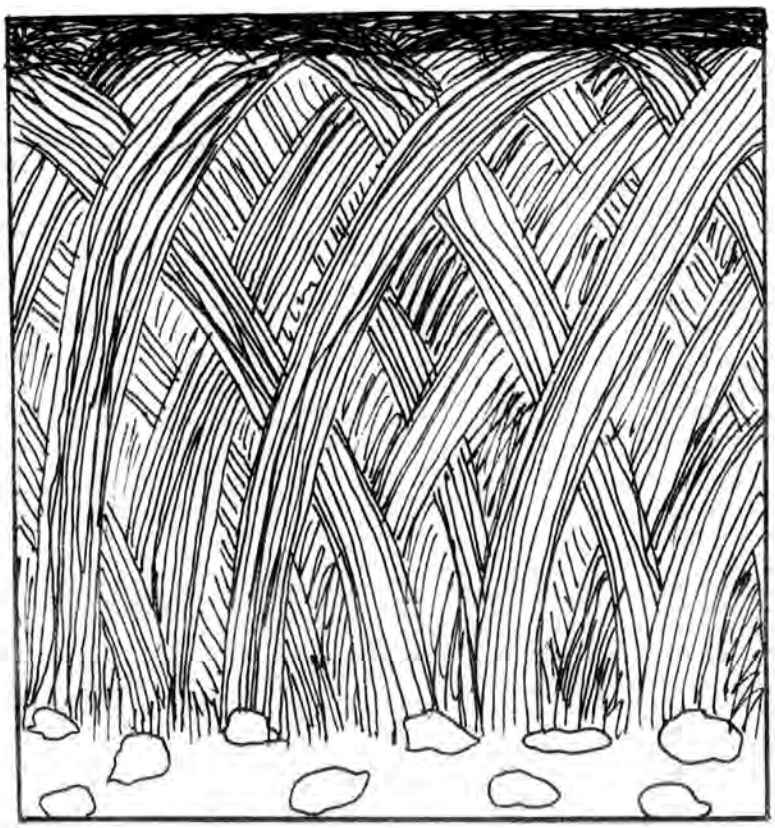


Fig3.1 Diagramatic representation of collagen fibre network

seems likely that the differences observed are due to the preparation technique used, as these will affect the surface of the specimen much more than the internal structure. Although it is generally agreed that the surface of articular cartilage is not perfectly smooth, the frequency and depth of the undulation is not universally agreed.

McCall (1968) and Walker et al (1969) observed that the surface of articular cartilage contained ridges or undulations of approximately 1 - 5 μm in height. They suggested that these ridges were due to the regular array of large collagen bundles. However, Clark (1971) concludes from his studies that these ridges, which he observed occurred parallel to the fracture edge of the specimen, are artefacts caused by the shrinkage of the cartilage during dehydration and that the dominant feature of the surface contours of articular cartilage are oval shaped depressions of approximately 20 - 40 μm diameter. He suggests that these depressions correlate with the positions of underlying cells.

Mow et al (1974) observed ridges perpendicular to the fracture edge of their specimens but did not observe collagen bundles. They suggest that these ridges are not artefacts and that they are due to the interaction of articular cartilage and the synovial fluid.

Cameron et al (1976) investigated the effect of sixteen different preparation techniques on the appearance of articular cartilage in the SEM.

They concluded that any technique which used air drying or freeze drying alone was likely to introduce artefacts and that the methods least likely to introduce artefacts were critical point drying or freeze drying of tissue which had been previously dehydrated through increasing strengths of solvents. They also state that even using these techniques the results can not be taken as absolute, as the response of articular cartilage to each method of drying is dependent on the age of the articular cartilage specimen.

3.3 SEM of Wet Tissue

Due to the problems of distortion of the specimen which can result from drying, several workers have modified the SEM to enable

observation of either frozen or wet biological specimens.

Cameron et al (1976) used a cold stub to examine frozen articular cartilage but found that even when using a large beam diameter, to reduce electron noise, the quality of the image they obtained was very poor.

Turner and Smith (1974) however, obtained very good results with a variety of biological tissues using this technique

The use of environmental control cells has been shown to be extremely useful in the observation of biological tissue. (Robinson 1974, Parsons 1974, Fukami et al 1974). However, there are restrictions associated with this technique, in particular the size of the specimen must be small enough to fit into the cell and resolution is reduced due to scattering of the beam by the vapour.

The possibility of observing biological tissue in the SEM without either treating the tissue or using an environmental cell has been shown by Judge et al (1974) and Nixon (1974). In both cases the tissue observed was human skin.

Judge et al observed excised human skin washed with 1% aqueous detergent solution at room temperature and then placed on a specimen stub. They report that the skin withstood the effects of vacuum for up to 40 minutes, but did not say what their criterion for withstanding vacuum was.

Nixon, using a modified SEM, simply placed his thumb over a half inch hole in the specimen chamber, in line with the electron beam. He was able to make a video recording of the image with magnification of up to 1K. Still photographs could then be taken of the video recording.

3.4 SEM of Wet Articular Cartilage

The results described in 3.3 suggest that it might be possible to observe other human tissues, such as articular cartilage, in the SEM without previously drying the specimen. Although it is unlikely that this technique would be useful in the detailed study of the structure of articular cartilage, due to the large beam diameter and low voltages necessary to avoid damage to the tissue, it could be useful for observing the deformations which occur during mechanical testing

of specimens. The advantages of observing such tests using SEM rather than conventional light microscopy would be the large field of view and depth of focus which can be obtained in SEM.

Although human articular cartilage has a very high water content, approximately 70 - 80% of the wet weight, the permeability of normal articular cartilage is low and water flow through the matrix when it is placed in a vacuum correspondingly slow. This is not the case, however, with fibrillated or osteoarthrotic cartilage. In addition to an increase in water content, fibrillated cartilage has a much higher permeability than normal cartilage and water flow when the tissue is placed in a vacuum will therefore be much quicker.

To establish whether or not untreated cartilage, both normal and fibrillated, could be observed in the SEM, specimens were taken from a patella which had been assessed for normality using the Indian Ink test (Meachim 1972) Fig. 3.2.

It was found that with normal cartilage, taken from areas where there was little or no residual staining, the SEM reached working vacuum within three minutes and that the specimen could indeed be observed. Specimens taken from areas where there was residual staining, and which were therefore fibrillated, could not be observed however, as the SEM did not reach working vacuum within the fifteen minute pump down period.

The SEM used was a Cambridge Stereoscan 600 and the only modification made was to use a pump of 300 ℓ/min. capacity rather than 100 ℓ/min. pump originally fitted.

Once it had been established that working vacuum could be reached, if the specimen was normal, the effect of both the beam and the vacuum on the tissue was investigated.

The beam voltage was set at 7.5Kv and maximum beam diameter was used. Figs. 3.3 to 3.7 show examples of micrographs of the surface of the specimen with magnifications of up to x 500. Reasonable resolution could be obtained at these magnificiations and there was no visually apparent damage to the tissue. Higher magnifications than x 500 did cause visually apparent damage to the tissue, as can be seen in Fig. 3.7. This is a micrograph at magnification of x 500 of an area of tissue which has previously been examined at x 1K magnification

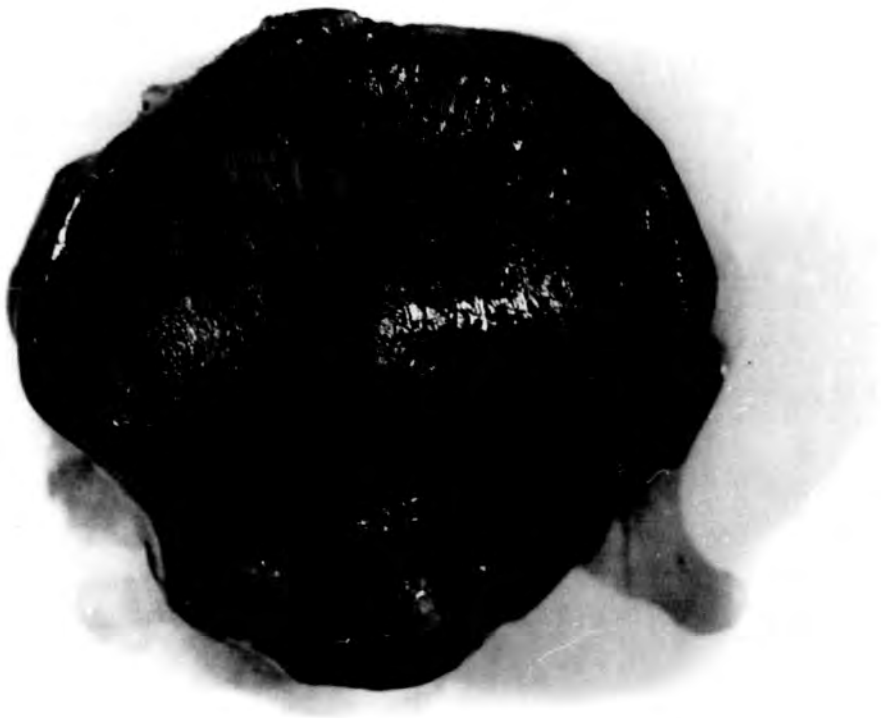


Fig. 3.2 Indian Ink staining of articular cartilage.

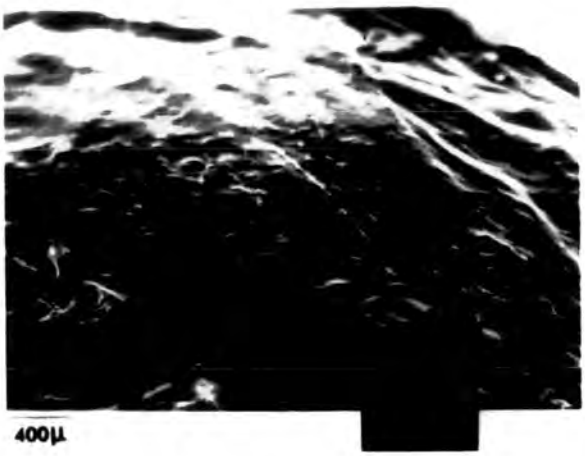


Fig. 3.3 Micrograph. X50.

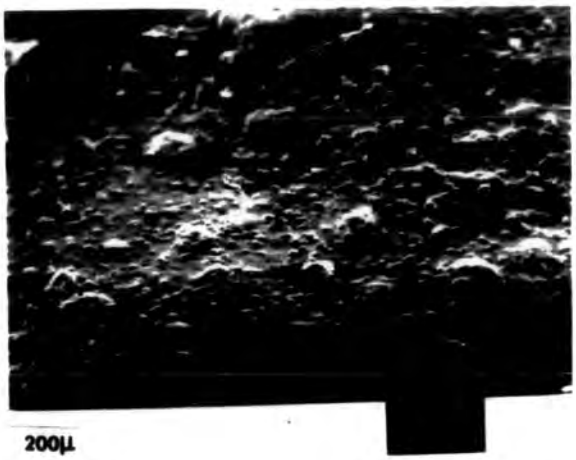


Fig. 3.4 Micrograph. X100.

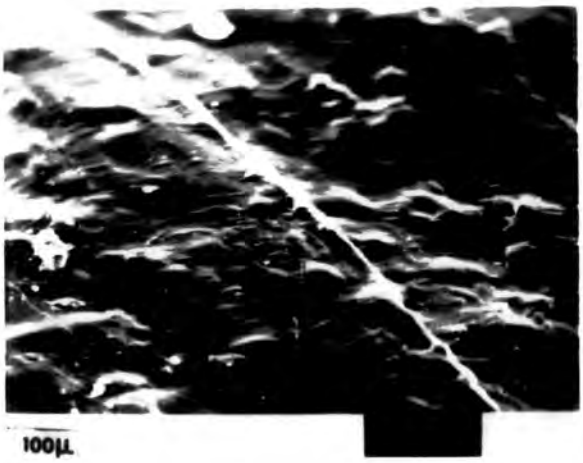


Fig. 3.5 Micrograph. X200

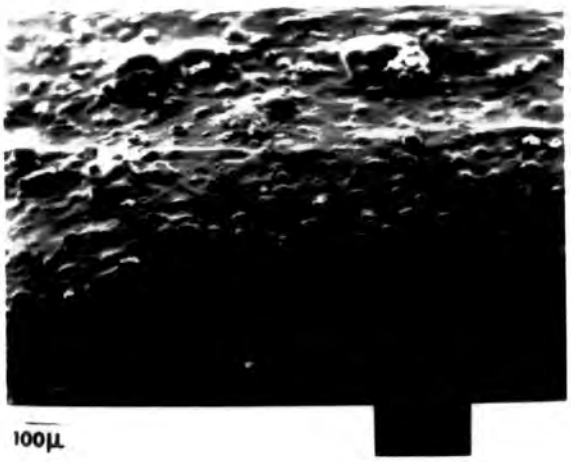


Fig. 3.6 Micrograph. X200

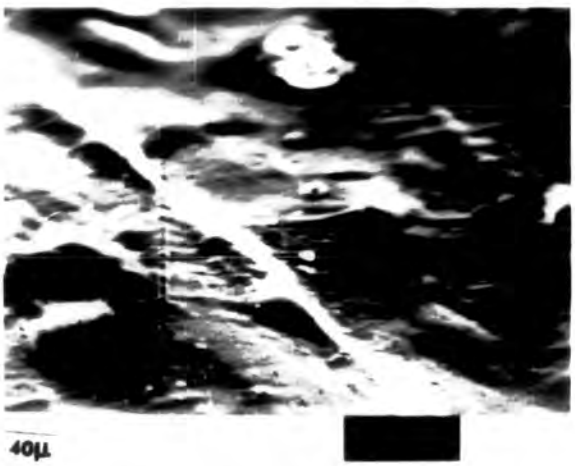


Fig. 3.7 Micrograph. X500.

The area previously scanned at 1K is clearly outlined in this micrograph indicating that damage had occurred to the tissue. It is concluded therefore that untreated articular cartilage can be observed in the SEM at magnifications of up to x 500 without apparent damage or alteration of the surface of the tissue due to the beam.

In addition to the visual examination as to the effect of vacuum and electron beam on the tissue, the effect of pump down and observation of the water content of the tissue, was also investigated.

Six specimens; two of just cartilage, two of cartilage plus underlying bone and two of just underlying bone, were immersed in Ringers solution and left for 30 minutes to reach equilibrium. The specimens were then removed from the Ringers solution, excess fluid was removed with a tissue and the specimen was weighed. Immediately after weighing the specimen was placed in the specimen chamber of the SEM and the microscope was pumped down to working vacuum. The length of time taken for the microscope to reach working vacuum was recorded and the specimen was removed from the microscope and re-weighed.

Each specimen was then re-immersed in Ringers solution until the original weight had been reached, which usually took about ½ hour, and replaced in the SEM and left in for five minutes at working vacuum. The specimens were then weighed and the whole procedure repeated this time leaving the specimens at working vacuum for ten minutes.

The total water content of the specimens was determined by drying to constant weight <u>in vacuo</u>.

Fig. 3.8 shows the results for the six specimens of loss of water content, expressed as a percentage of wet weight, against time in the microscope.

As can be seen, loss of water as a percentage wet weight is highest in the two specimens of just cartilage and lowest in thoseof just bone. This is not surprising given the much higher water content of cartilage compared with bone.

Fig. 3.9 shows the percentage of the original water content which was lost during the time in the microscope and it can be seen that

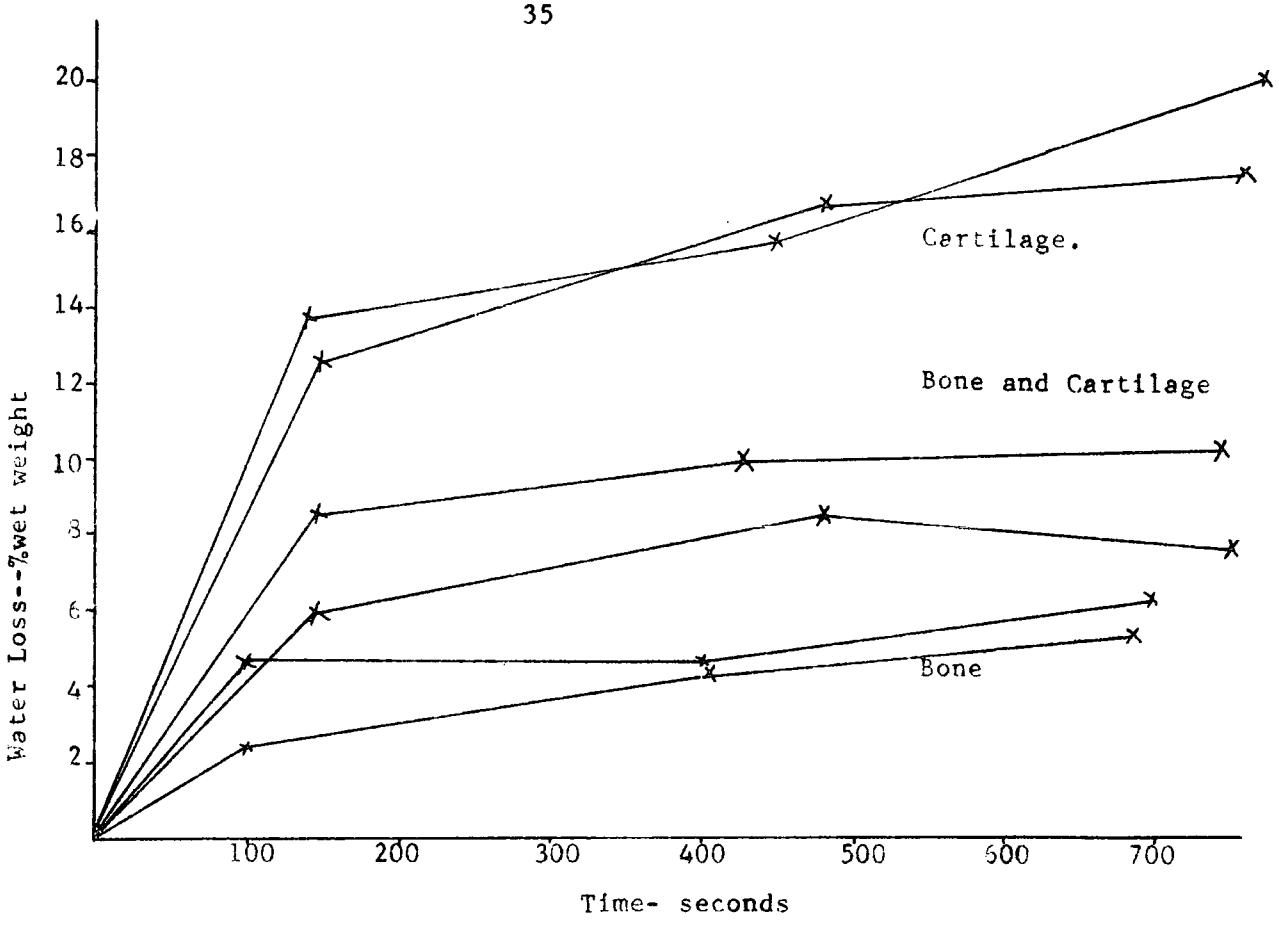


Fig3.8 Water loss as a percentage of wet weight.

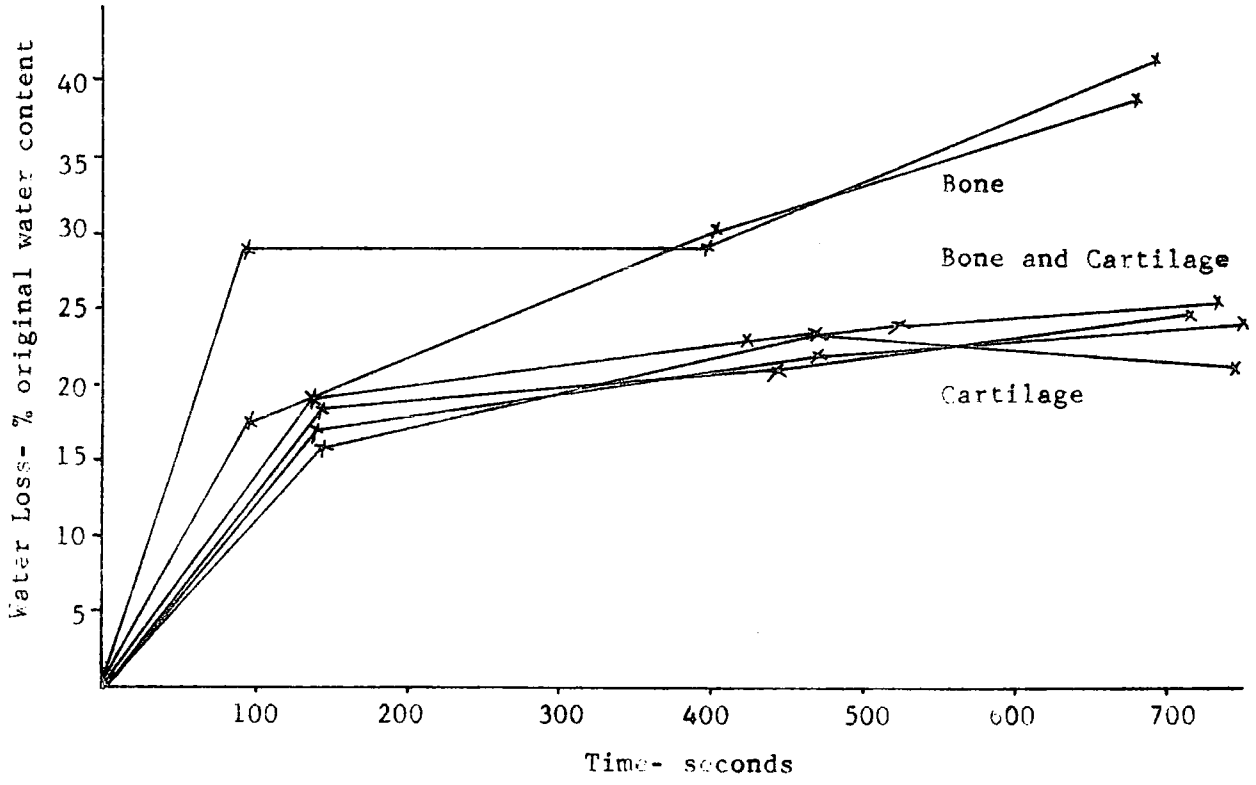


Fig3.9 Water loss as a percentage of original water content.

a much larger percentage of the original water content is lost from bone (more than 40%) than either the cartilage or cartilage plus bone specimens. This is due to the extremely low permeability of cartilage compared with bone.

These results show that if cartilage is only examined for about five minutes after pump down has been reached the wet weight will be reduced by about 16%, which is equivalent to a loss of about 20% of the original water content. It seems likely from the short time taken for the cartilage to return to its original weight when re-immersed in saline that a large proportion of the water loss measured is due to removal of superficial water. It is possible therefore that the water content of the bulk of the tissue is unaffected or only affected very slightly, whilst in the SEM. If this is the case then the mechanical properties of the tissue when tested in the SEM may well be the same, or very similar, to those when it is tested outside the microscope immersed in Ringers.

From these results it was decided that the SEM could be usefully used to observe the deformations which occur during compression of small plugs of cartilage.

CHAPTER 4

EQUIPMENT DESIGN AND INSTRUMENTATION

4.1 Introduction

As explained in Chapter 1 the main purpose of this investigation was to try to observe, and measure, the deformation of articular cartilage under physiological loading conditions.

In Chapter 2 the various studies that have been performed to determine the loading which occurs in human joints in vivo were described. Although the results of these studies vary quite considerably, they do indicate that during normal walking, at frequencies of approximately one cycle per second, the maximum stress that occurs in articular cartilage is between 2 and 6 MN/m^2.

The load cycle that occurs each time a step is taken is not a simple sinusoidal variation (Paul 1967). However, because of the difficulty of exactly simulating the load cycle previous workers who have studied cyclic loading of articular cartilage have approximated the physiological load cycle by applying a sinusoidally varying load.

In this investigation the response of articular cartilage during a single load application is studied and it was decided that a near approximation to physiological loading could be achieved by applying a constant displacement rate, which would result in stresses of up to 10 MN/m^2 in 2 to 3 seconds. Based on previous investigations into the mechanical properties of articular cartilage, described in Chapter 2, it was calculated that if a displacement rate of approximately 0.3 mm/sec was applied the levels of stress and strain achieved within 2 to 3 seconds would be approximately those that occur in vivo.

The equipment used in this investigation was therefore designed to operate at a range of constant displacement rates with 0.3 mm/sec as the mid-point of the range in each case. Facilities to measure stresses of up to 10 MN/m^2, and displacements of up to 3 mm were also incorporated in the rig designs. It was decided that the specimens would have a diameter of 5 mm and therefore the loads measured would be up to approximately 250 N.

4.2 SEM Test Rig and Instrumentation

Given the ranges of load and displacement required for the testing of the specimens, the main criterion for the design of the SEM rig was that of size. The load cell, displacement transducer, specimen holder and compression device all had to be small enough to fit into the specimen chamber of the microscope.

Although small load cells are commercially available they are rather expensive and therefore a small load cell was designed and built specifically for this study. The load cell is shown in Fig. 4.1. As can be seen the load cell enables the measurement of both compression and shear loads by recording the change in resistance of strain gauges attached to two pairs of lever arms. Gauges 1 and 2 are coupled together to measure compressive loads whilst 3 and 4 measure shear loads. Each pair of strain gauges were connected to form a ½ bridge input into a portable strain indicator (Manufactured by Peekel, Rotterdam, Holland). The output of the strain indicator was connected to an Ultra Violet recorder. The load cell was calibrated using static loading and the results are shown in Appendix B. It can be seen that within the range of loads required for the tests the relationship between the load cell output and the applied load is linear.

The displacement transducer used in this rig is a commercially available linearly variable differential transducer (LVDT). The output of the LVDT was connected to a direct reading transducer meter (Type C52 Manufactured by Boulton and Paul, Wolverhampton) and the output from this was recorded on a second channel on the U.V. recorder.

The device which enables the constant displacement rate to be applied to the specimen is simply a specimen stub attached to a brass block which can move in a channel cut in a steel mounting. The brass block has an internal screw thread and is linked via a worm and wheel to a constant speed Parvalux motor. The output from the motor can be varied using a rheostat and with the worm and wheel arrangement chosen, the displacement rate can be varied from 0.1 mm/sec to 0.8 mm/sec .

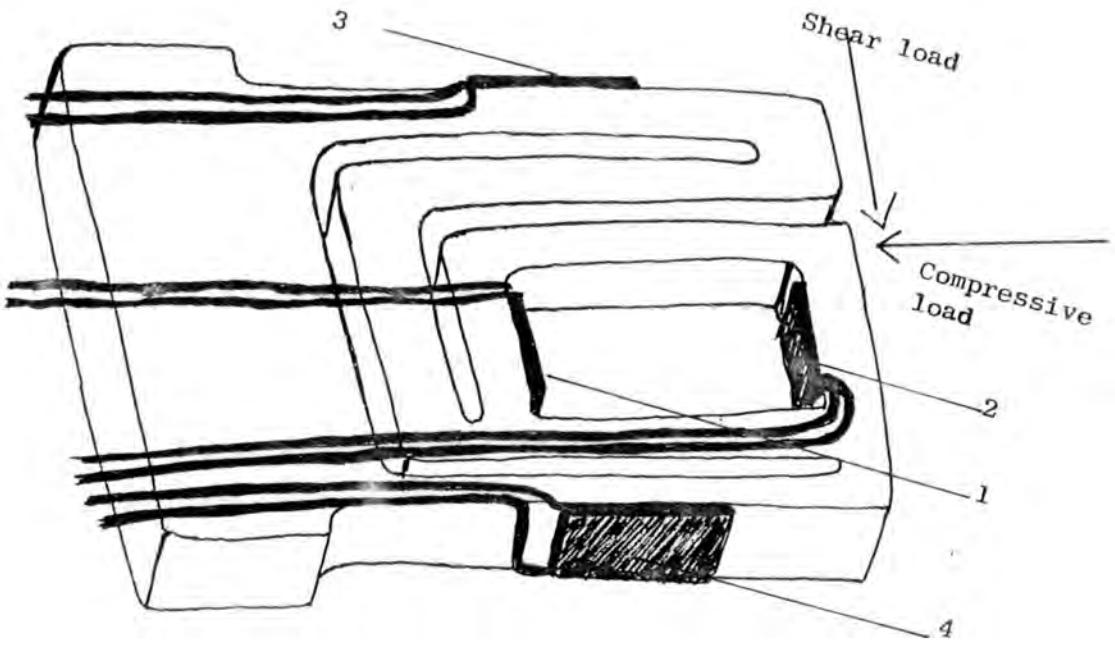


Fig4.1 S.E.M load cell.

The load cell, LVDT and compression device were mounted on a base platten supplied by Cambridge Instruments as shown in Fig 4.2.

The next step in the design of this equipment was to arrange for the leads from the load cell and LVDT, and the drive shaft of the compression device to be connected to the recording equipment and the motor outside the SEM, whilst maintaining an airtight seal. A standard front plate for the SEM vacuum chamber was modified for this purpose. The tilt and rotate controls normally mounted on the front plate were removed and in their place were inserted an airtight pin connector for the electrical connections and a drive coupling sealed with O rings as shown in Fig. 4.3.

Once the SEM rig had been designed and built one further calibration was carried out before testing began. The design of the load cell is dependent on bending of lever arms under either compressive or shear loading producing a change in the resistance of the attached strain gauges. At each particular load therefore, there will be a small displacement of the specimen stub due to the bending of the levers. By loading the rig with no specimen present this displacement can be measured and subsequently subtracted from the overall displacement measured when a specimen is actually tested. The result of the displacement–load calibration of the SEM rig is shown in Appendix B.

4.3 Dartec Test Rig

The Dartec testing machine can be operated in either tension or compression but the load cell used for these tests was suitable only for tension. For the series of tests performed in the Dartec it was therefore necessary that a compression cage should be used which would enable the machine to be operated in the tensile mode whilst applying compressive loads to the specimen. Although compression cages are commercially available for Dartec test machines they are not suitable for testing at the loads used in this investigation, as the frictional resistance is of the same order of magnitude as the loads required to compress articular cartilage. A low friction compression cage was therefore designed

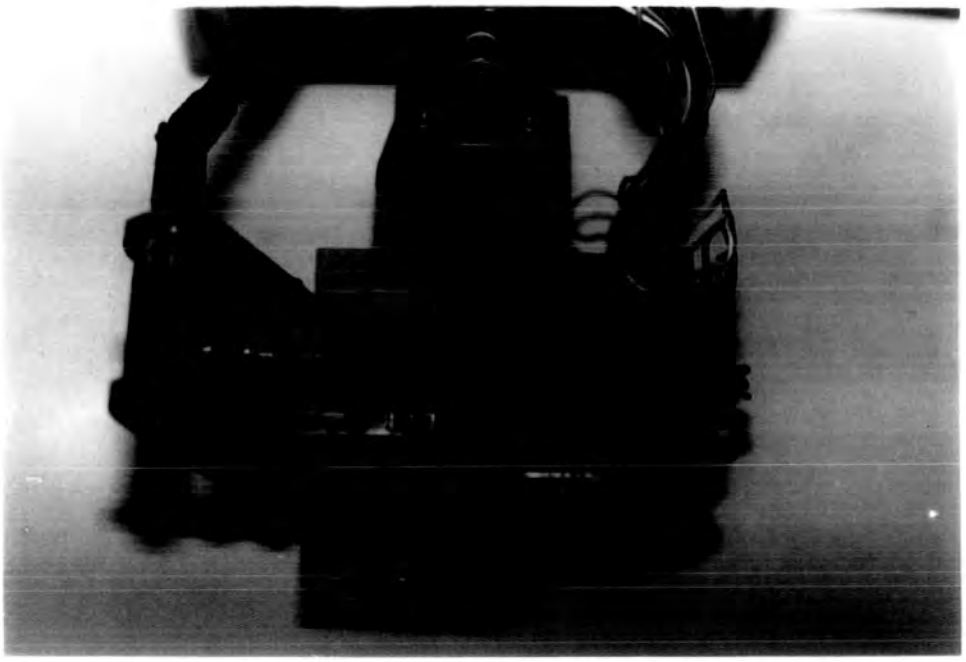


Fig. 4.2 Base Platten.

Fig. 4.3 Front Plate.

for this investigation as shown in Fig. 4.4. The bearings and shafts of the cage were supplied by E.W. Bearings Limited, Brough Park, Fossway, Newcastle upon Tyne, and the frictional resistance of the cage is less than 10N. The external extensometer of the Dartec was mounted on the cage and the plunger was positioned resting on an adjustable screw which could be locked in position as shown in Fig. 4.5. This adjustable screw enables the extensometer to be accurately zeroed during the test procedure which, as will be described in detail in Chapter 5, is necessary for the operation of the machine.

In the centre of the bottom plate of the compression cage a hole was drilled for the specimen holder to locate in. As the specimens were to be tested immersed in saline a 2 mm circular channel was cut in the bottom plate of the cage and during testing a small tube of perspex was placed around the specimen in this channel. The join between the perspex tube and the plate was watertight and once the specimen had been positioned the tube could be inserted and the specimen immersed in saline.

As with the SEM rig, the deformation–load characteristic of the Dartec rig with no specimen present was determined and this is shown in Appendix B.

4.4 Instron Rig

The Instron test machine was used in the compressive mode and the equipment needed for the tests was therefore very simple. A small brass plate, with a hole for the specimen holder and a channel for the perspex tube, was made to position on the top plate of the load cell. On the underside of the brass plate a small circular ridge which locates with a recess in the top plate and enables accurate centring of the specimen. A 10 mm diameter brass cylinder with a screw thread to fit the crosshead mounting of the Instron was the only other piece of equipment needed. Both these pieces of equipment are shown in position in the Instron in Fig. 4.6

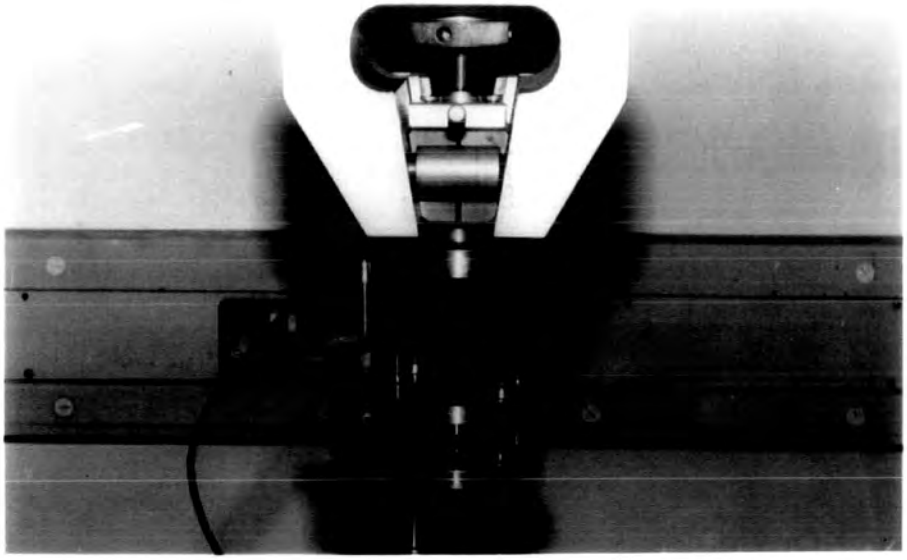


Fig. 4.4 Dartec Compression Cage.

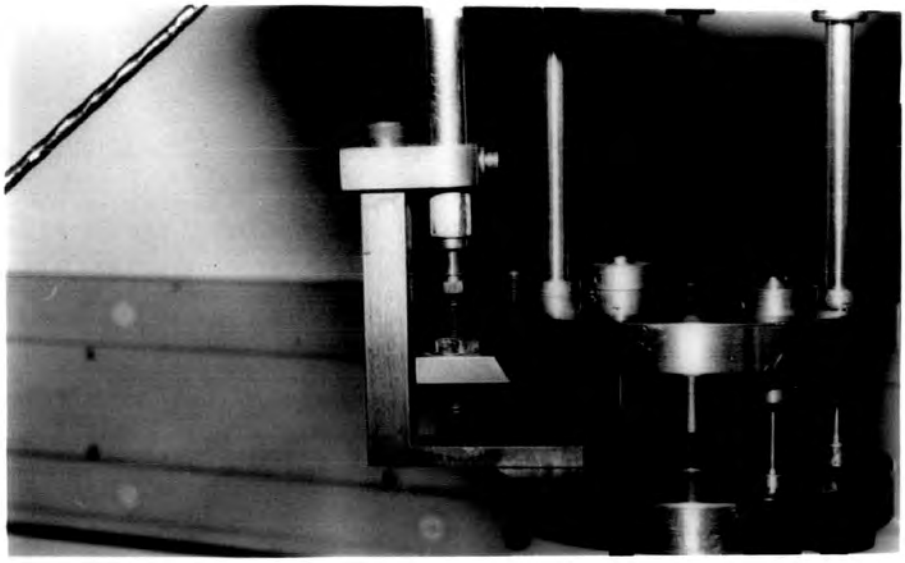


Fig. 4.5 Cage Zeroing Screw.

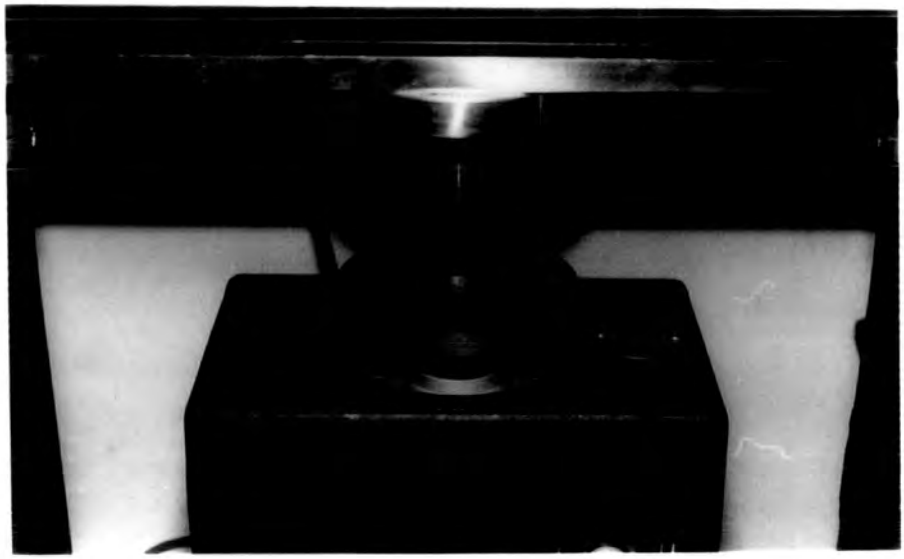


Fig. 4.6 Instron Test System.

4.5 Microtome

The Microtome used during this investigation was manufactured by MSE of London. The device was modified as it was thought that the slices of cartilage removed would be more likely to be parallel to the surface of the specimen if the remaining cartilage was confined and unable to 'bend'. Fig. 4.7 shows the equipment used in place on the microtome. The brass rod can be moved up inside the brass holder, which has a hole slightly larger than 5 mm drilled through it. The specimens were placed in the holder and by raising the rod the required thickness of specimen could be pushed up and sliced off. It was necessary to make the hole slightly larger than the diameter of the specimens as it was found that the underlying bone was often of slightly larger diameter than the cartilage. The technique used to remove the slices using this equipment is described in Chapter 5.

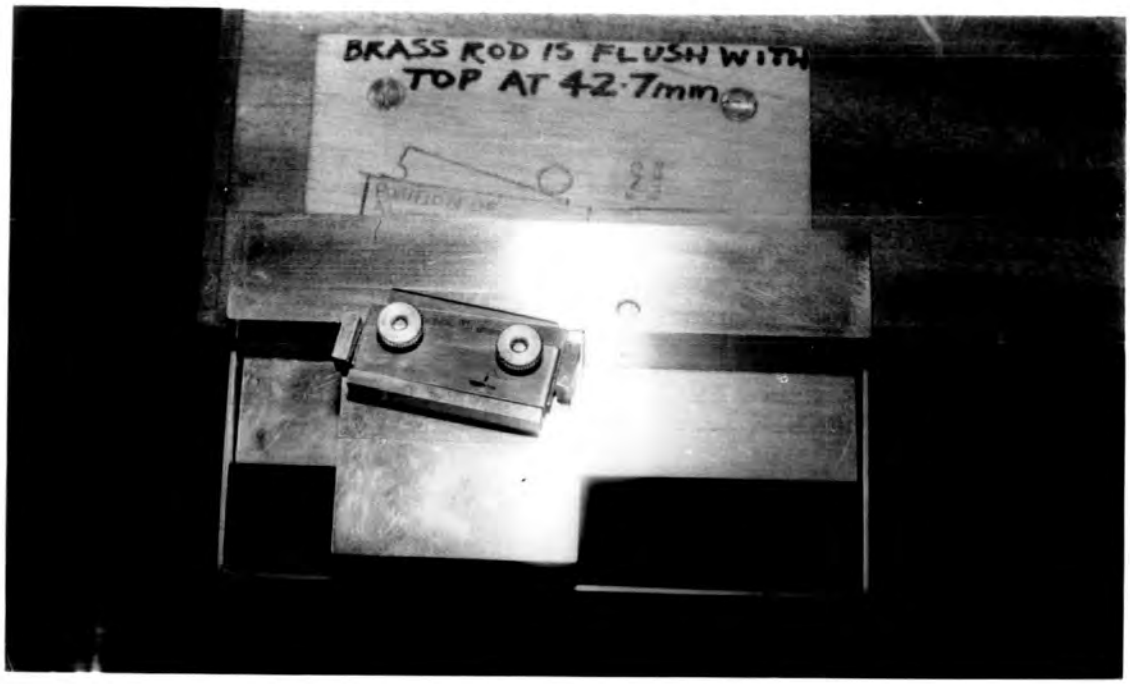


Fig. 4.7 Modified Microtome.

CHAPTER 5

STORAGE AND PREPARATION OF SPECIMENS AND EXPERIMENTAL PROCEDURE

5.1 Collection and Storage of Specimens

All the specimens used in this study were from adult human patellae. The patellae were obtained at post mortem and stored in airtight glass containers at either + $4^{o}C$ or - $20^{o}C$ until used. A small amount of saline solution was present in the containers which prevented dehydration of the tissue, whilst avoiding the problem of leaching out of the ground substance which occurs if cartilage is stored totally immersed in saline solution.

The effect of storage procedures on the mechanical properties of tissue has been studied by several workers. However, as was mentioned in Chapter 2, the results of these investigations have been conflicting. In order to gain more information as to the significance of storage procedures the two procedures mentioned earlier were adopted during this study. 23 patellae were used in the study, 7 of which were stored frozen at - $20^{o}C$ and 16 unfrozen at + $4^{o}C$. This was taken into account during the analysis of the mechanical testing and is discussed further in Chapter 7.

The ages of the patellae ranged from 25 to 82 years.

5.2 Preparation of Specimens

As each patella was needed it was removed from the fridge and those stored at - $20^{o}C$ were left to defrost at room temperature.

The patellae were then carefully dissected to remove any soft tissue and washed with saline to remove any synovial fluid. The Indian ink test (Meachim 1972) was used to assess visually the extent and degree of fibrillation which was present in each patella. This test was not used to assess the normality of the tissue quantitatively as this was done later by biochemical analysis, but was used to give an indication as to whether or not a specimen removed from a particular site would be normal. This was particularly important when preparing specimens for testing in the SEM as fibrillated specimens could not be used, due to the much higher permeability they exhibit.

To enable easy handling and positioning of the patella it was placed in a stainless steel dish which had four screw holes through the side wall. Pointed end screws were introduced through these holes and screwed into the bone of the patella, thus holding it firmly within the dish. The dish was attached to an adjustable stand so that each area of cartilage from which a specimen was to be removed could be positioned with the articular surface in a horizontal plane. Once the patella had been clamped into the dish it was covered with a wet tissue to prevent dehydration of the articular cartilage. This tissue was removed and replaced as each individual specimen was prepared.

The specimens used in all the tests in this investigation were 5 mm diameter cylindrical plugs of cartilage with underlying bone which were removed from the patella using a hardened steel cutter and press. The cutter was pressed through the cartilage into the underlying bone and by sharply twisting the patella the bone could be fractured, leaving the specimen in the cutter. The adjustable stand, cutter and press used are shown in Fig. 5.1.

The original cutter used had only a small diameter hole running through it and the specimens were ejected from the cutter by a small plunger which was positioned inside the cutter and which could be pushed against the cartilage surface to force the specimen out. Although this method proved satisfactory for normal tissue it was unsuitable for use with fibrillated or osteoarthrotic tissue and the cutter was therefore modified. The diameter of the hole running through the cutter was increased to a little more than 5 mm with the cutting edge remaining at 5 mm diameter. The specimens could now be ejected from the cutter by a plunger pressed on the underlying bone thus avoiding any possibility of damage to the articular cartilage.

The site on the patella which each specimen was removed from was recorded diagramatically and the specimens were placed in small screw-top jars with a small amount of saline to prevent dehydration. By careful positioning of the patella on the adjustable stand the specimens could be cut so that the surface of the cartilage plug was perpendicular to the sides of the plug. The bone end of the

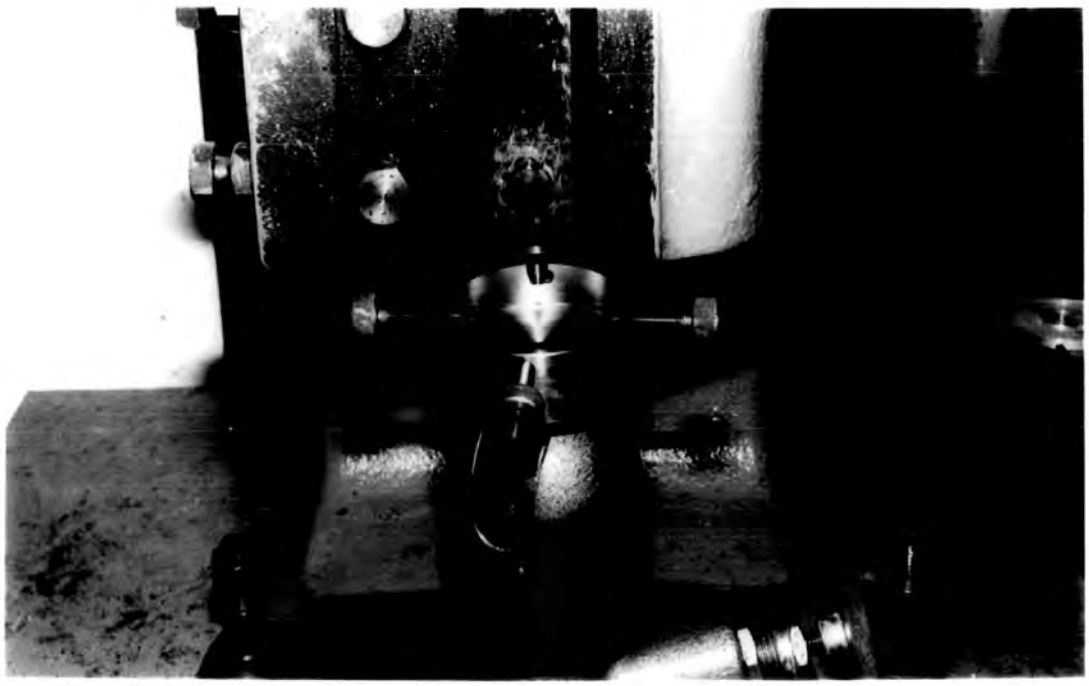


Fig. 5.1 Adjustable Stand, Cutter and Press.

specimen did not always fracture perpendicular to the sides of the plug and therefore the bone end was subsequently trimmed flat in a watch-makers lathe. The specimens were then stuck onto small brass specimen stubs using cyno-acrylate adhesive. This adhesive sets very quickly, usually in about ten seconds, and once the specimen was firmly attached to the specimen holder it was placed, with the holder into its own individual jar. This again prevented dehydration of the articular cartilage. Just prior to testing, each specimen was immersed in saline solution for one hour to ensure that it was fully hydrated. The specimens were not removed from the same sites on each patella but were taken from sites which had a large enough flat area to be positioned horizontally under the cutter. This obviously depended on the contours of each patella. The maximum number of specimens obtained from one patella was twelve but usually eight or nine could be prepared.

5.3 Experimental Procedure

5.3.1 Introduction

As described in Chapter 3, the equipment designed for use in the SEM applied a constant rate displacement to the specimen. The test mode adopted in the Dartec and Instron tests was also a constant rate displacement to enable easy comparison between the three series of tests.

The majority of full thickness specimens and specimens with slices removed tested in the Dartec were tested at a displacement rate of 0.3 mm/sec . As discussed in Chapter 2, it is likely that this rate will fall within the normal physiological range. In addition to the main series of tests the effect of strain rate was also determined in both the SEM and Dartec.

The individual slices tested in the Instron were tested at a displacement rate of 0.083 mm/sec which meant that the strain rate was of the same order of magnitude as in the full thickness tests.

5.3.2 Experimental Procedure Using SEM

The SEM used in this investigation was a Cambridge Stereoscan S600. The operating voltage was 7.5 Kv and during the test the magnification was set on X50. The scan speed had to be set at TV to enable a video recording to be made.

At the beginning of each test session the test rig was placed inside the specimen chamber, without a specimen present, and the microscope was pumped down to working vacuum. The beam was focused and any astigmatism present was compensated for. At this stage the test rig could be approximately positioned so that the specimen would be in the field of view and only a small amount of adjustment would be necessary when the specimen was actually inserted. When the best possible picture had been obtained the microscope was set to 'air' and the tests could be started.

As stated earlier each specimen was soaked in saline for 1 hour before testing. Before placing in the microscope the specimens were therefore wiped lightly with a tissue to remove any surplus saline. The specimen was then inserted into the test rig, placed in the specimen chamber and the microscope was pumped down to working vacuum. While the microscope was pumping down the motor and belt drive were positioned and the motor was clamped to the table top.

Once working vacuum had been reached the specimen could be positioned exactly within the field of view and then the test rig stage was clamped to the front plate. During the test the stage was left clamped to the front plate and was in its lowest position. This minimised the movement of the rig itself during the test, but it could not be eliminated altogether. The microscope was then focussed accurately at mag x 200 and scan speed 0.5 frame seconds. When these were reset to x 50 and TV scan the gain and contrast were adjusted.

The compression stub was moved into the field of view and was set just before contact with the specimen. The video recorder

and UV recorder were then switched on and then the motor was switched on. The compression test was allowed to continue until the load recorded reached approximately 100N. The time taken varied therefore for each test, depending on the 'stiffness' of the specimen but usually the motor was on for 3 or 4 seconds. Immediately the test was finished the UV recorder was switched off and the microscope was set to standby. This meant that if the vacuum deteriorated due to fluid being expelled from the tissue the HT voltage did not cut out, due to trip mechanisms, which can cause damage to the screen as the beam decays. The motor was then reversed until the compression stub was in its original position, as indicated by the LVDT reading. The microscope could then be brought back up to air and the video recorder switched off.

The specimen was then removed from the test rig, re-immersed in saline solution and a new specimen tested.

Each test took about five minutes to perform including the time taken for the microscope to reach working vacuum. Once a specimen had been tested in the SEM it was re-immersed in saline and either re-tested in the SEM or the Dartec or prepared for biochemical analysis.

If the specimen was to be re-tested it was left to recover for at least 4 hours. It was found that by leaving the specimens for this length of time the response under identical circumstances was, within experimental error, identical to the original response. Based on this fact, specimens were tested three times at three different displacement rates, 0.1 mm/sec, 0.3 mm/sec, 0.8 mm/sec. These were the minimum, median and middle displacement rates that could be obtained without changing the motor or gears used. The results of these tests are discussed in Chapter 7.

5.3.3 Experimental Procedure Using Dartec

Although the Dartec is a fairly complicated machine to set up, the test procedure once the trips and ramp speed have been set is quite simple. A detailed step by step procedure for the

tests performed is therefore given in Appendix C and the information given here will be of a more general, descriptive nature.

As has been stated earlier the purpose of using the Dartec test machine was to enable tests to be performed on 'wet' specimens, that is specimens immersed in saline, as a comparison to those performed at vacuum in the SEM. In addition, by removing slices of cartilage from the specimens and re-testing the remaining cartilage it was hoped that an estimate of how each slice contributed to the overall deformation could be made.

The first fact to establish was therefore whether or not repeatable results could be obtained from the same specimen if it was left to recover, immersed in saline, between tests.

As with the SEM tests it was found that the response of each specimen was repeatable as long as the specimen had been left for at least 4 hours. A similar series of tests to those performed in the SEM were carried out to establish the effect of strain rate on the response of the tissue. This was particularly important with the Dartec tests as the strain rate would obviously be higher once a slice had been removed from a specimen if it was re-tested at the same displacement rate. The displacement rates applied ranged from 0.03 to 3 mm/sec and the results of the tests are described more fully in Chapter 7. Briefly the results indicated that there was no significant difference in response within this range of displacement rates. A fundamental assumption was then made which was that if the response of the full thickness of tissue was repeatable at different strain rates then the response at each level through the thickness of the tissue would be the same when the tissue was re-tested. Based on this assumption it was presumed that the difference in response of a specimen when re-tested with a slice removed was due solely to the removal of that slice.

The test procedure for specimens tested in the Dartec was therefore as follows -

The specimen was placed, immersed in saline, in the test rig and a constant rate displacement was applied until the recorded load reached approximately 100N. The specimen was then unloaded,

removed from the rig and left to recover for 4 hours. After 4 hours the specimen was removed from the holder, placed in the microtome holder and a slice of cartilage approximately 500 μm thick was sliced off parallel to the articular surface. The thickness of the slice was measured using a micrometer and the remaining cartilage plug was re-glued to the specimen holder. The specimen could then be re-tested and this procedure could be continued until all the cartilage had been removed from the specimen.

In some cases the specimens were only tested at full thickness in which case after the first test they were removed from the specimen holder and left to recover immersed in saline before being prepared for biochemical analysis.

5.3.4 Experimental Procedure In Instron

The experimental procedure in the Instron was essentially very similar to that of the Dartec. Each specimen was placed in the test rig, immersed in saline and loaded at a constant rate of displacement up to approximately 100N. The specimen was then removed from the rig and left to recover for at least 4 hours.

Once the specimen had recovered it was sliced into layers of approximately 500 μm thickness. Each slice was measured and then individually tested using the same test rig. Because of the much higher strain rates which would have resulted if the slices were tested at 0.3 mm/sec the displacement rate was reduced to 0.083 mm/sec for these slices. As with the other test procedures the slices were left to recover immersed in saline before being prepared for biochemical analysis.

5.3.5 Measurement of Thickness

The thickness of all the specimens tested in the SEM and those tested only at full thickness in the Dartec were measured after they had been tested.

After the test had been carried out the specimens were left immersed in saline to recover. The cartilage was then sliced

off the bone using a scalpel and the thickness of cartilage measured using a micrometer. In some cases, where the bone cartilage interface was not perpendicular to the sides of the plug, an average thickness for the whole specimen was estimated. In each case care had to be taken not to apply any compressive force to the cartilage during the measuring as the resulting deformation would obviously introduce errors into the thickness measurement. It was decided before any experiments were carried out that the easiest way to measure the thickness would be after testing when the specimens could be removed from the underlying bone. This obviously assumes that the specimens do in fact recover their original thickness. Measurements taken from the video recordings of the specimens before testing support this assumption as they agree closely with the micrometer measurements taken after testing.

In the case of the sliced specimens the overall thickness was estimated by adding together the thicknesses of the individual slices.

Once the cartilage thickness had been measured it was then ready to be analysed biochemically.

CHAPTER 6

BIOCHEMICAL ANALYSIS AND RESULTS

6.1 Introduction

Any study of the mechanical properties of a tissue must include a method of assessing the normality of the tissue being tested. In the case of articular cartilage this is particularly important as the effect of other variables, apart from the biochemistry, such as age of the specimen and site within the joint, on the mechanical properties is still not known. If any sense is to be made of the mechanical results obtained then it must be possible during the analysis to restrict the number of variables to a minimum.

As discussed in Chapter 2, although fibrillation of the articular surface is a clear indication that disruption of the cartilage has occurred it seems likely that disruption of the surface of the articular cartilage is in fact preceded by alterations in the water and uronic acid content of the tissue. Measurements of these two variables would therefore appear to be the most accurate method of determining the normality of the tissue.

In this study therefore, the water and uronic acid content of each specimen tested was measured biochemically. In this chapter the biochemical techniques used are described and the relationships between these two variables and age and site of specimen are discussed. The relationship between the biochemistry and the mechanical properties of the tissue will be discussed in Chapter 7.

6.2 Water Content

The water content of the specimens was determined by drying to constant weight in vacuo. Each specimen was taken from its storage tube, surplus saline was removed with a soft tissue and the wet weight of the specimen was measured. The specimens were then placed in a desiccator and left with the pump running until a constant dry weight had been reached. 48 hours was usually sufficient for this. The water content of the tissue was then calculated as the difference between the wet and dry weight.

It is possible that some intracellular water is not removed using this drying technique and that the true water content of the tissue is slightly higher than measured. However, it is unlikely that the intracellular water content has any significant effect on the mechanical properties of the tissue as there are few cells in articular cartilage, whereas the water content as measured, that is the matrix water content, may be directly related to the mechanical properties. It is also unlikely that the increase in water content seen in the initial stages of osteoarthrosis is due to an increase in intracellular water and therefore, as the purpose of measuring water content was to assess the normality of the tissue and determine the relationship, if any, between water content and mechanical properties of the tissue, it was decided that the technique described should be used in this study.

6.3 Uronic Acid Content

The Uronic Acid content of each specimen was determined by the method of Bitter and Muir (1962). After the water content of each specimen had been determined, as described in 6.3, the specimens were digested in papain solution (0.2 ml papain in 2 ml 1% $NaHCO_3$/Cyst buffer Ph 8.0) at $60^{o}C$ for 48 hours. The volume was made up to 5 ml with distilled water and, depending on the dry weight of the specimen, either 0.1 ml or 0.3 ml aliquots were taken for chemical analysis.

6.4 Biochemical Results

The water content of the samples ranged from 66% to 86% of the wet weight. The variation in water content with age is shown in Fig. 6.1. There is no apparent relationship between the water content and the age of the sample although there is an increase in the range of water content with age. This

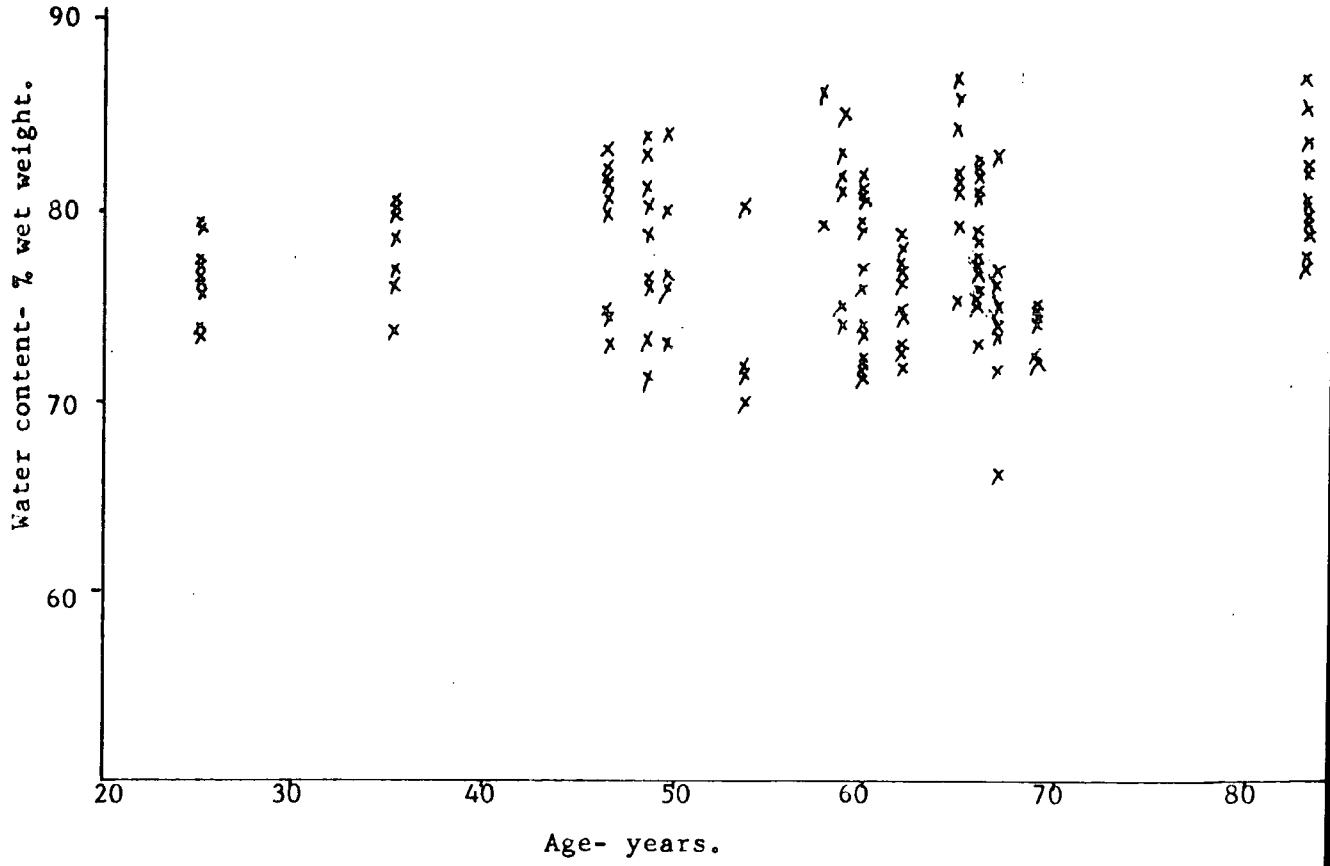


Fig6.1 Variation in water content with age.

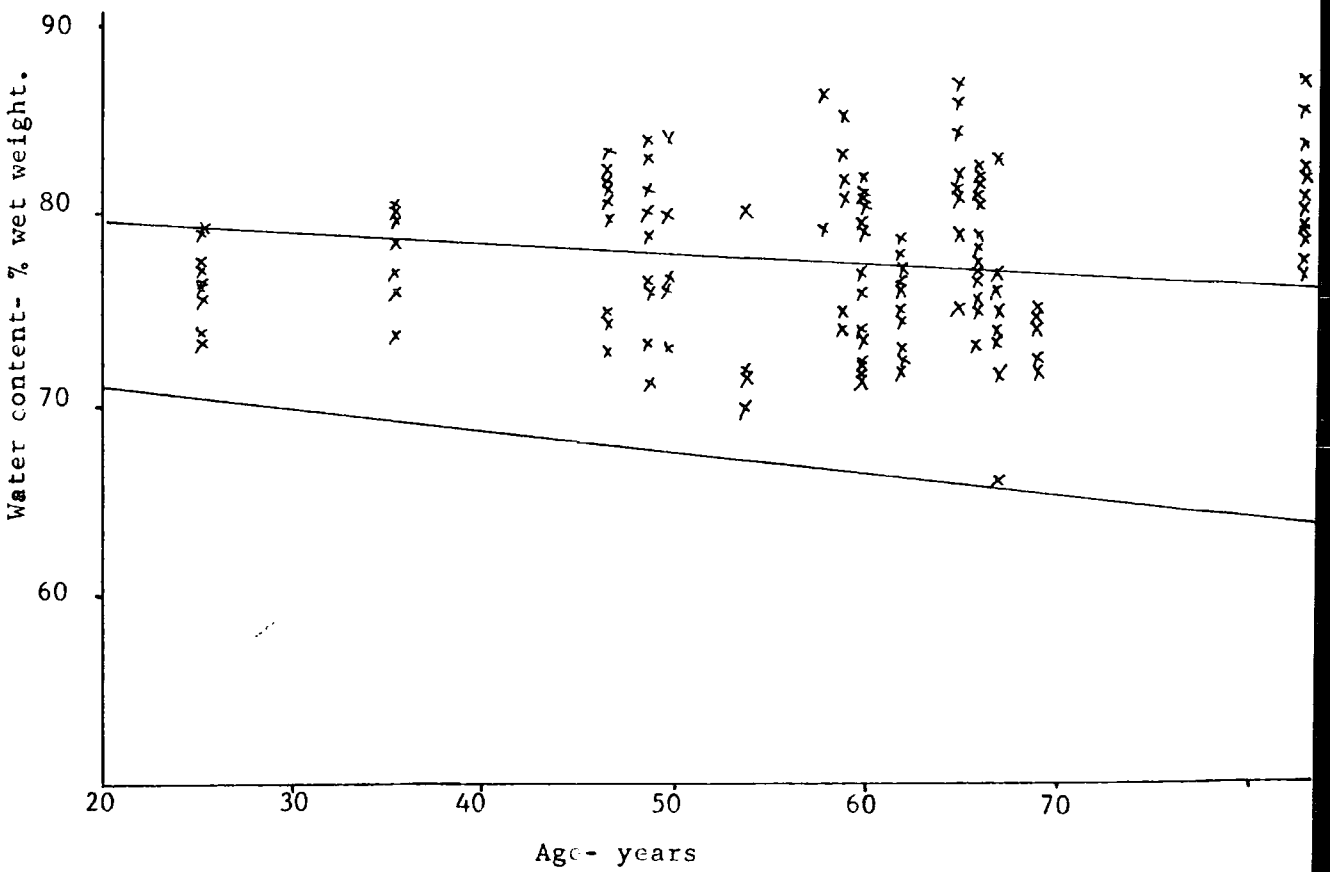


Fig6.2 Variation in water content with age plus normal range.(Venn 1977).

is consistent with previous observations that the incidence of fibrillation and osteoarthrosis, indicated by increased water content, increases with age whereas the water content of normal cartilage decreases slightly with age (Venn 1977). If the variation in range of water content with age for normal articular cartilage as determined by Venn(1977) is superimposed on the results (Fig. 6.2) it can be seen that approximately 50% of the samples fall within the normal range while approximately 50% have water content higher than the normal range. The number of specimens at any particular age with an above normal water content increases with age, with all the specimens from the 25 year old patella falling within the normal range and all the specimens from the 82 year old patella being above the normal range.

Using the ranges for normal water content determined by Venn, the specimens in this study can therefore be divided into two groups; those where there has been no alteration in biochemistry and can be classified as normal, and those where the biochemistry would indicate disruption of structure is present to some degree. This classification into normal and abnormal specimens is discussed further in Chapter 7 in relation to mechanical properties of the specimens.

The variation in water content with site of the specimen is shown in Fig. 6.3 and 6.4. In Fig. 6.3 the actual values measured are shown and in Fig. 6.4 the distribution of normal and abnormal specimens, as determined by age and water content, is indicated for those patellae where specimens from both groups were present. These results indicate that there is no systematic variation in water content with site.

The Uronic Acid content of the specimens ranged from 0.5% to 4.7% on a dry weight basis and 0.1 to 1.04% on a wet weight basis. These values are consistent with the values reported by previous workers for normal articular cartilage (Maroudas et al 1969, Ficat and Maroudas 1975, Sweet et al 1977).

The variation in Uronic Acid content with age and site are shown in Figs. 6.5 to 6.8 and there is no correlation between Uronic Acid content and either of these two variables.

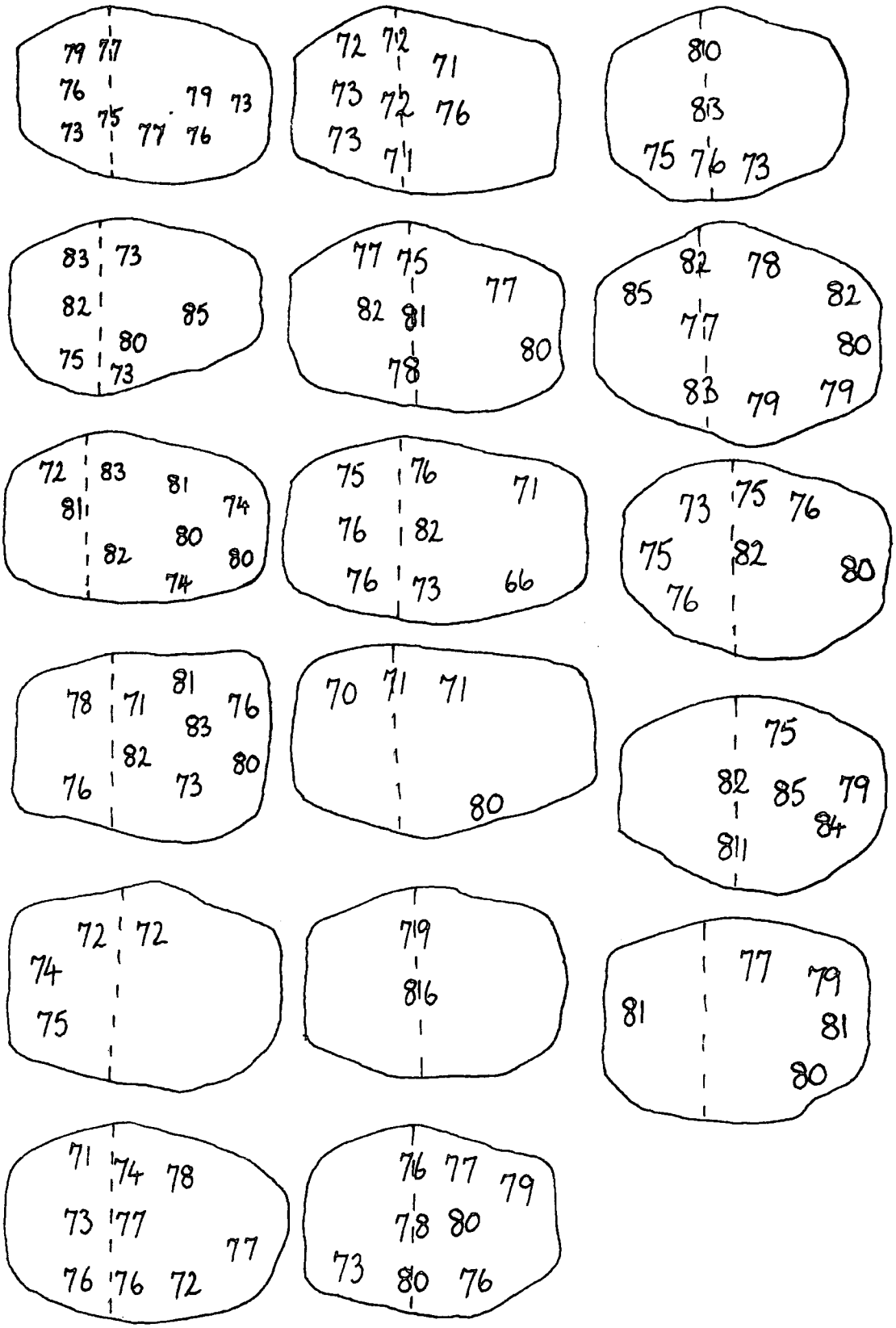


Fig6.3 Variation in water content(as a % of wet weight) with site.

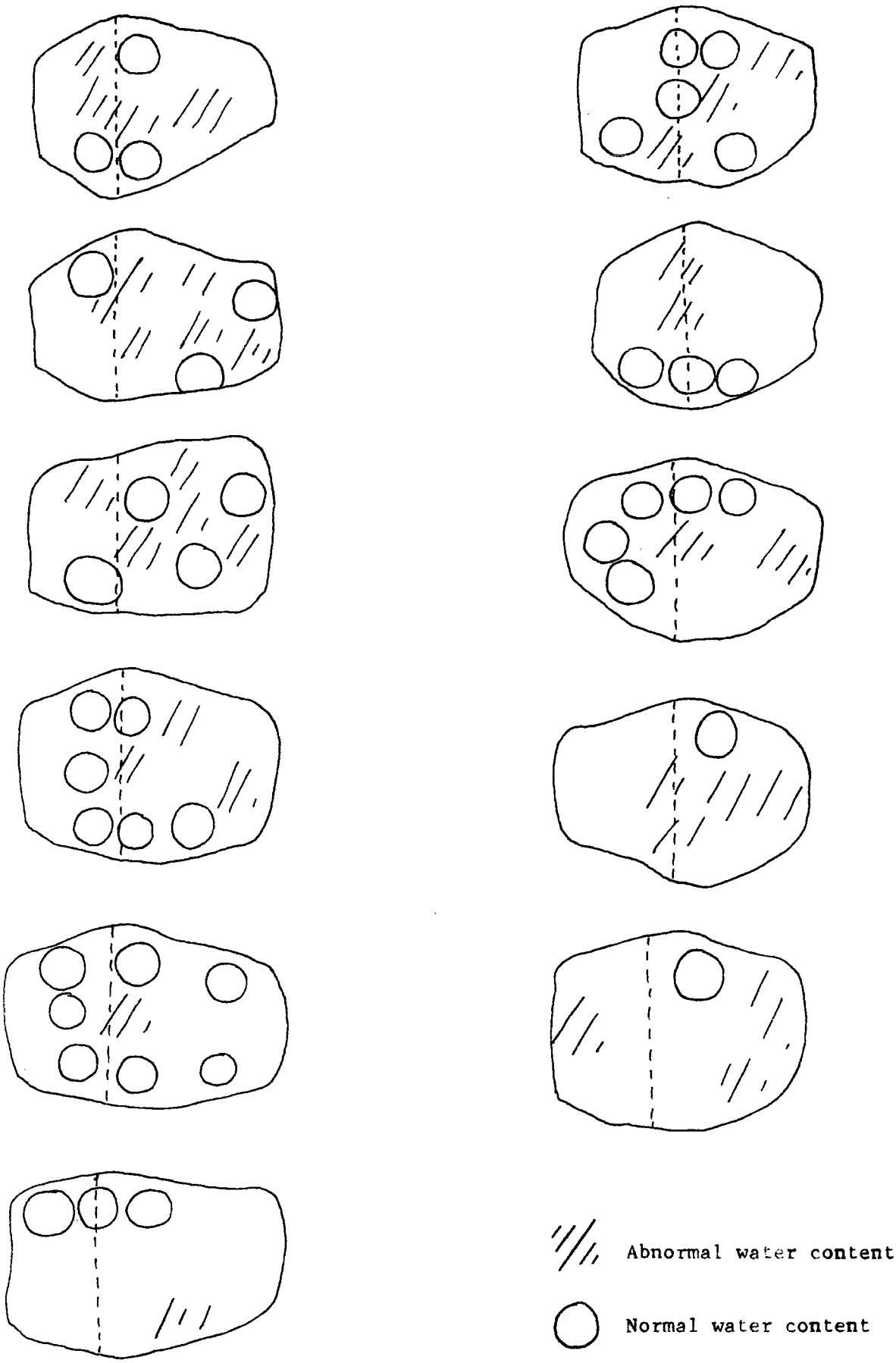


Fig6.4 Distrobution of normal and abnormal tissue.

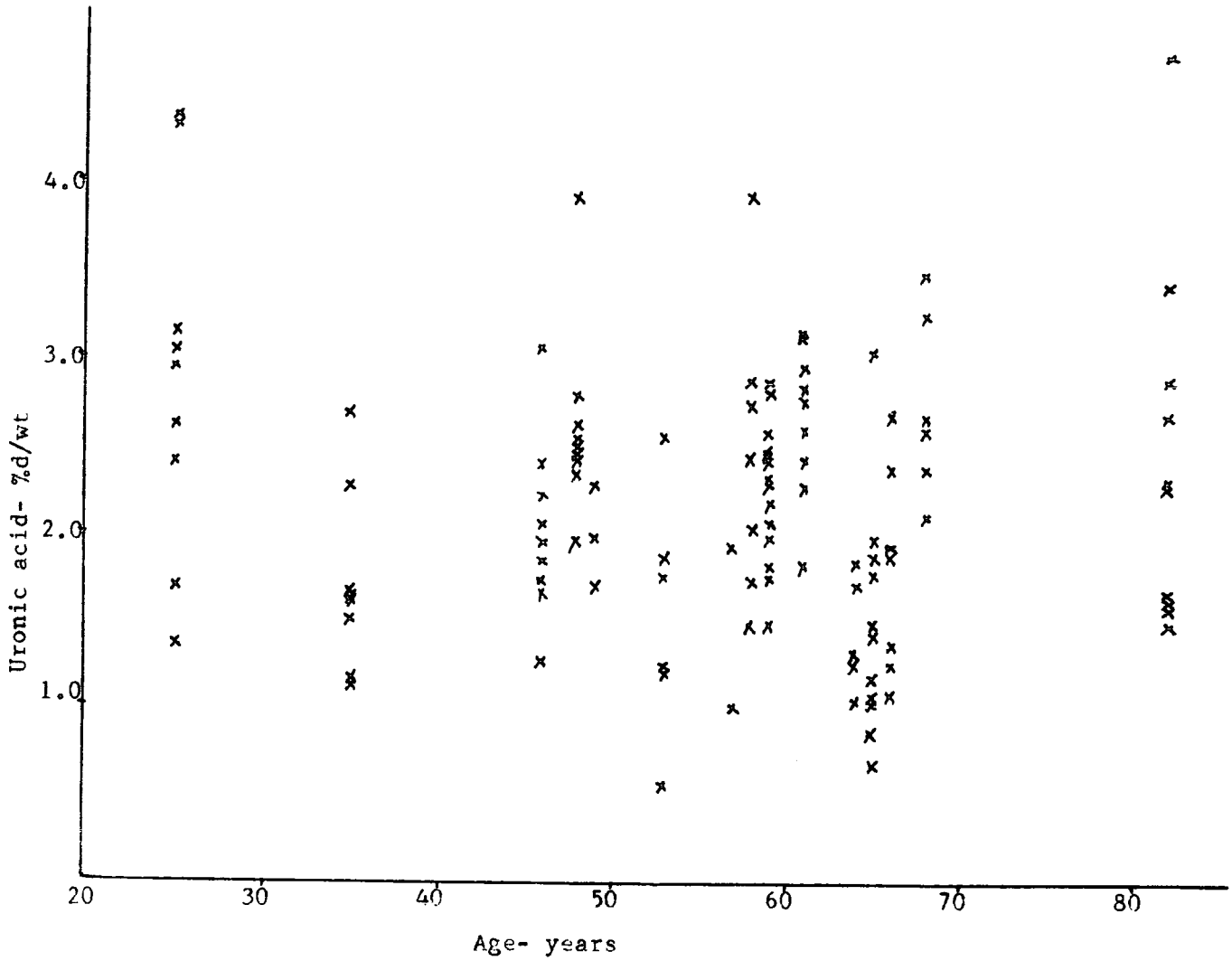


Fig6.5 Variation in Uronic Acid content (% dry weight) with age.

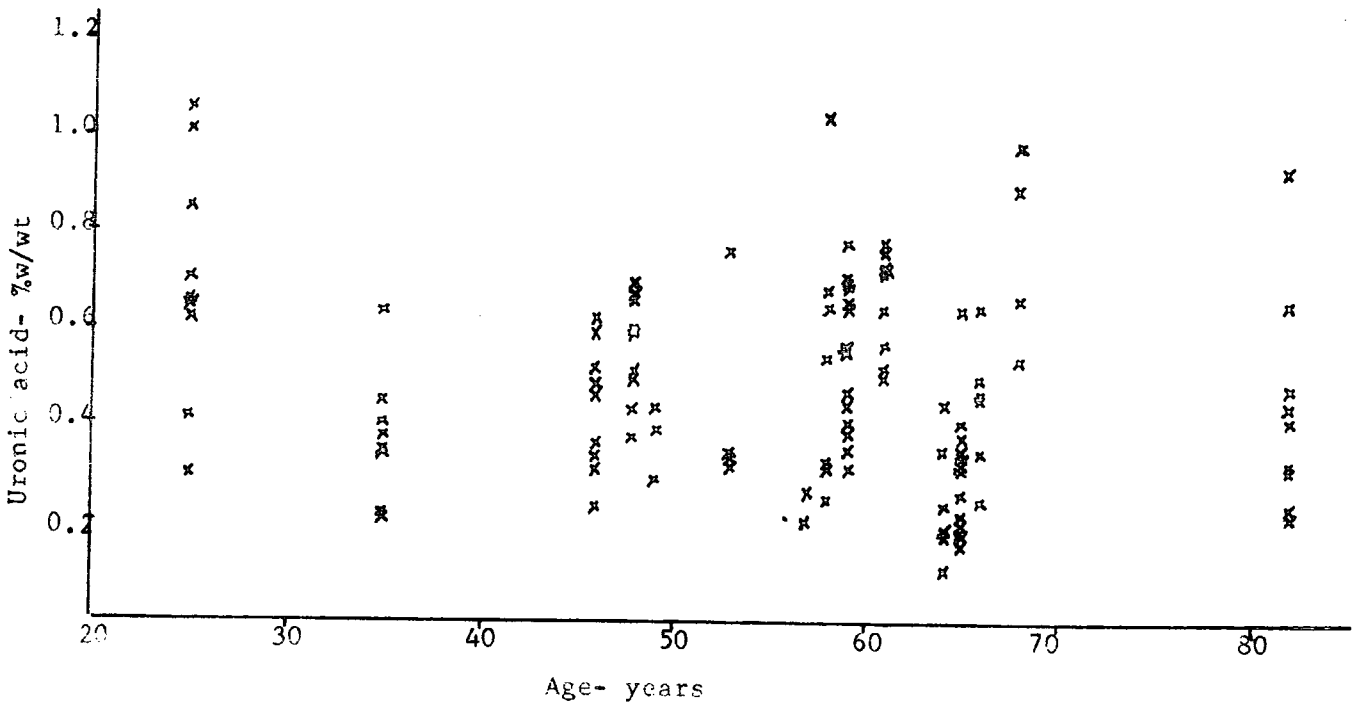


Fig6.6 Variation in Uronic Acid content (% wet weight) with age.

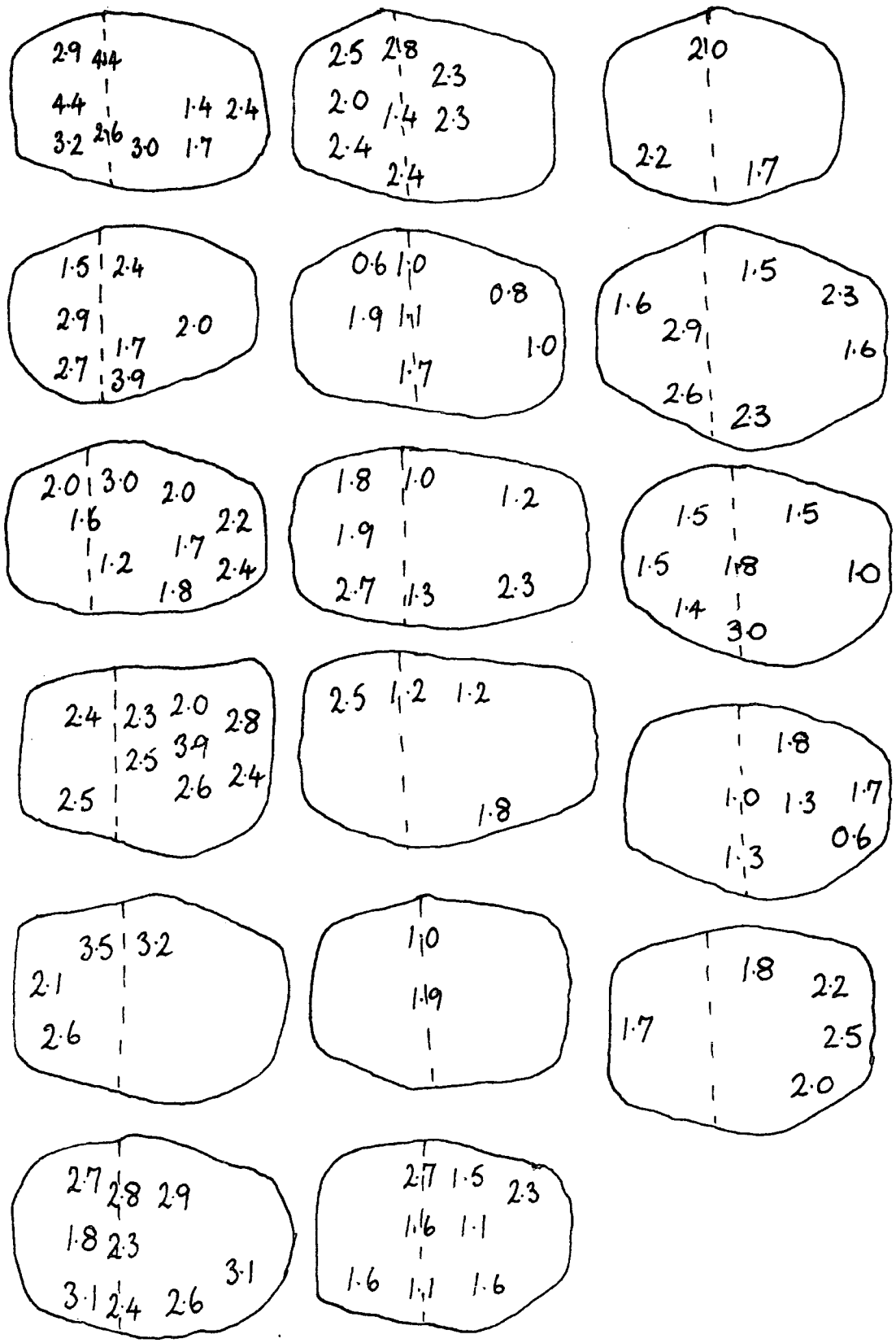


Fig6.7 Variation in Uronic Acid content (% d/wt) with site.

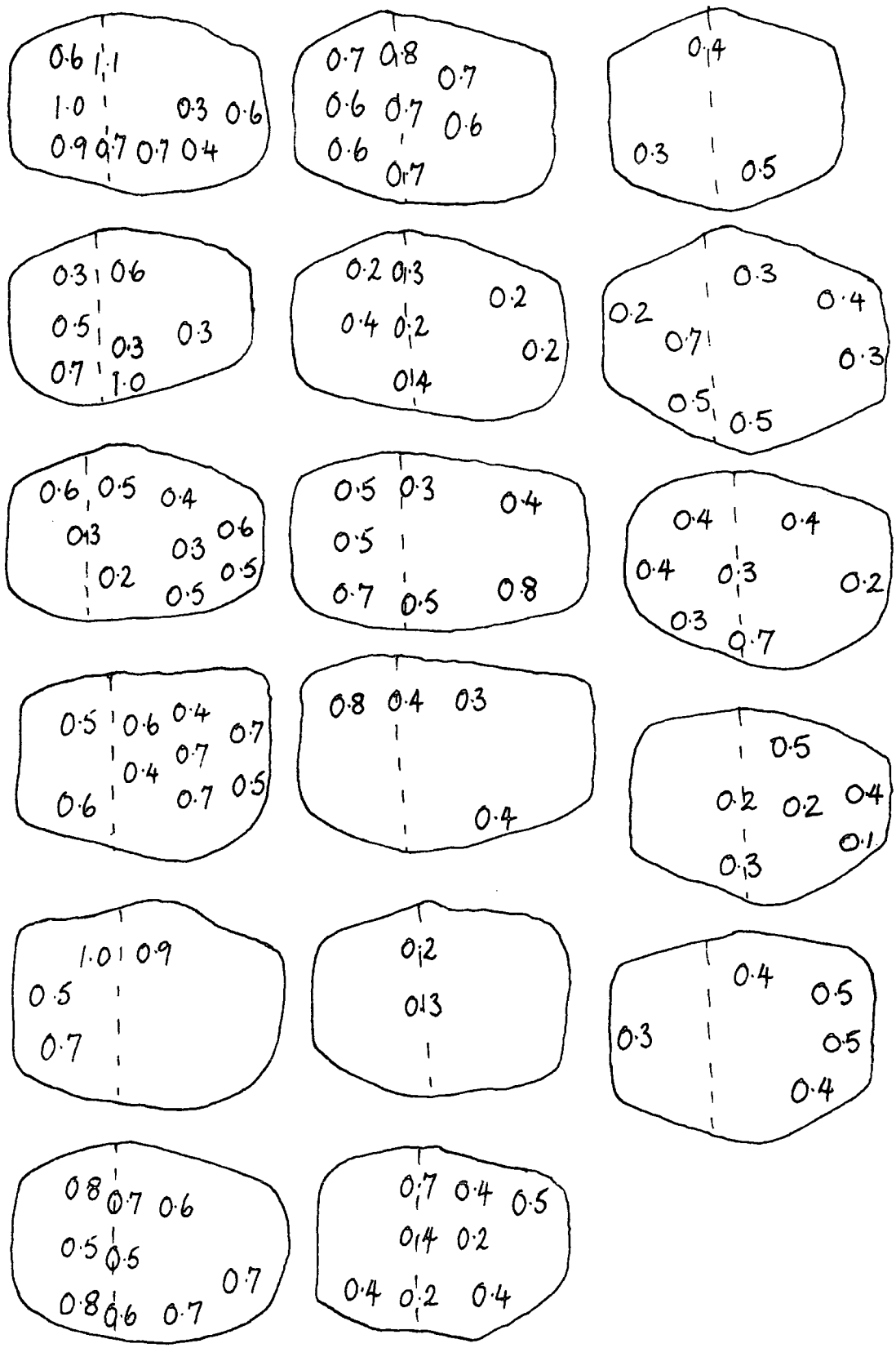


Fig6.8 Variation in Uronic Acid content (% w/wt) with site.

CHAPTER 7

MECHANICAL RESULTS; ANALYSIS AND DISCUSSION

7.1 Introduction

As described in Chapter 5, three test procedures using three different test machines were used during this investigation. In each case a constant rate displacement was applied to the specimen and the resultant load measured.

Before the main bulk of the experimentation was started three short series of tests were performed to establish two criteria for the subsequent analysis.

The first series of tests was to establish whether a specimen tested more than once responded in the same manner each time, if it had been left to recover immersed in saline between tests. The second series of tests was to establish the effect of varying the strain rate on the response of the tissue. The information from these tests is most important to the analysis of the results from the Dartec tests as it is during these tests that not only is the same specimen tested more than once but the thickness of the specimen is reduced between successive tests. It is also necessary to enable analysis of all the full thickness tests as it was proposed that all the tests be performed at the same displacement rate and therefore, as the thickness of the specimens was not the same in each case, at slightly different strain rates.

The third series of tests was to establish whether or not the full thickness response recorded under vacuum in the SEM was the same as the response recorded for the same specimen, when immersed in saline. This information is obviously required before any comparison between different specimens tested under the two conditions can be made. The results of these three preliminary investigations are presented first in this chapter and based on these results the subsequent analysis of the main series of tests is then described. The analysis of results is divided into two sections: variation in response with depth, and response of full thickness tissue.

7.2 Repeat Testing of Specimens and Effect of Varying Strain Rate

Several specimens were tested more than once in both the SEM and Dartec. It was found that if the specimens were left immersed in saline for about 4 hours in between tests the response, although not identical, was essentially the same for repeat tests.

Figs 7.1 to 7.4 show four examples of specimens tested more than once in the SEM and Figs 7.5 to 7.8 show four examples of specimens tested more than once in the Dartec. In some cases specimens were compressed to less than half their original thickness during the tests and it is therefore apparent that articular cartilage has a quite remarkable ability to recover from the effects of compressive loading. Figs 7.9 to 7.14 show the load-deformation response of six specimens each tested at five different displacement rates; 0.03, 0.06, 0.3, 0.6, 3 mm/sec in the Dartec and Figs 7.15 to 7.17 show the response of three specimens tested at displacement rates of 0.1, 0.3 and 0.8 mm/sec in the SEM.

The thickness of these specimens ranged from 2.15 mm to 3.5 mm which, with the displacement rates used, gives a range of strain rates from 0.86% per second to 140% per second.

As can be seen with the exception of the specimen shown in Fig. 7.13, there is very little variation in response within the range of strain rates tested. There is no consistent increase or decrease in the slope of the response with increasing strain rate.

The exception to the results is the specimen shown in Fig. 7.13. This was the thickest of the specimens tested and therefore the applied strain rates were at the lowest end of the range tested. It seems likely that the increase in deformation of the tissue shown at the slowest strain rates is due to the increased significance of water flow during these tests. This would result in larger deformations at the same load as in effect the response measured is a combination of the elastic and creep response of the tissue.

Based on these results it was decided that all full thickness specimens in the SEM and Dartec could be tested at the same displacement rate of 0.3 mm/sec. This would mean that the

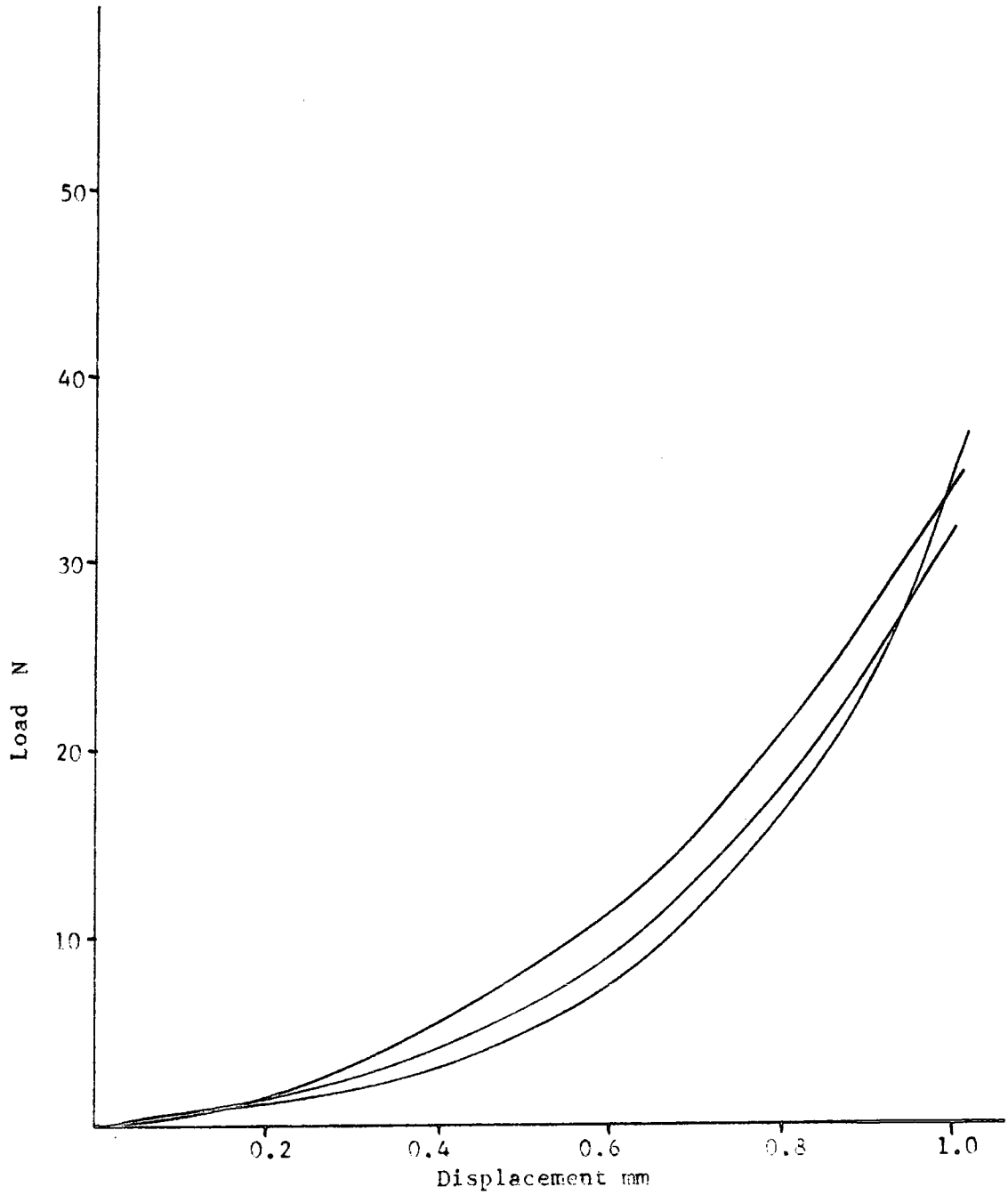


Fig7.1 Repeated test (SEM).

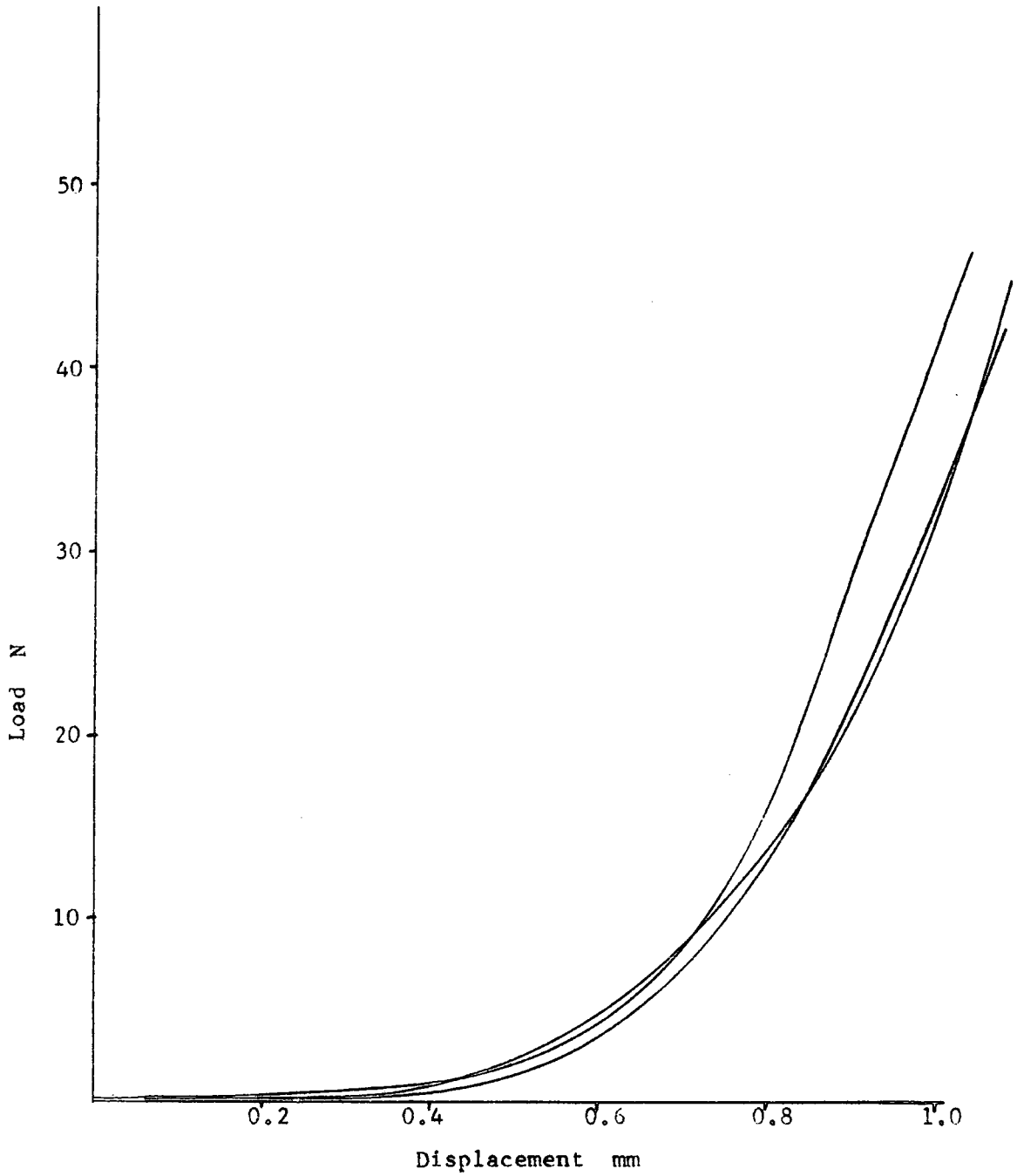


Fig7.2 Repeated test (SEM).

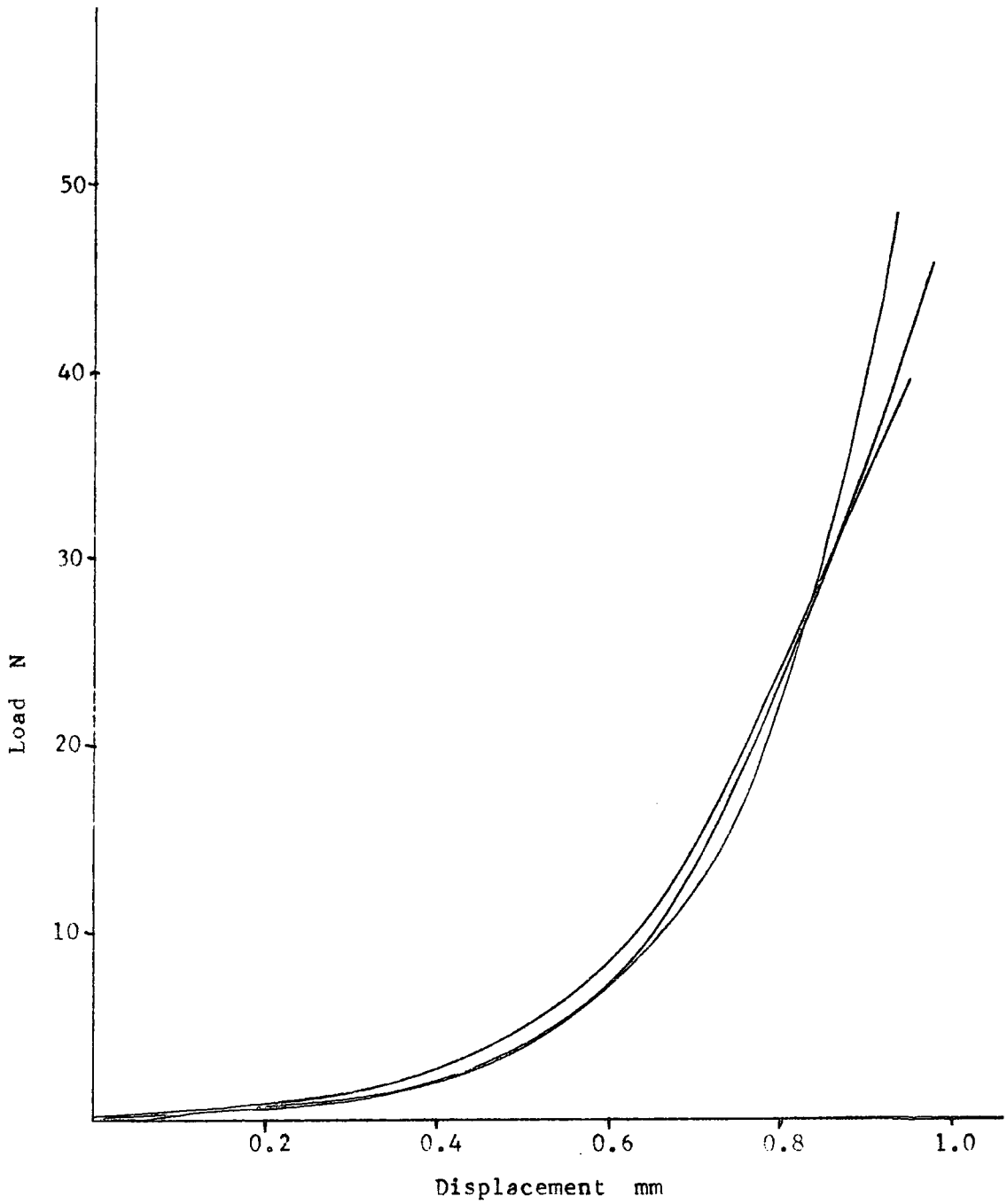


Fig7.3 Repeated test (SEM).

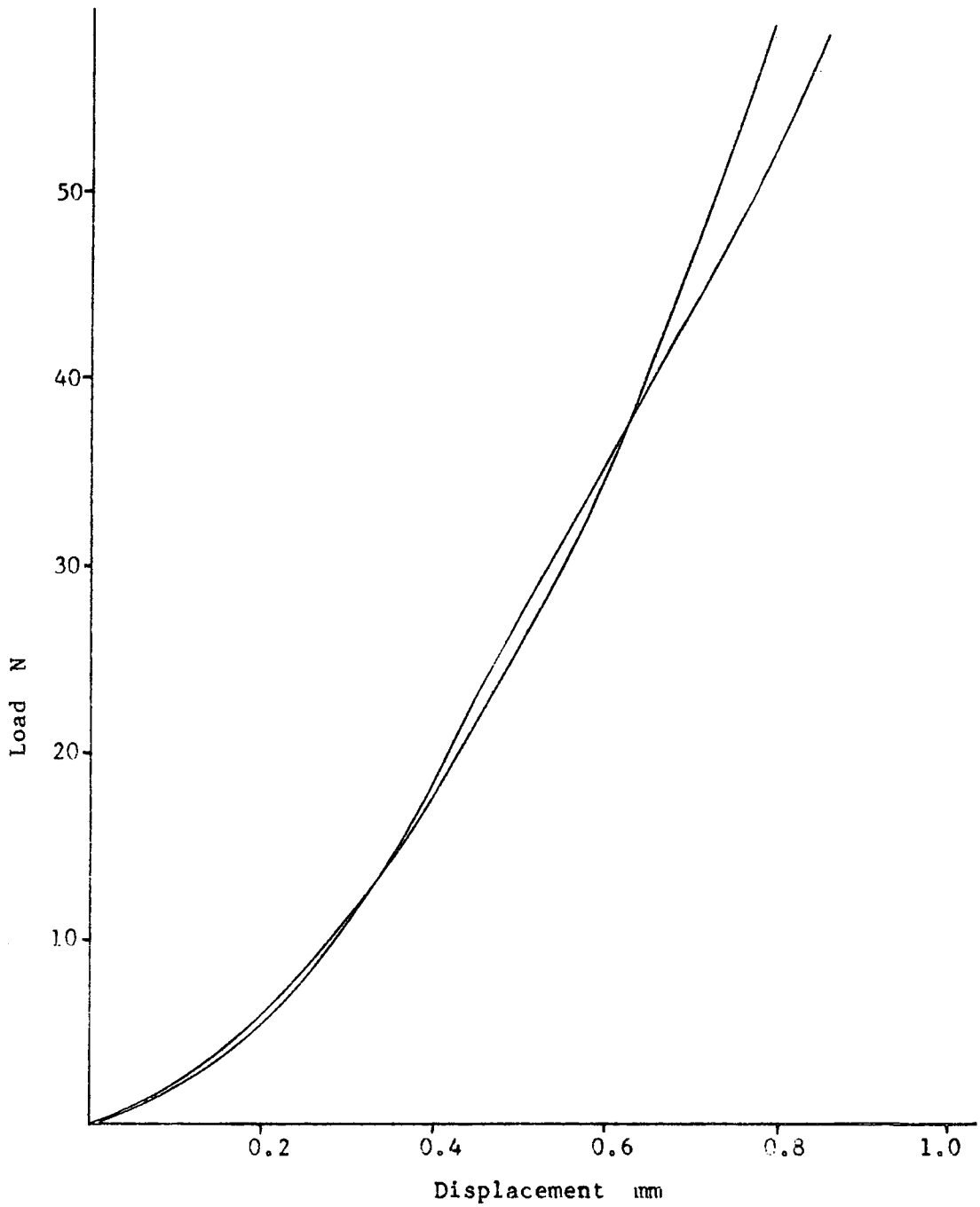


Fig7.4 Repeated test (SEM).

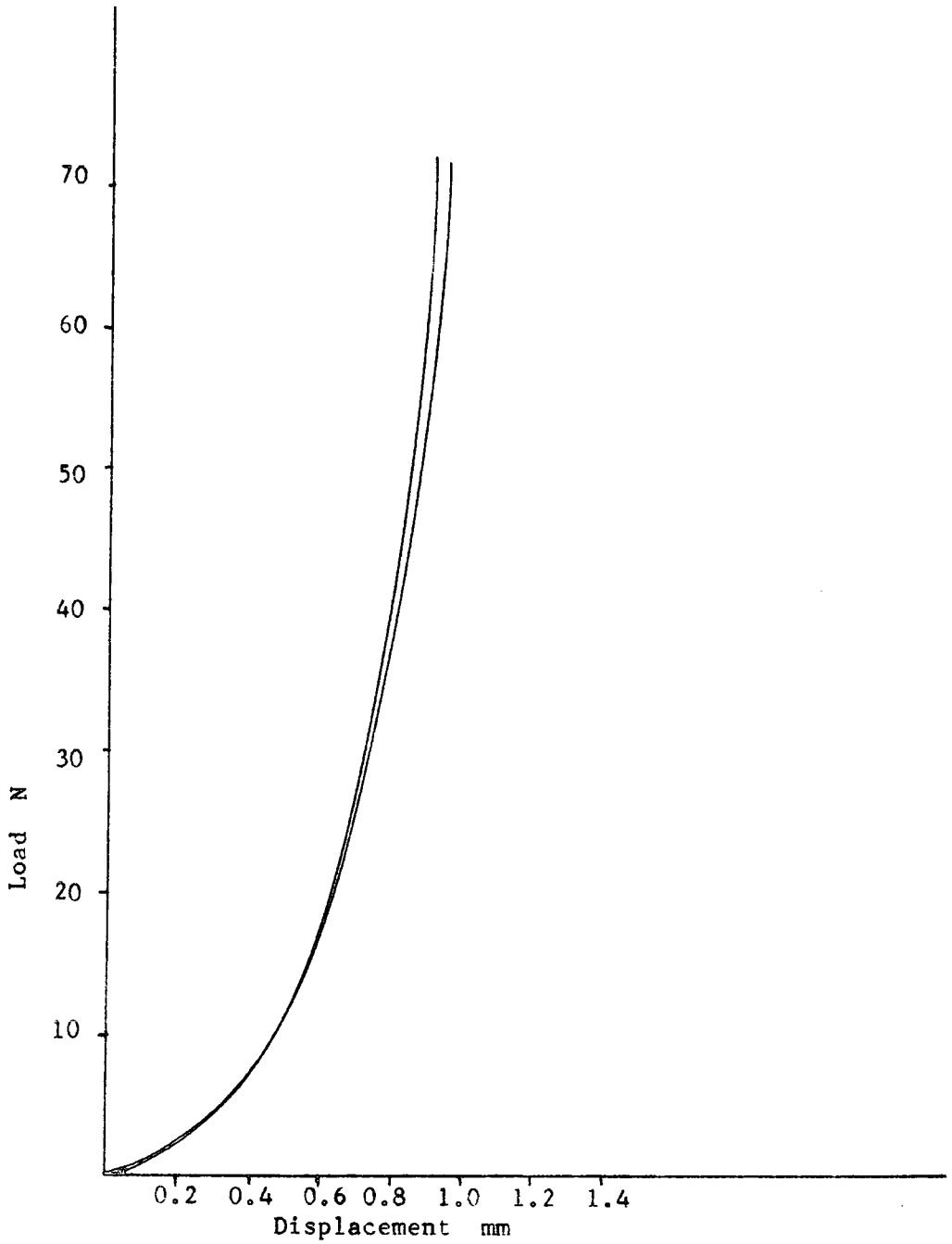


Fig7.5 Repeated test.(Dartec)

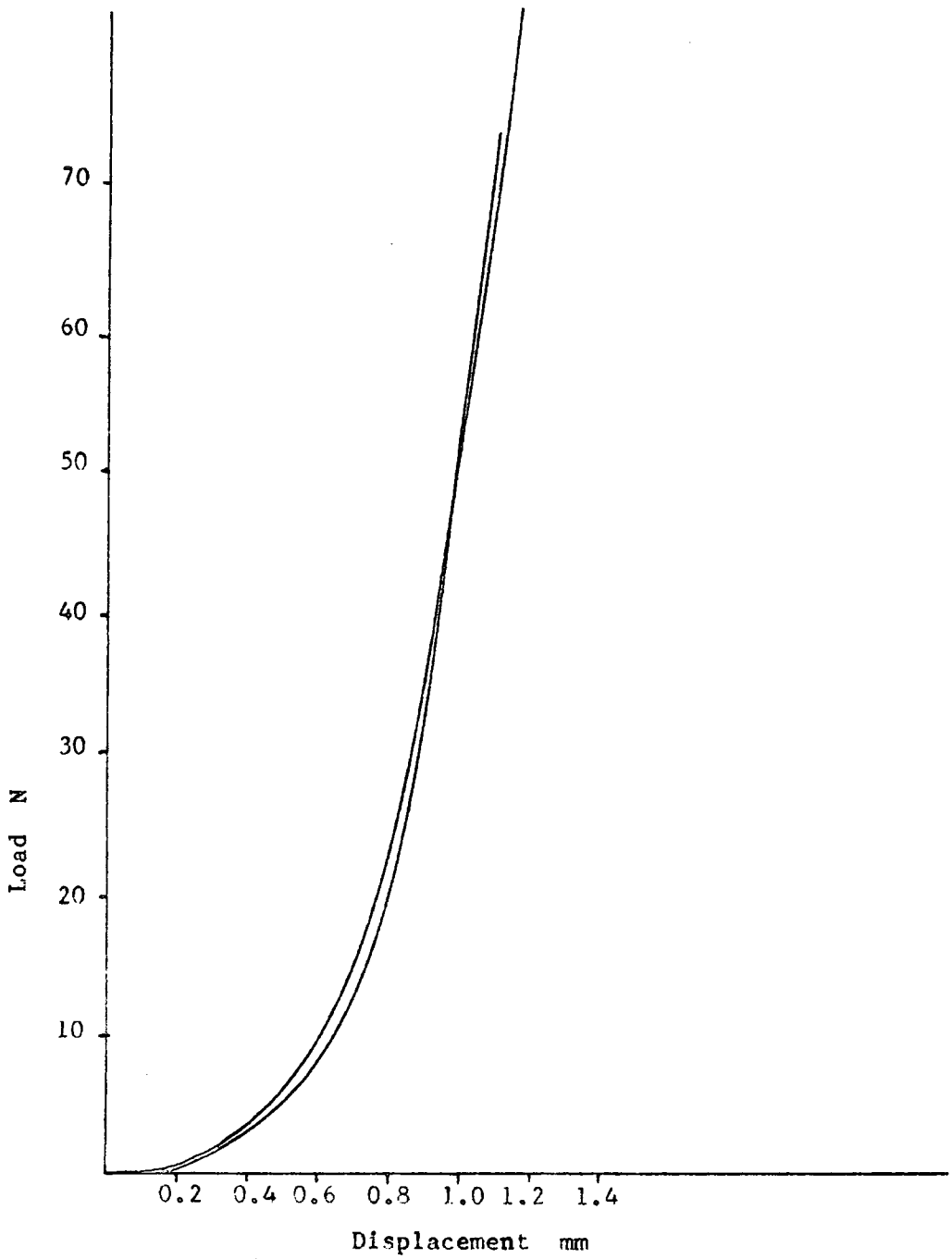


Fig7.6 Repeated test.(Dartec)

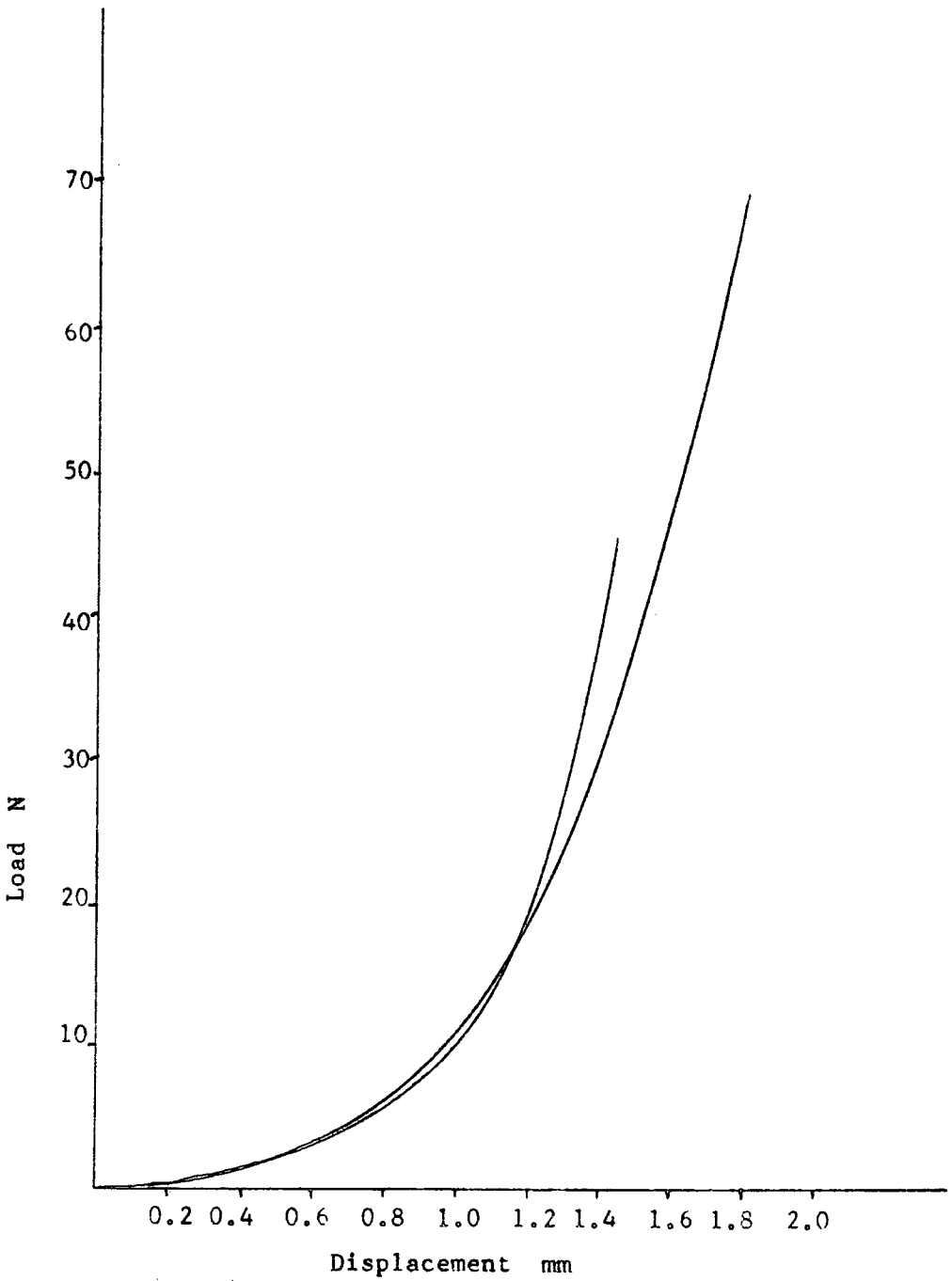


Fig7.7 Repeated test.(Dartec)

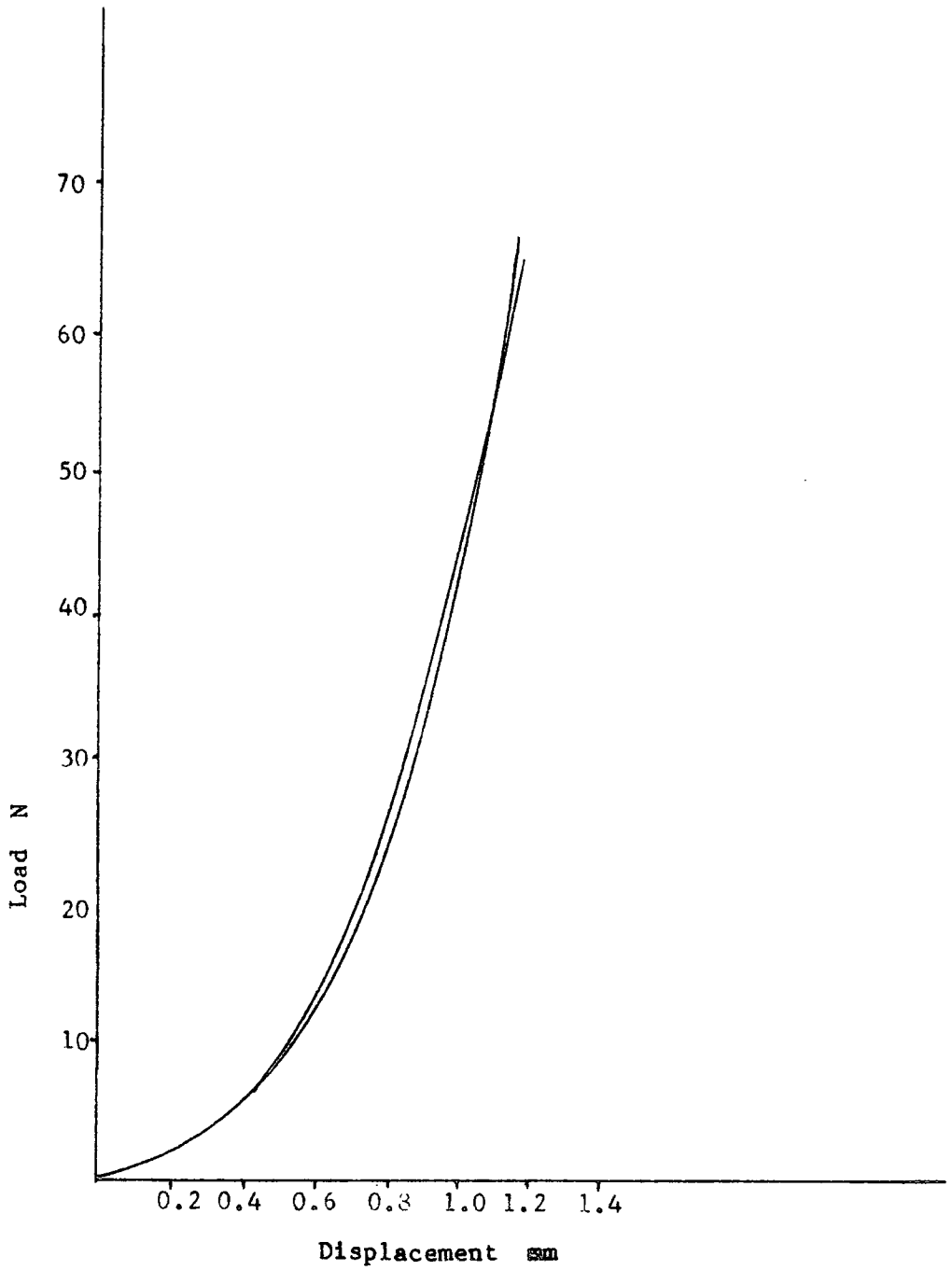


Fig7.8 Repeated test.(Dartec)

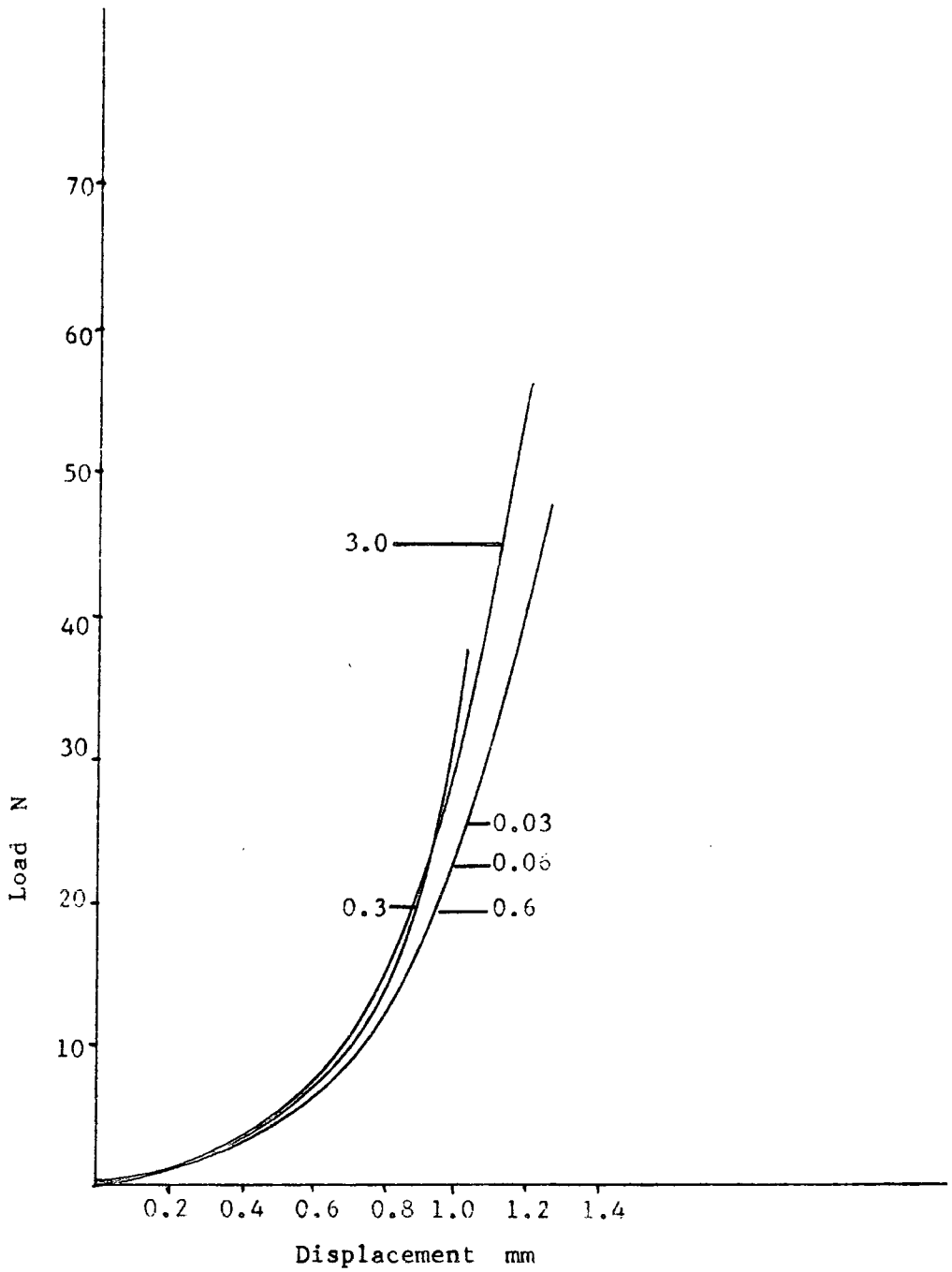


Fig7.9 Effect of varying displacement rate (Dartec).

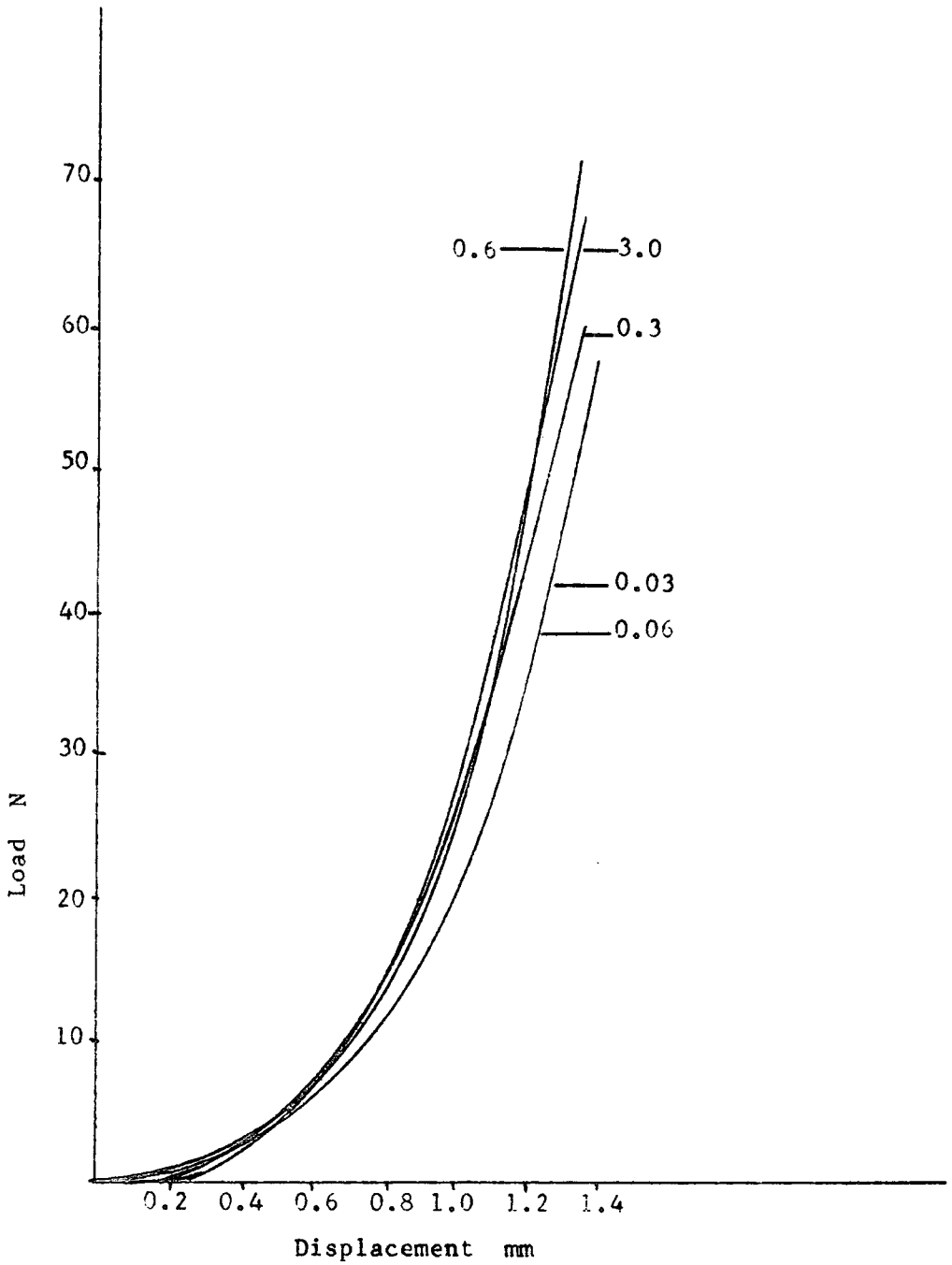


Fig7.10 Effect of varying displacement rate (Dartec).

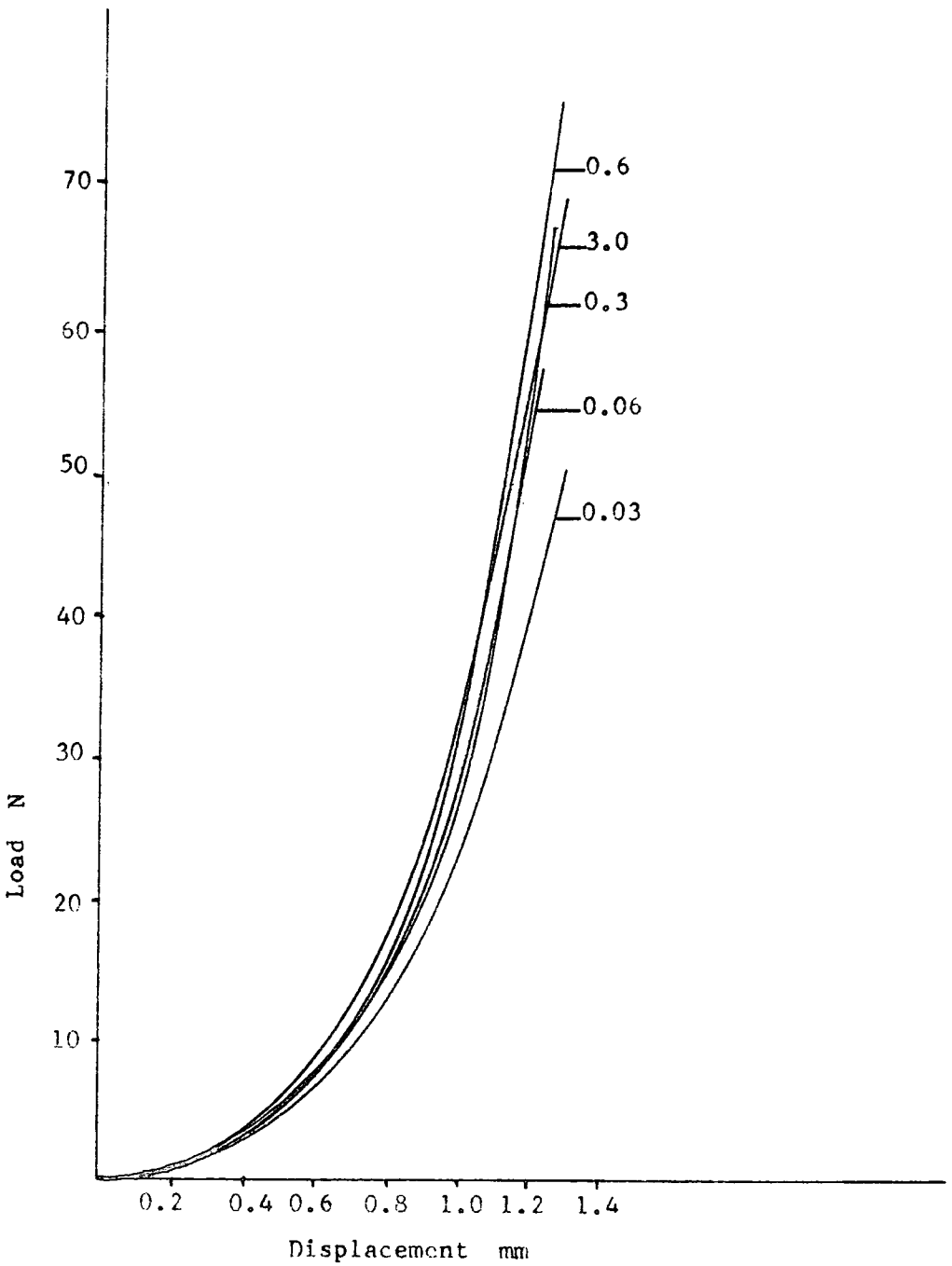


Fig7.11 Effect of varying displacement rate (Dartec).

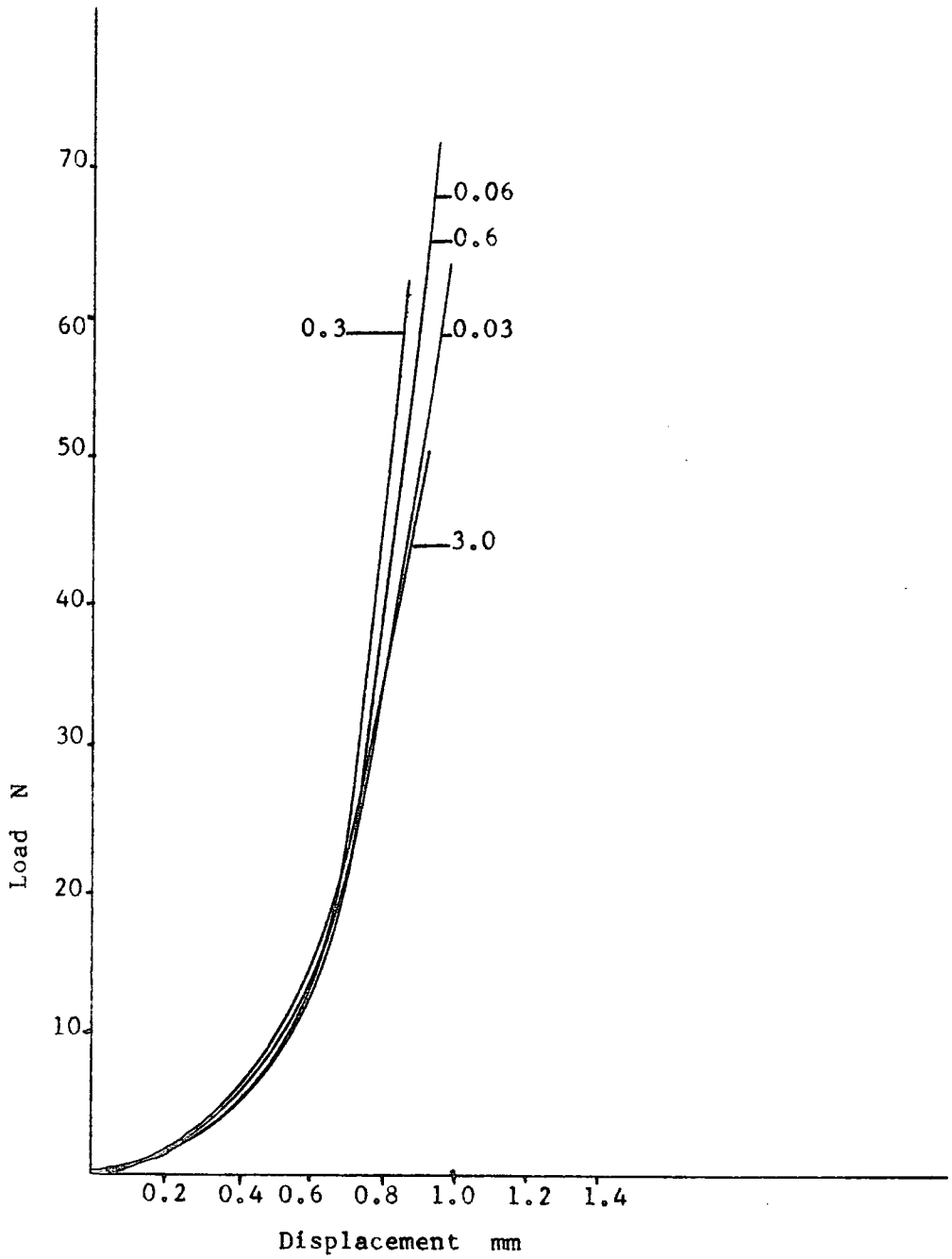


Fig7.12 Effect of varying displacement rate (Dartec).

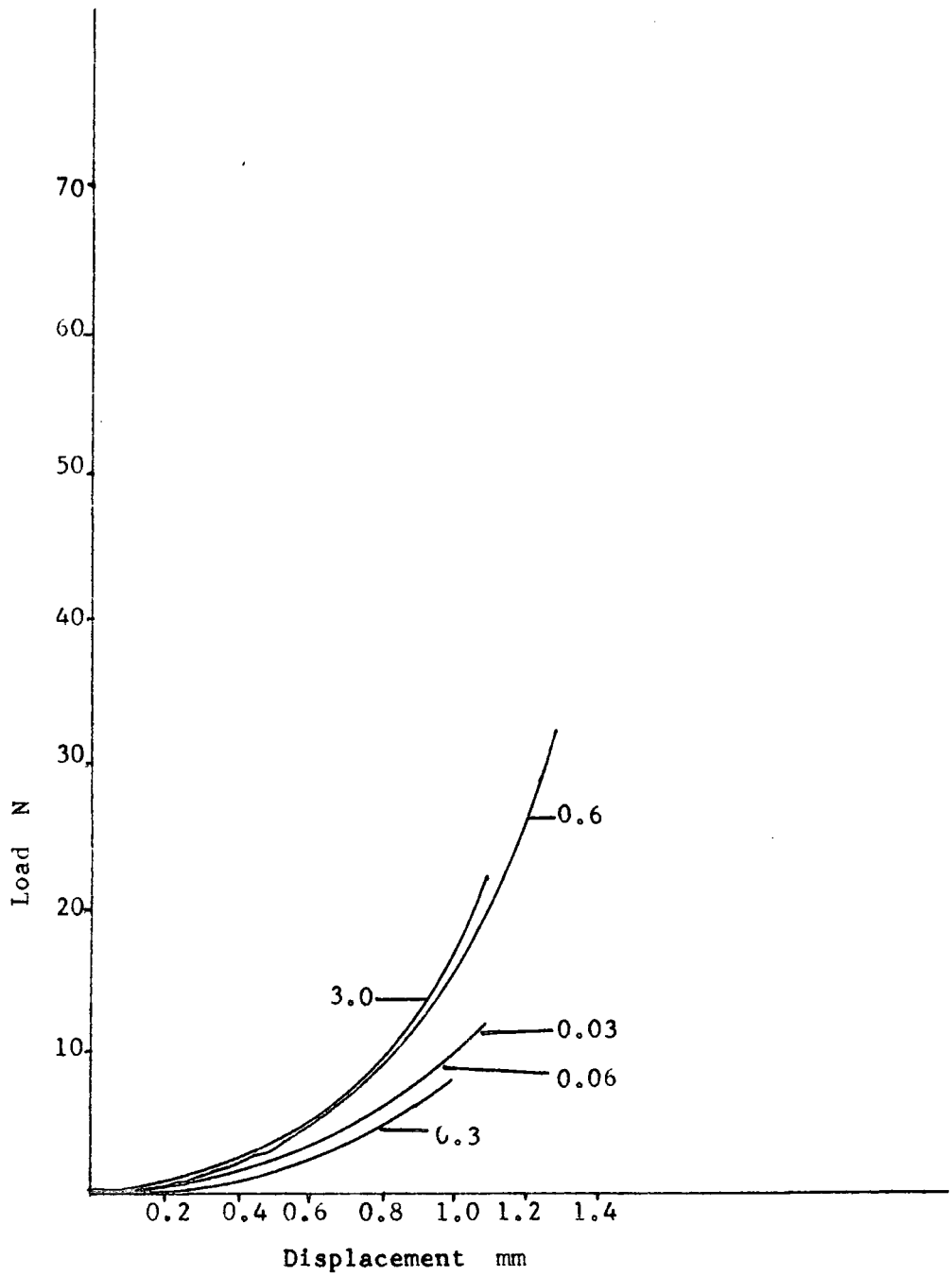


Fig7.13 Effect of varying displacement rate (Dartec).

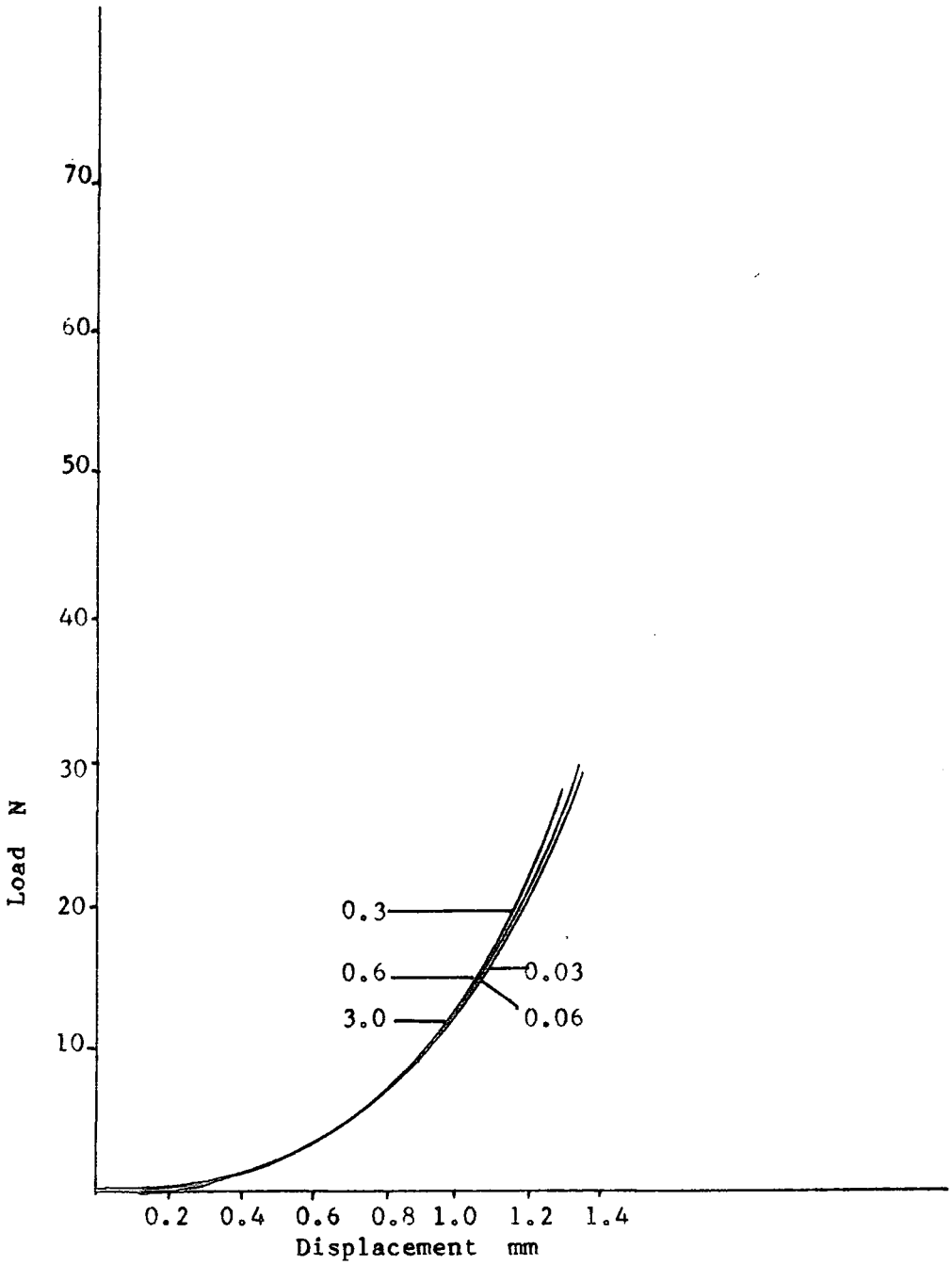


Fig7.14 Effect of varying displacement rate (Dartec).

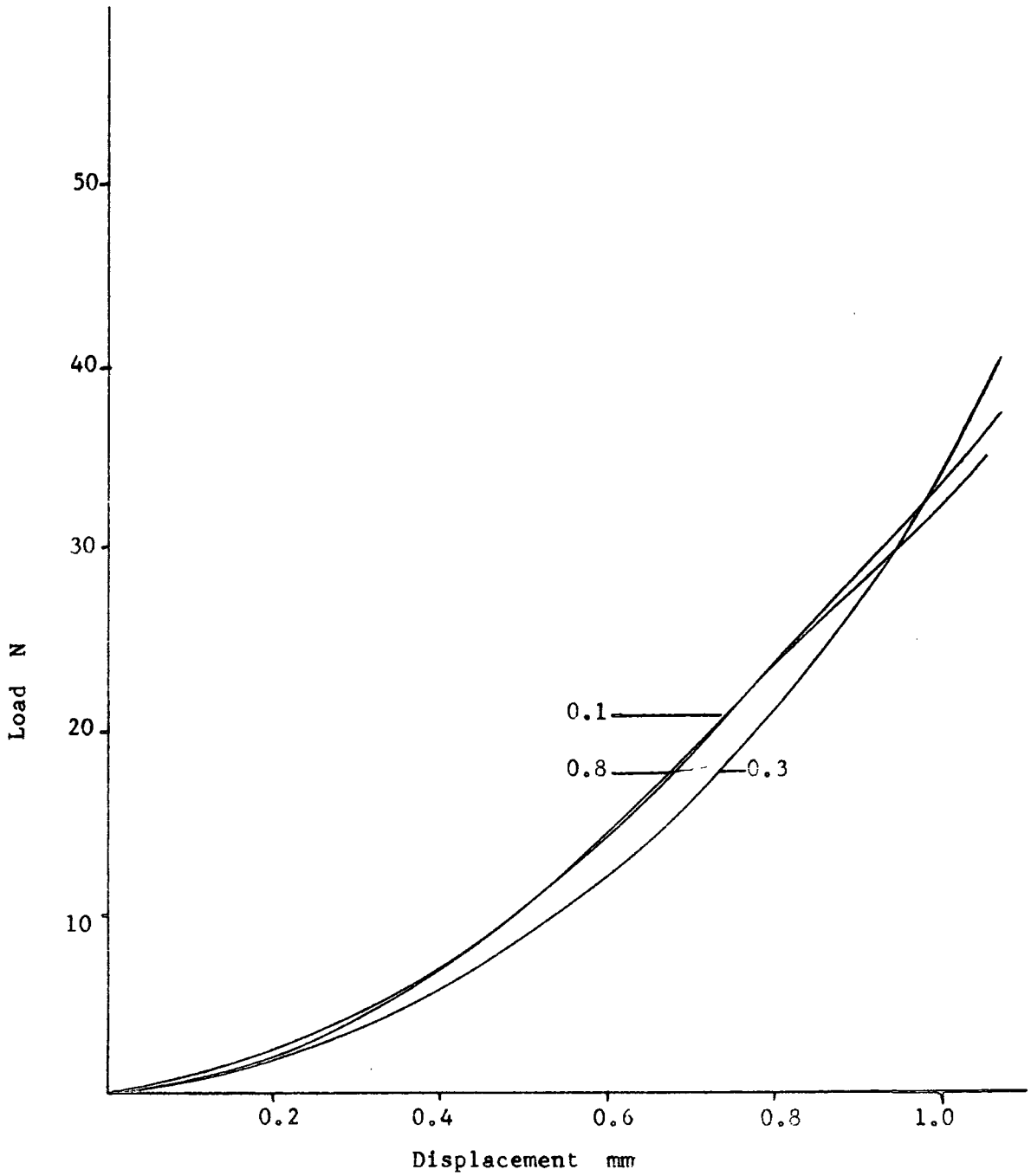


Fig7.15 Effect of varying displacement rate (SEM).

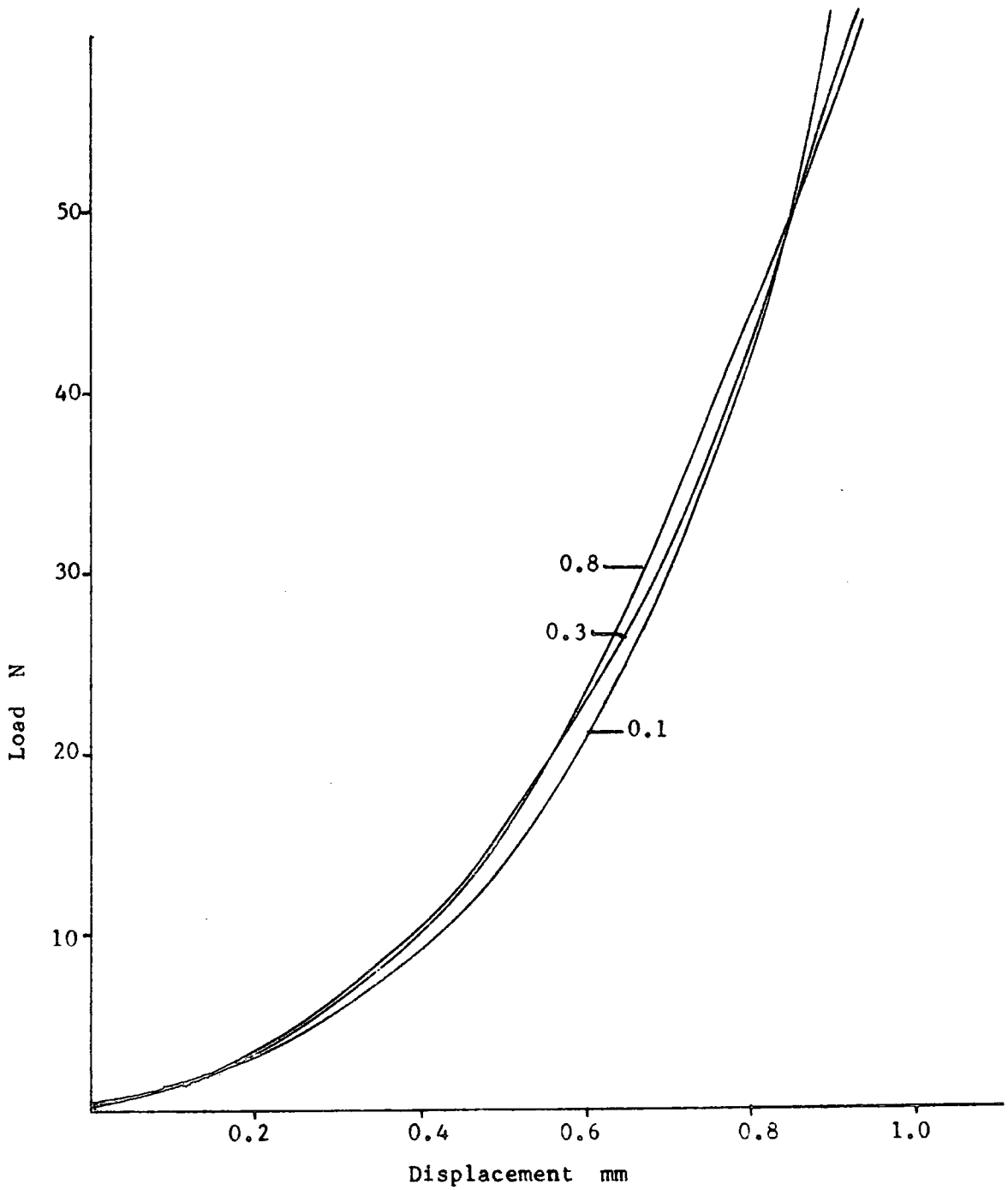


Fig7.16 Effect of varying displacement rate (SEM).

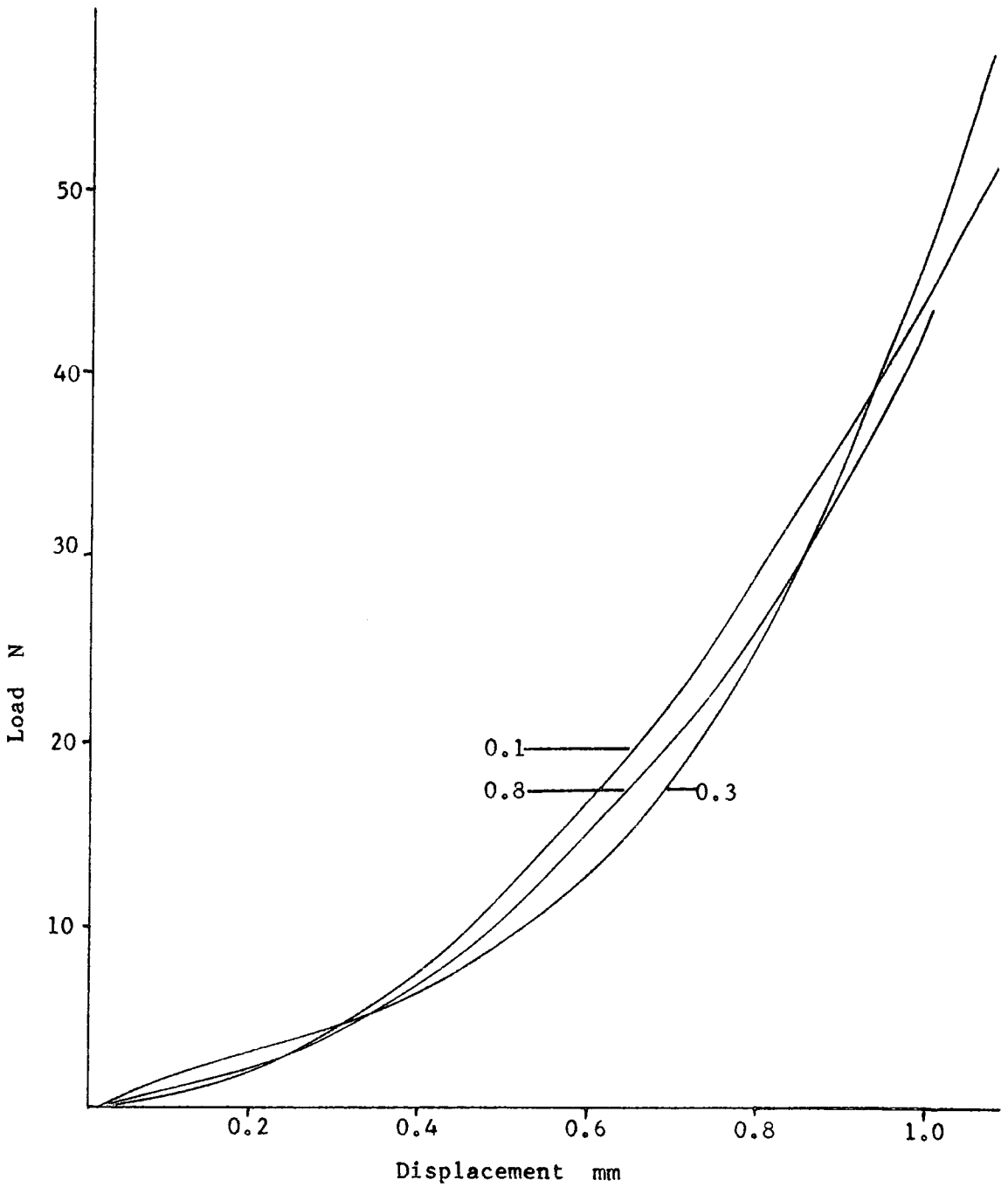


Fig7.17 Effect of varying displacement rate (SEM).

applied strain rate was well within the range tested during this series of preliminary tests. For the testing of slices in the Instron the applied displacement rate was chosen as 0.083 mm/sec. As the slices were nominally 0.5 mm thick this results in a strain rate of approcimately 17% per second, which is again well within the range tested and approximately the same as during the full thickness tests.

It was also decided that the tests in the Dartec, where slices of cartilage are removed and the remaining tissue retested, could all be performed at 0.3 mm/sec and it would be reasonable to assume that any difference in the response was due to the removal of the tissue, rather than either repeat testing or the slight increase in strain rate.

7.3 Comparison Between Response in SEM and Dartec

To establish whether or not the response of articular cartilage is affected by the specimen being in a vacuum during these tests 21 full thickness specimens were tested both in the SEM and the Dartec. The results obtained by the two different methods are shown in Figs 7.18 to 7.38. The analysis used to convert the load–displacement measurements to stress–strain and the definition of compliance are described in detail in Section 7.4.

As can be seen, in the majority of cases the responses recorded were very similar in both the SEM and Dartec. There is no consistent difference in the responses measured as shown by Fig. 7.39,which is a graph of compliance in SEM versus compliance in the Dartec.

The slight difference which was observed in some cases is therefore attributed to experimental error. On the basis of these results it is therefore assumed that during these short term tests, lasting only 2 or 3 seconds, articular cartilage deforms in essentially the same manner whether immersed in saline or in a vacuum.

In the analysis of the main series of tests therefore no differentiation is made between specimens tested in the SEM and those tested immersed in saline. For those specimens which were tested under both conditions only the response immersed in saline is included in the analysis.

These tests are of course of short duration and as discussed in 7.2 it is unlikely that fluid flow is a major contribution to the deformation which occurs during the test. If the tests were of long duration, more than a few seconds, it would be expected that a much greater difference in response would be apparent. During long duration tests the major contribution to the deformation is fluid flow and the rate of fluid flow is dependent on both the magnitude of the applied load and on the osmotic pressure difference which exists between the matrix and the surrounding medium (Elmore et al 1963). In the case of long duration tests cartilage tested under the vacuum would therefore be likely to appear much less stiff, i.e. it would exhibit greater deformation for the same applied load, than the same specimen tested in saline.

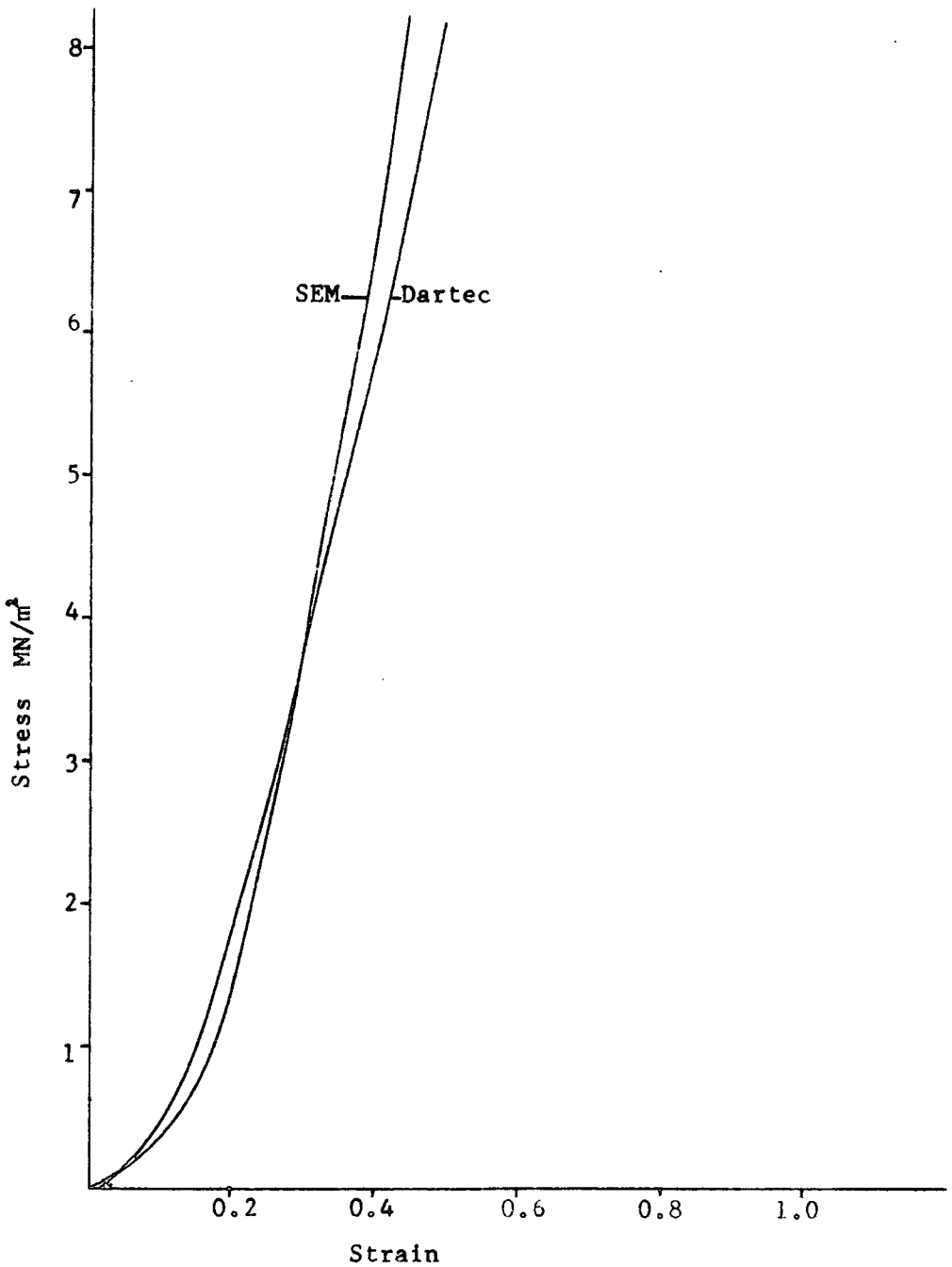


Fig7.18 Stress—strain response in SEM and Dartec.

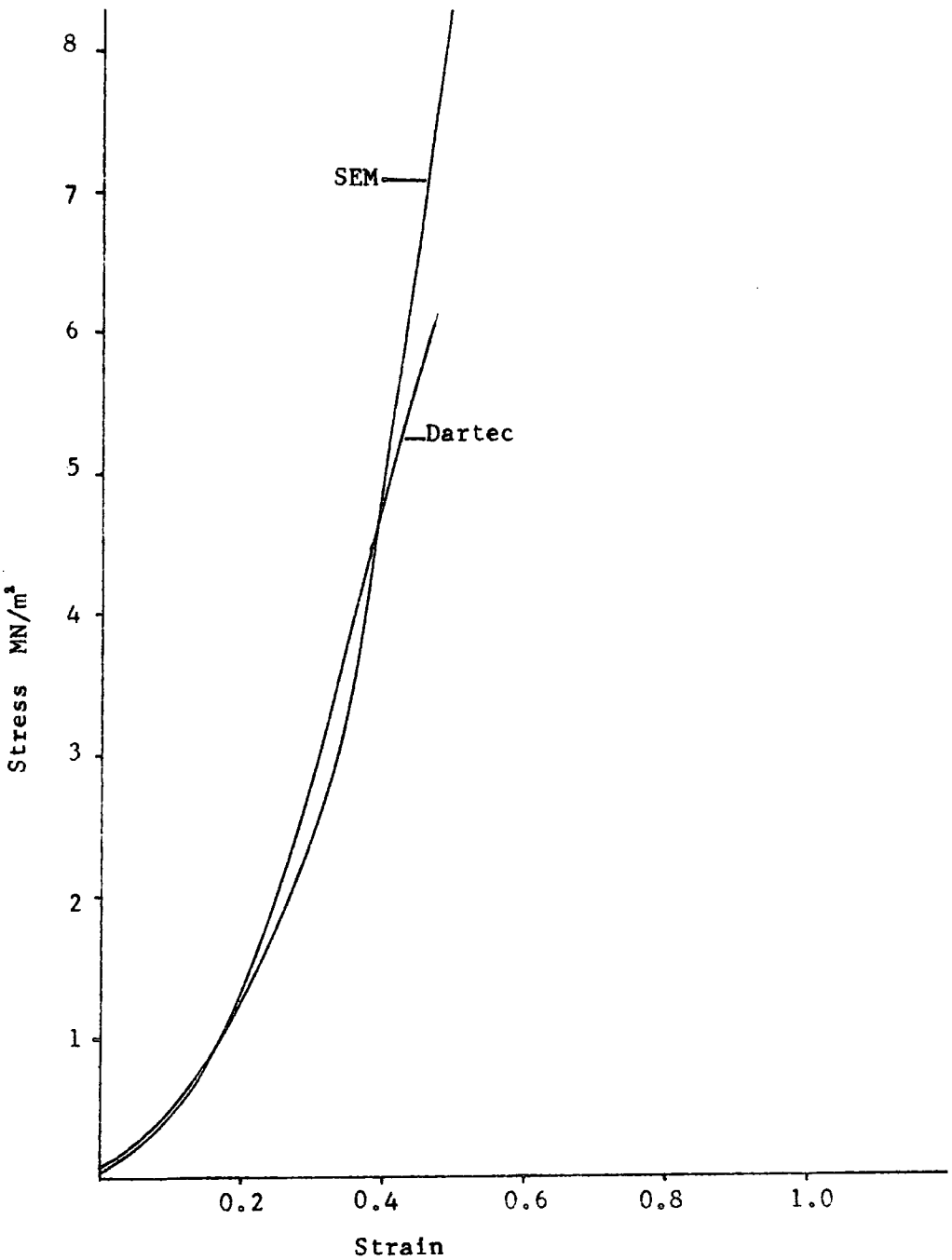


Fig7.19 Stress—strain response in SEM and Dartec.

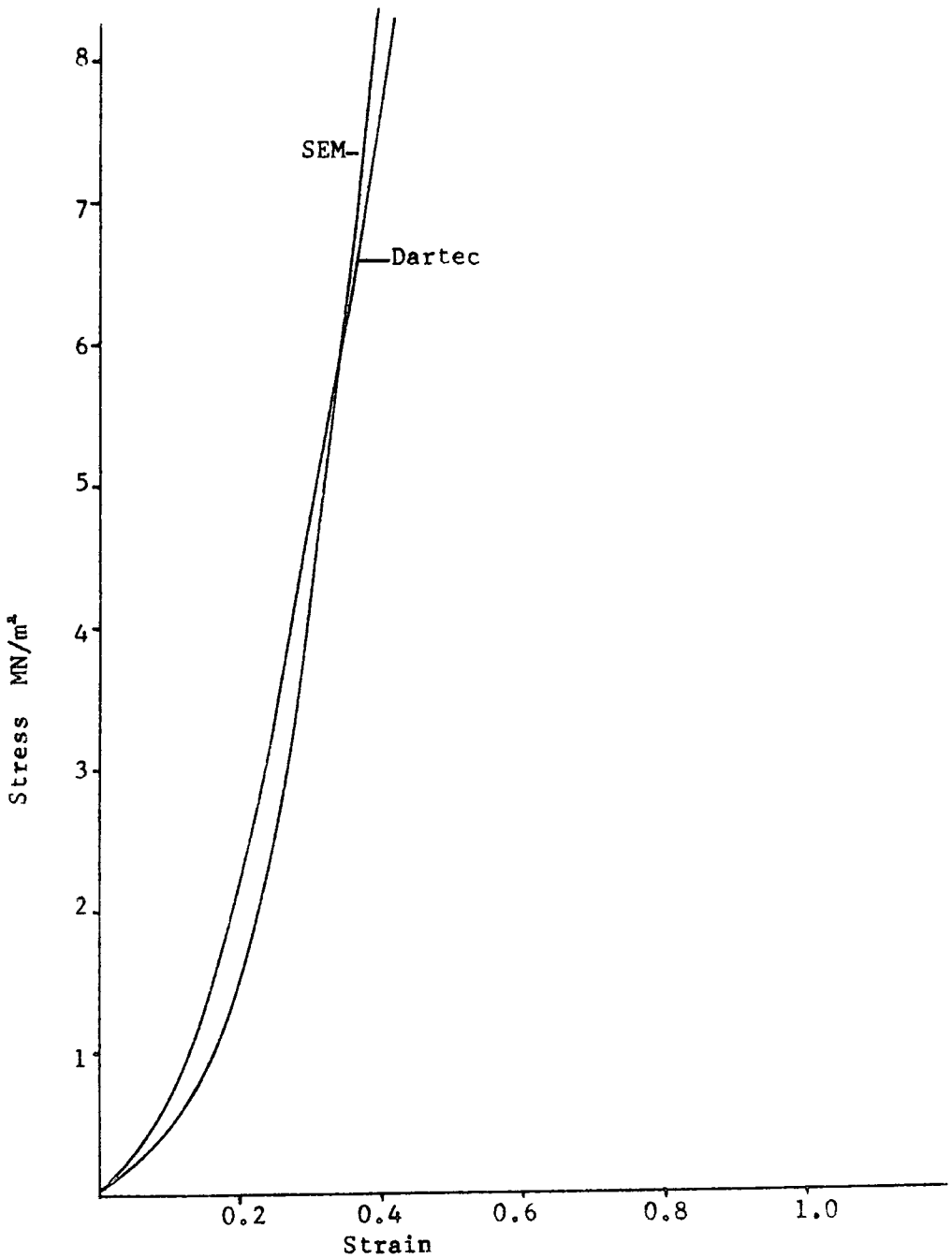


Fig7.20 Stress—strain response in SEM and Dartec.

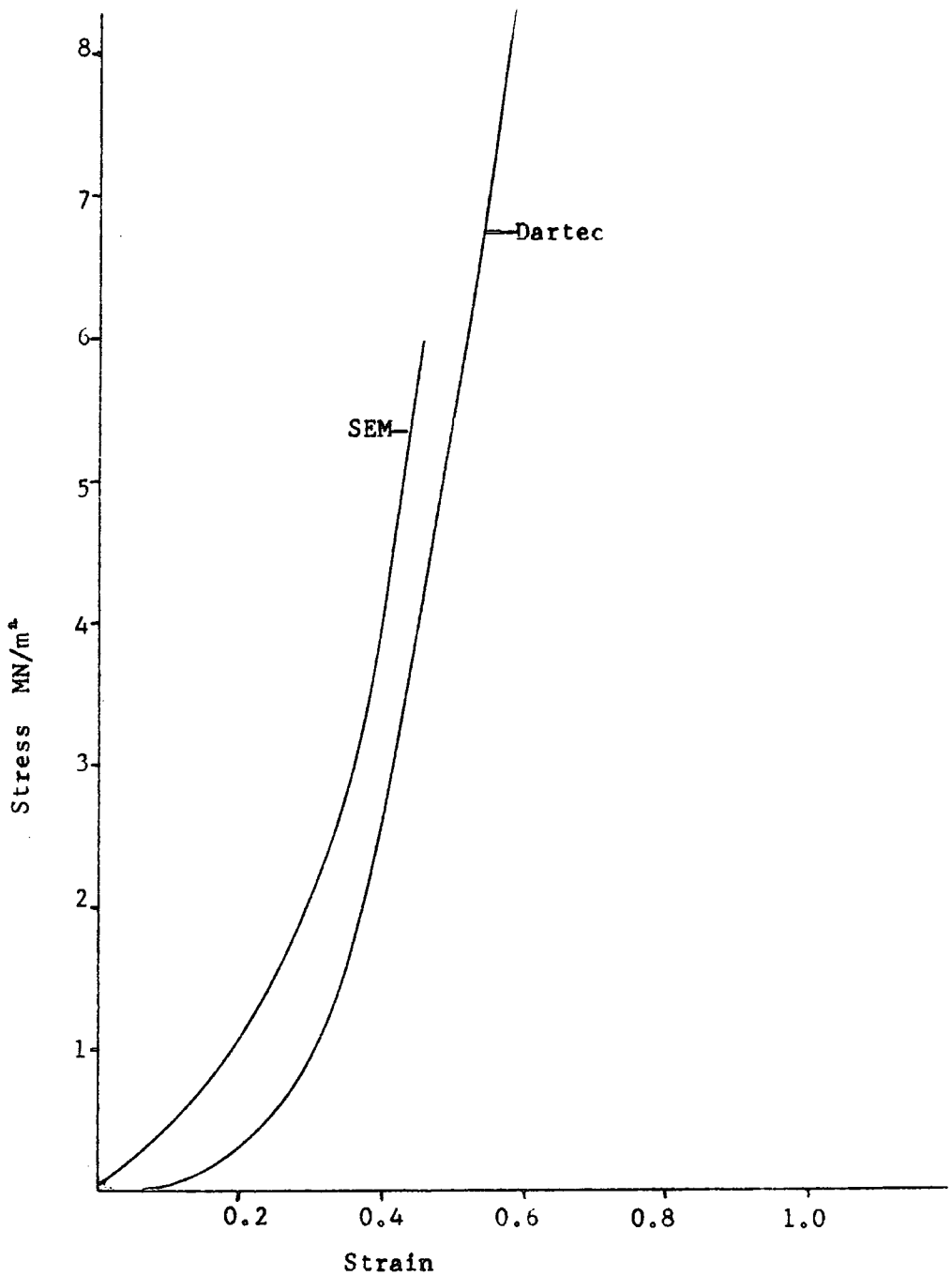


Fig7.21 Stress—strain response in SEM and Dartec.

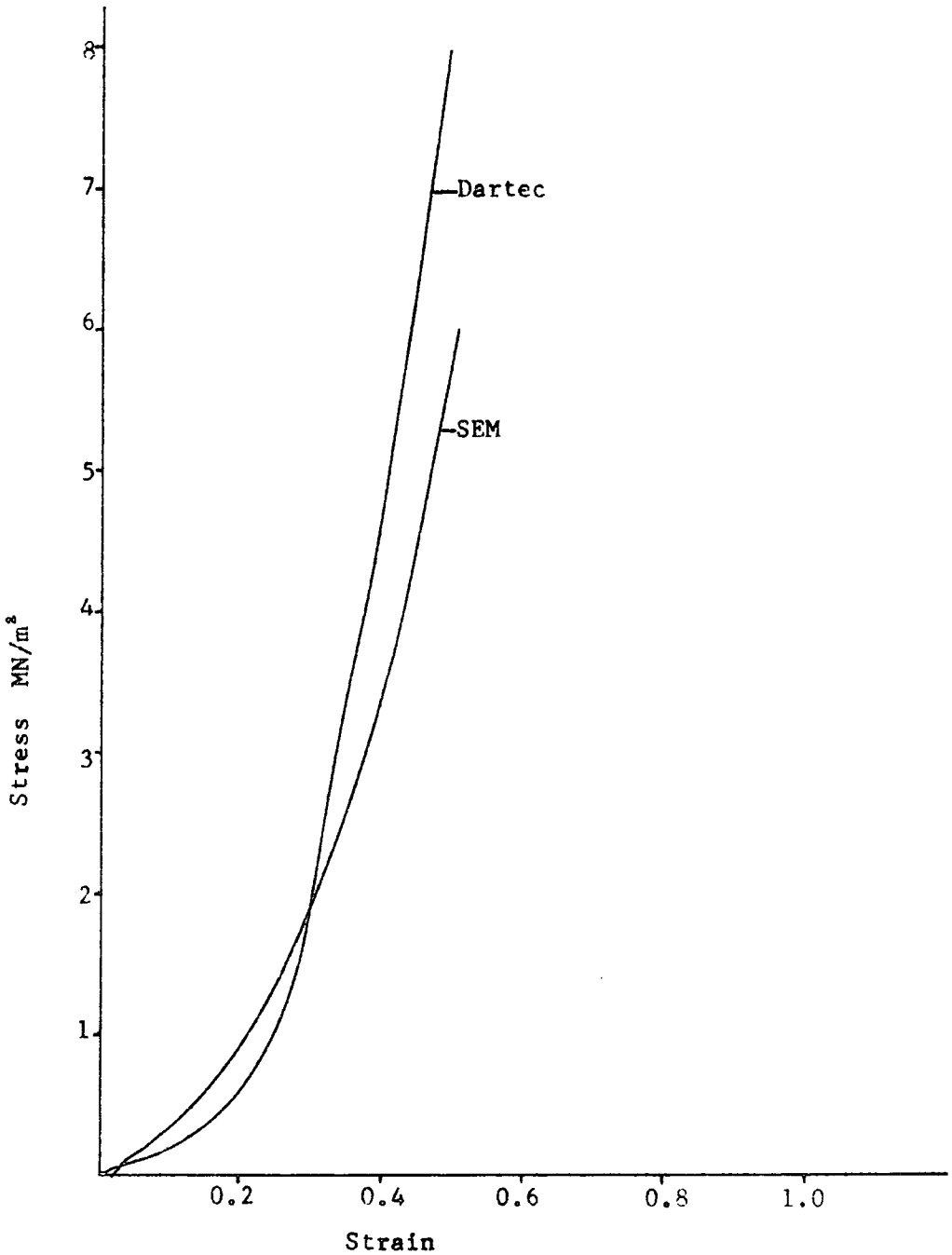


Fig7.22 Stress—strain response in SEM and Dartec.

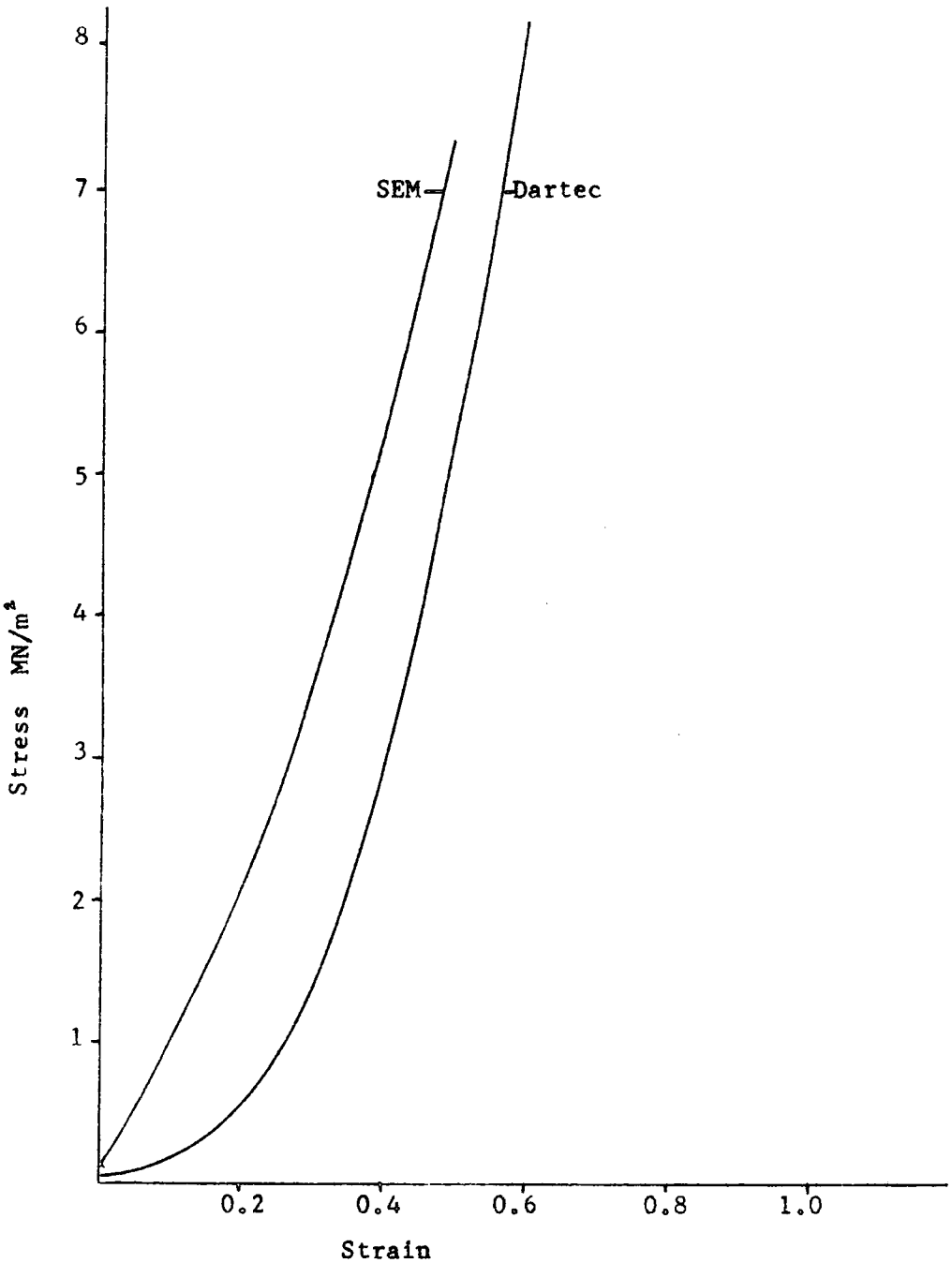


Fig7.23 Stress—strain response in SEM and Dartec.

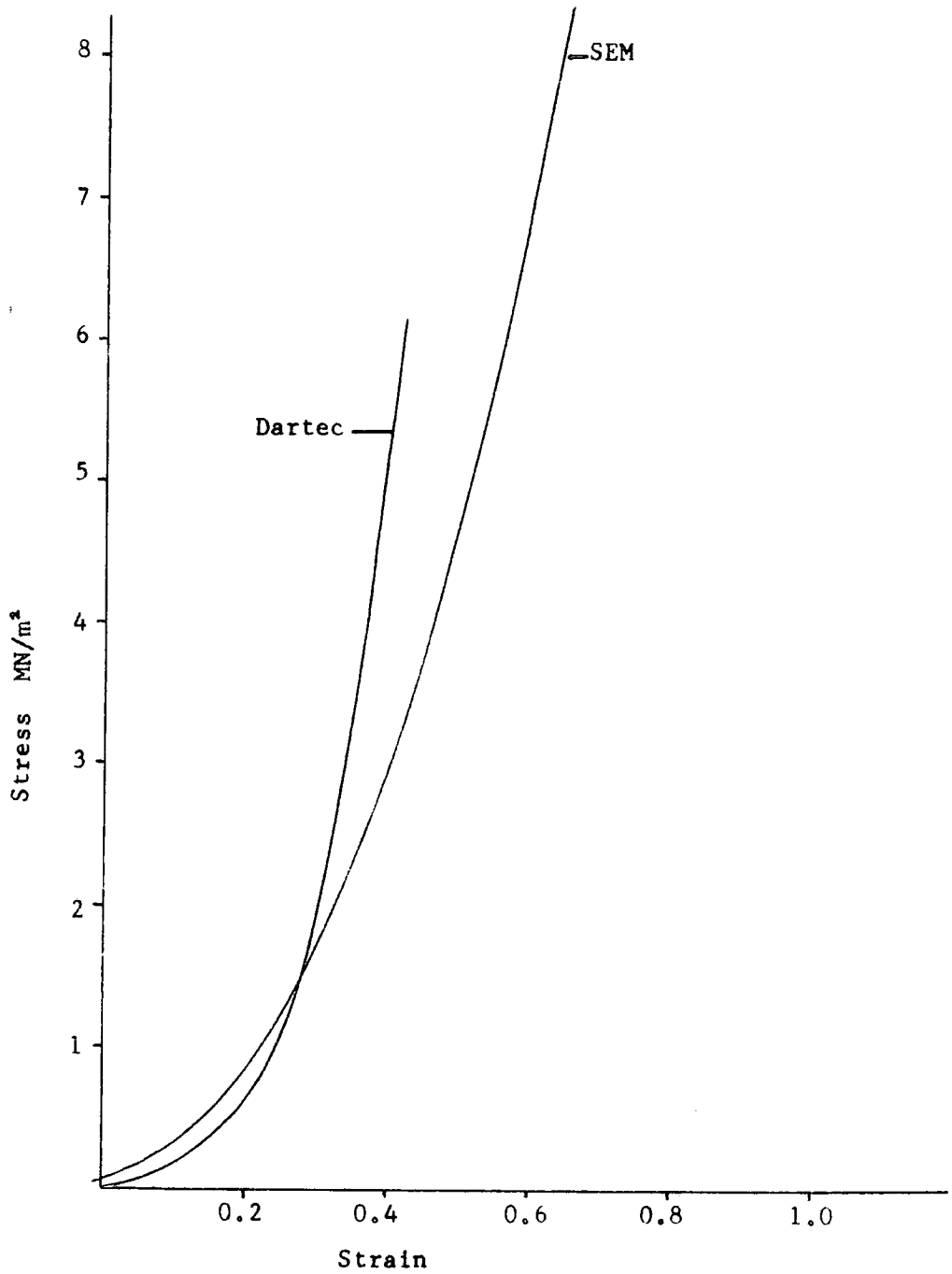


Fig7.24 Stress—strain response in SEM and Dartec.

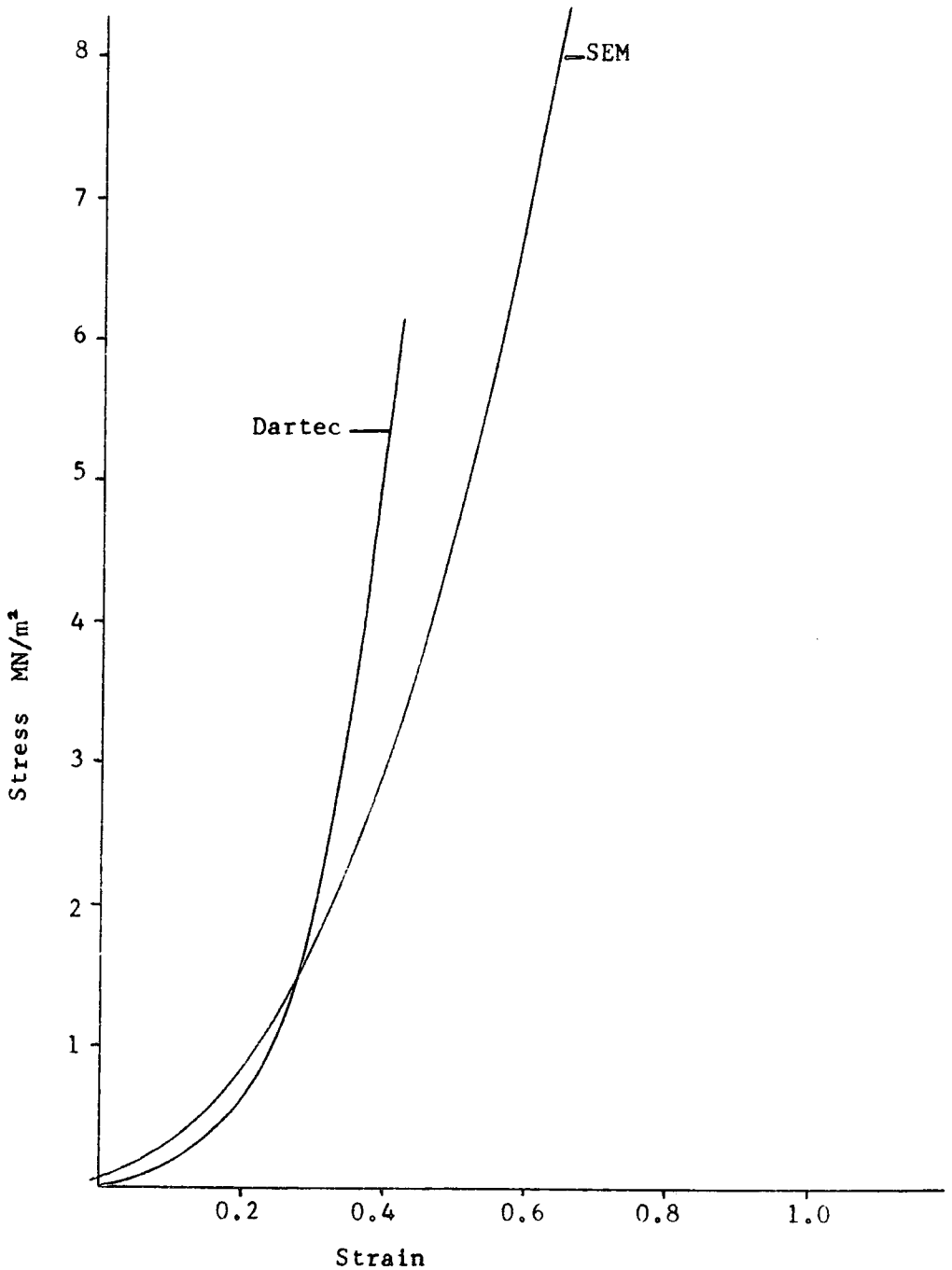


Fig7.24 Stress—strain response in SEM and Dartec.

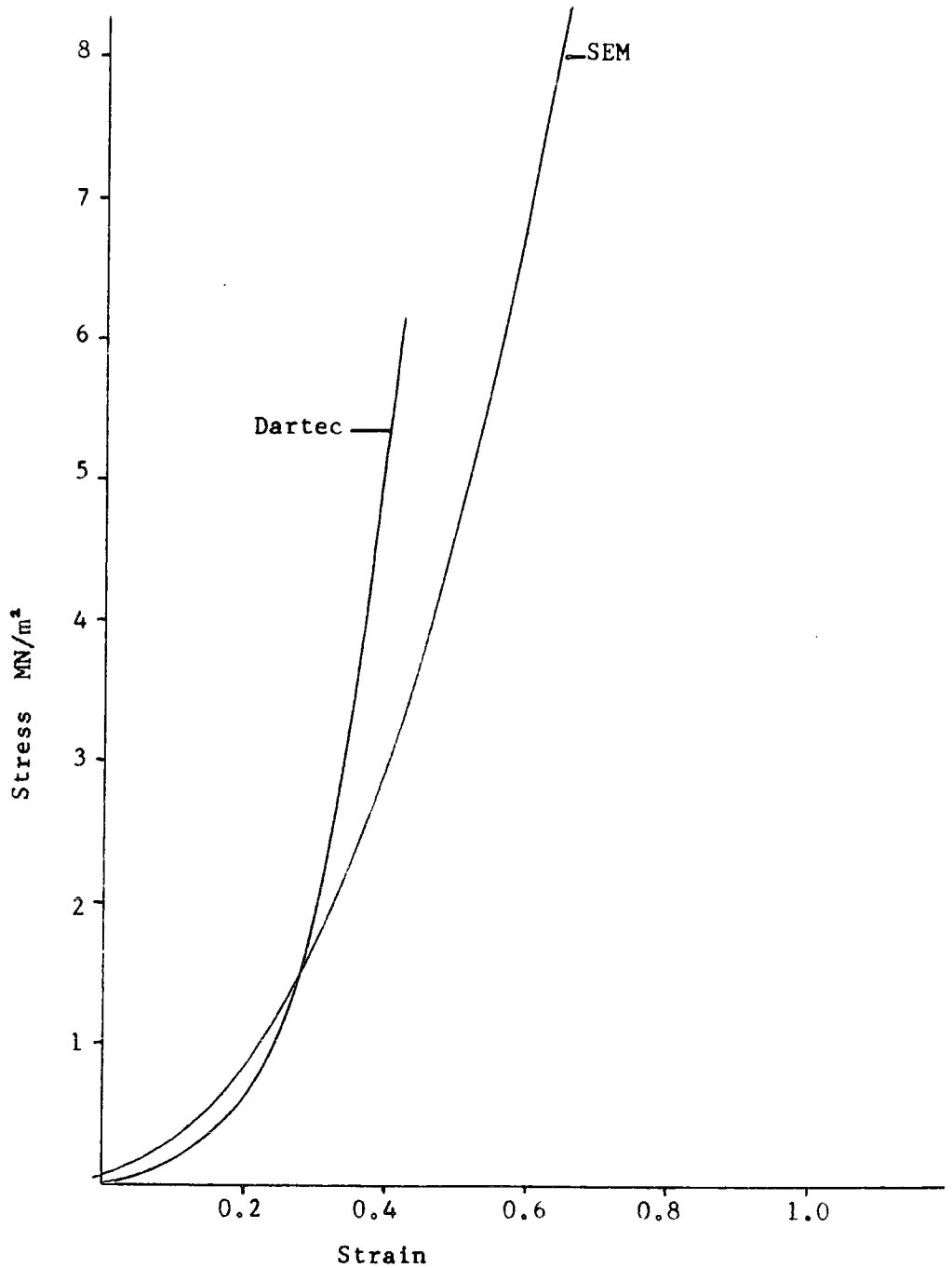


Fig7.24 Stress—strain response in SEM and Dartec.

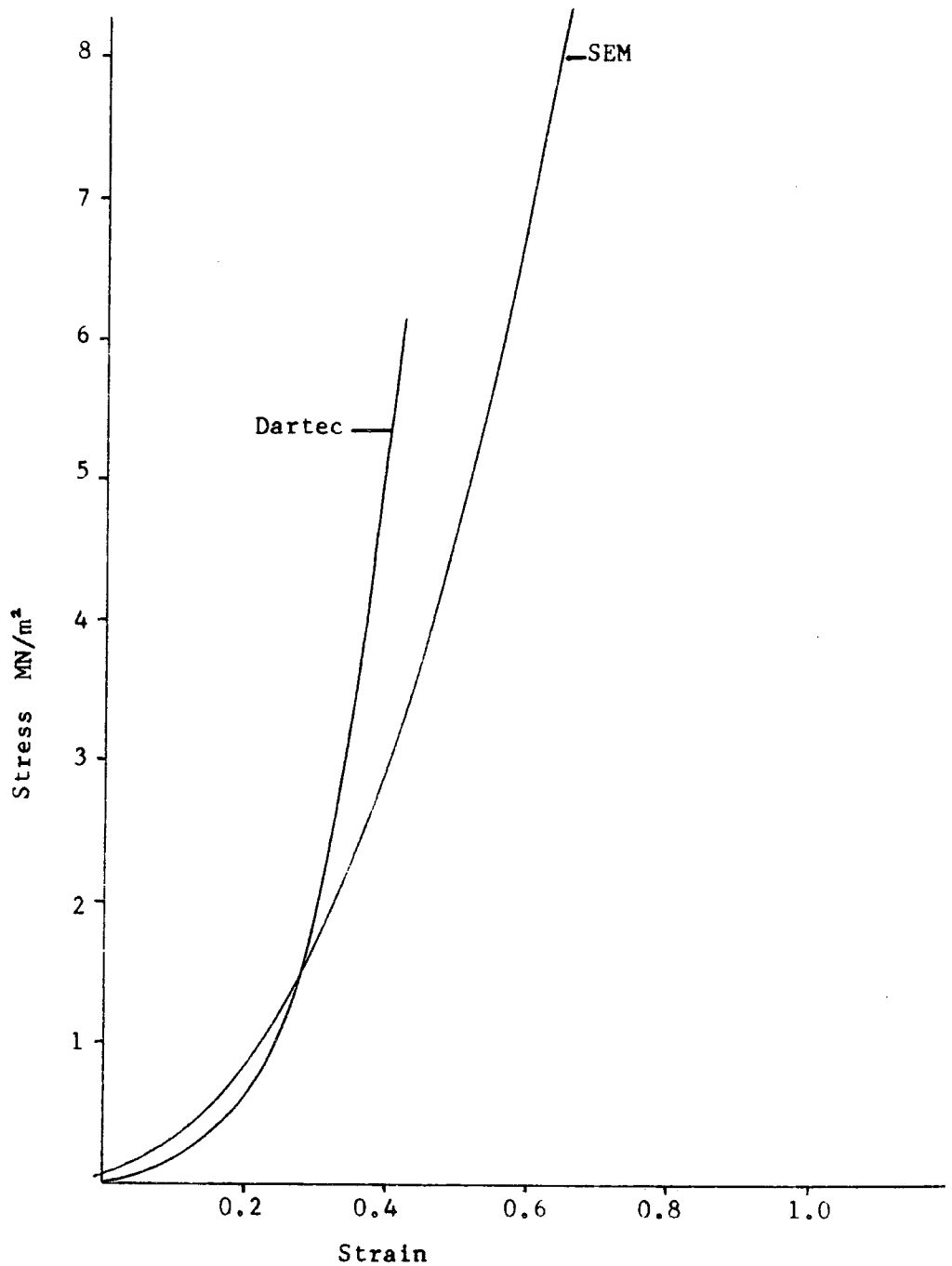


Fig7.24 Stress—strain response in SEM and Dartec.

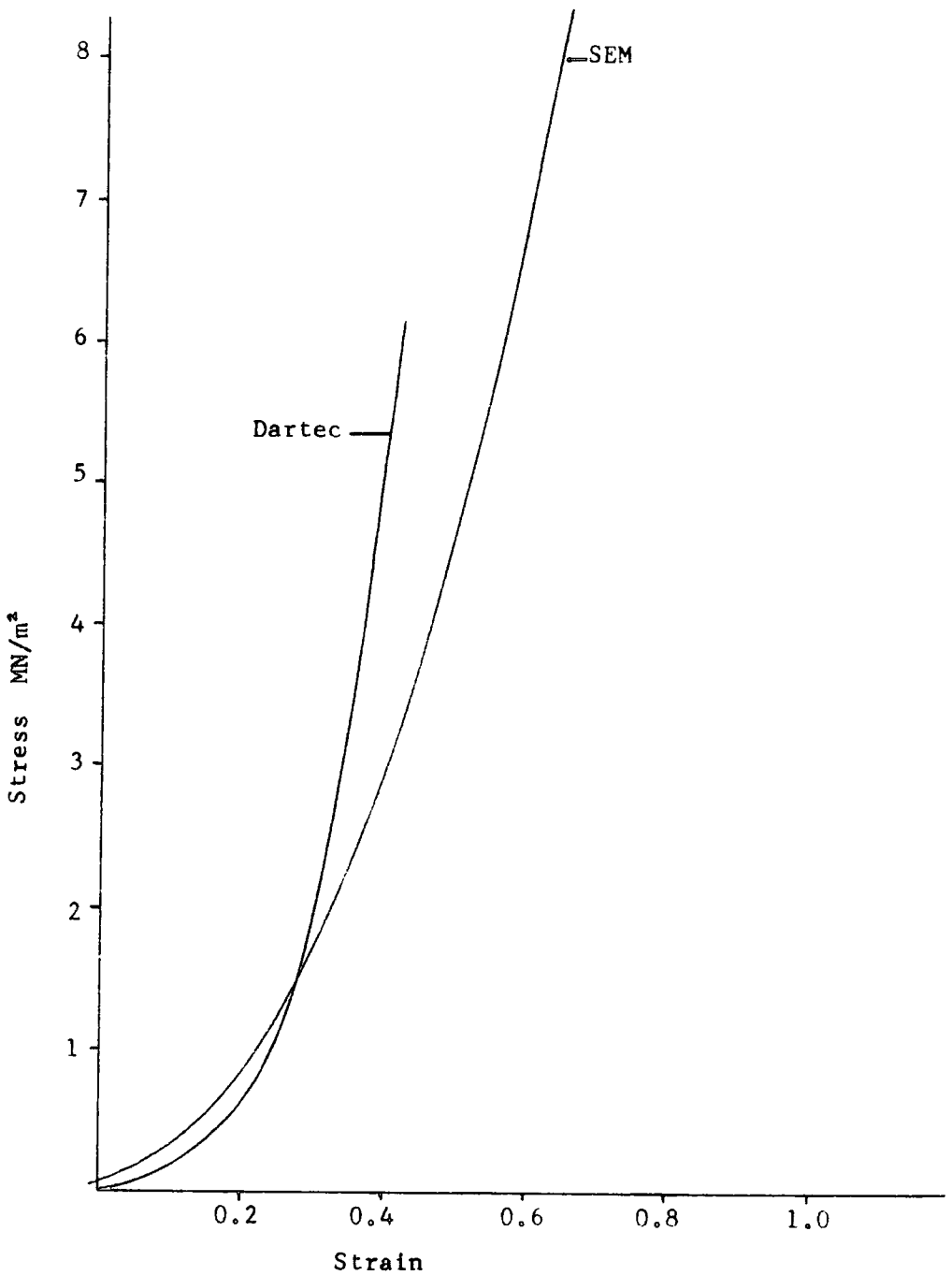


Fig7.24 Stress—strain response in SEM and Dartec.

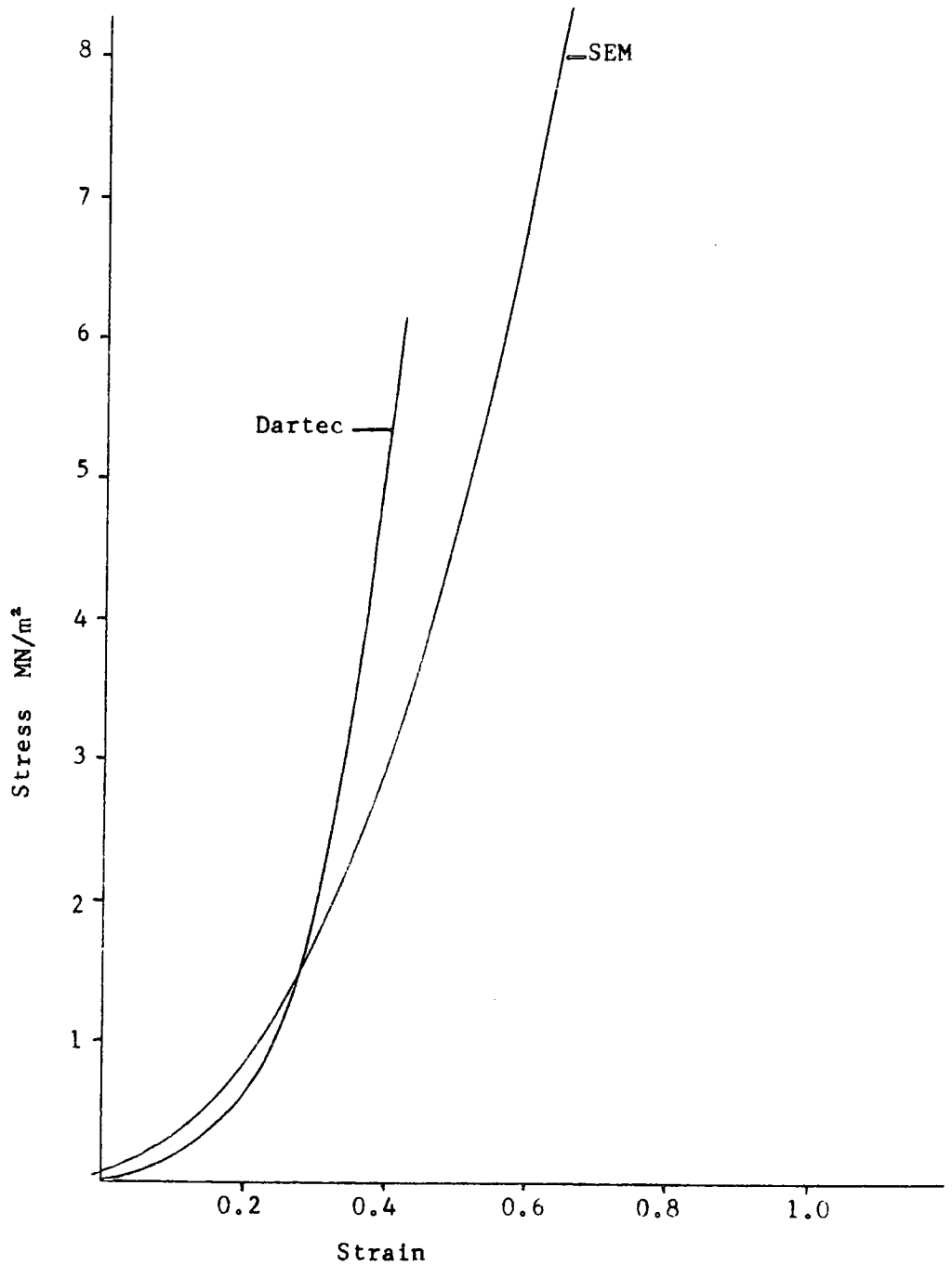


Fig7.24 Stress—strain response in SEM and Dartec.

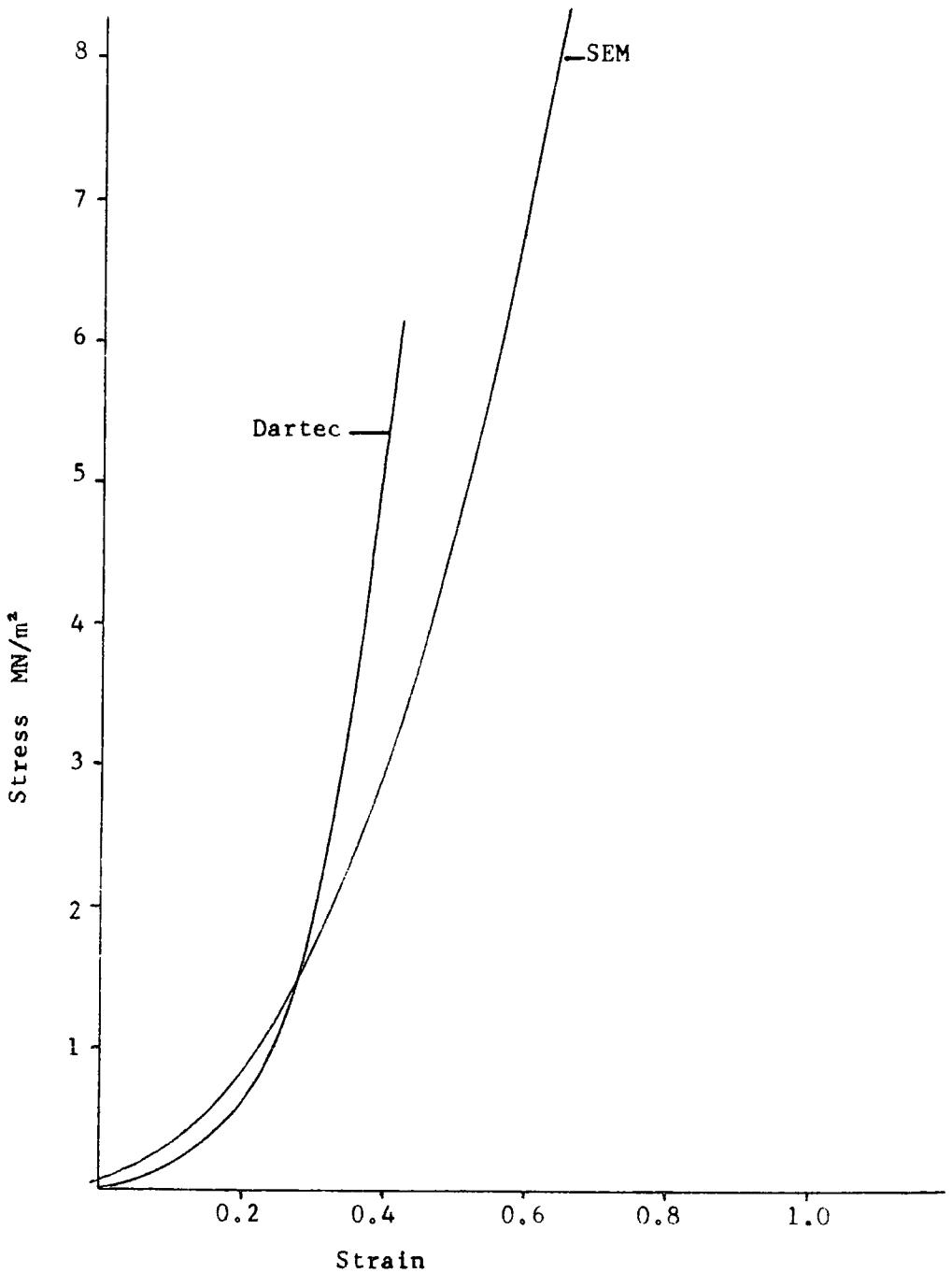


Fig7.24 Stress—strain response in SEM and Dartec.

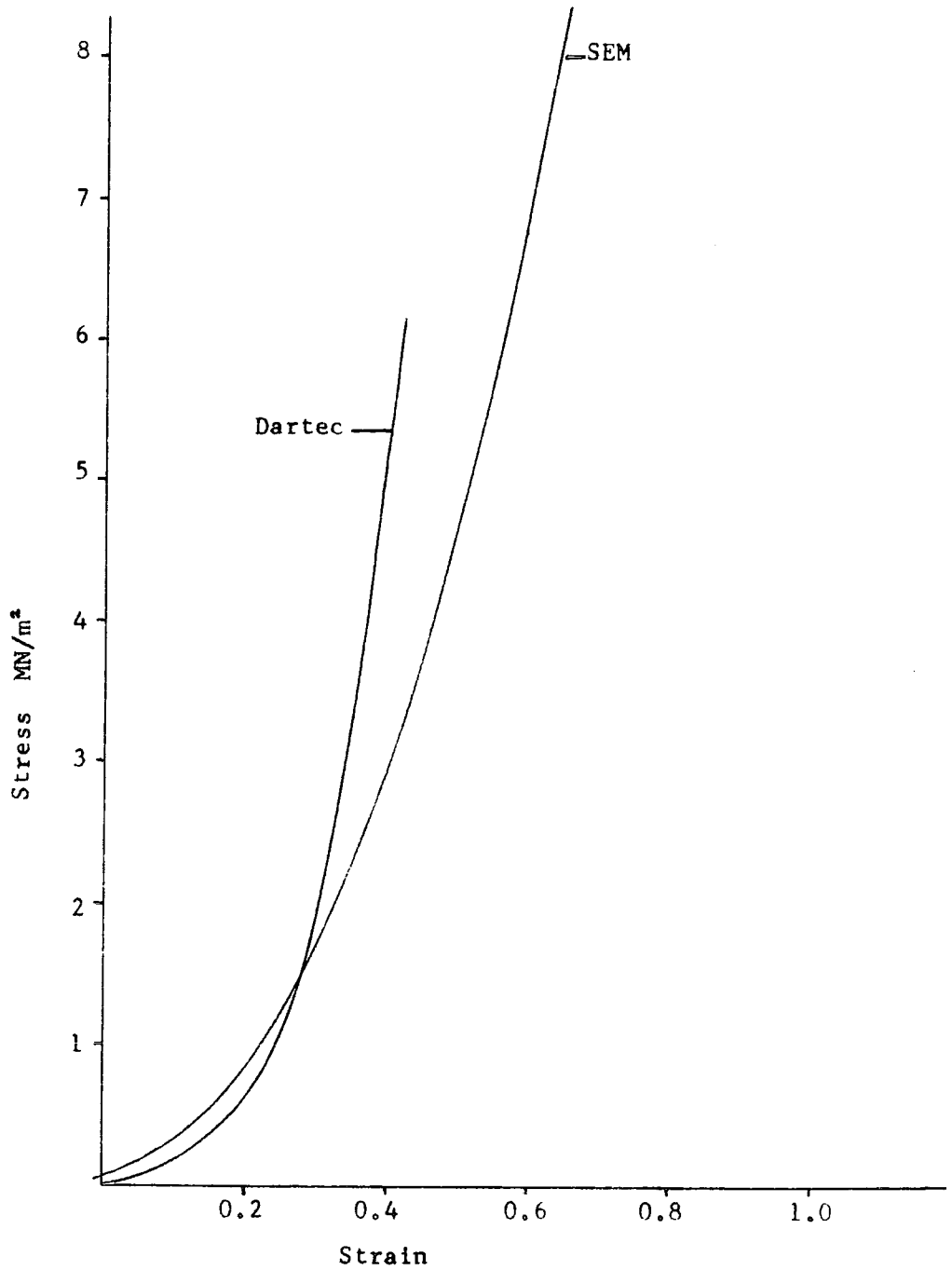


Fig7.24 Stress—strain response in SEM and Dartec.

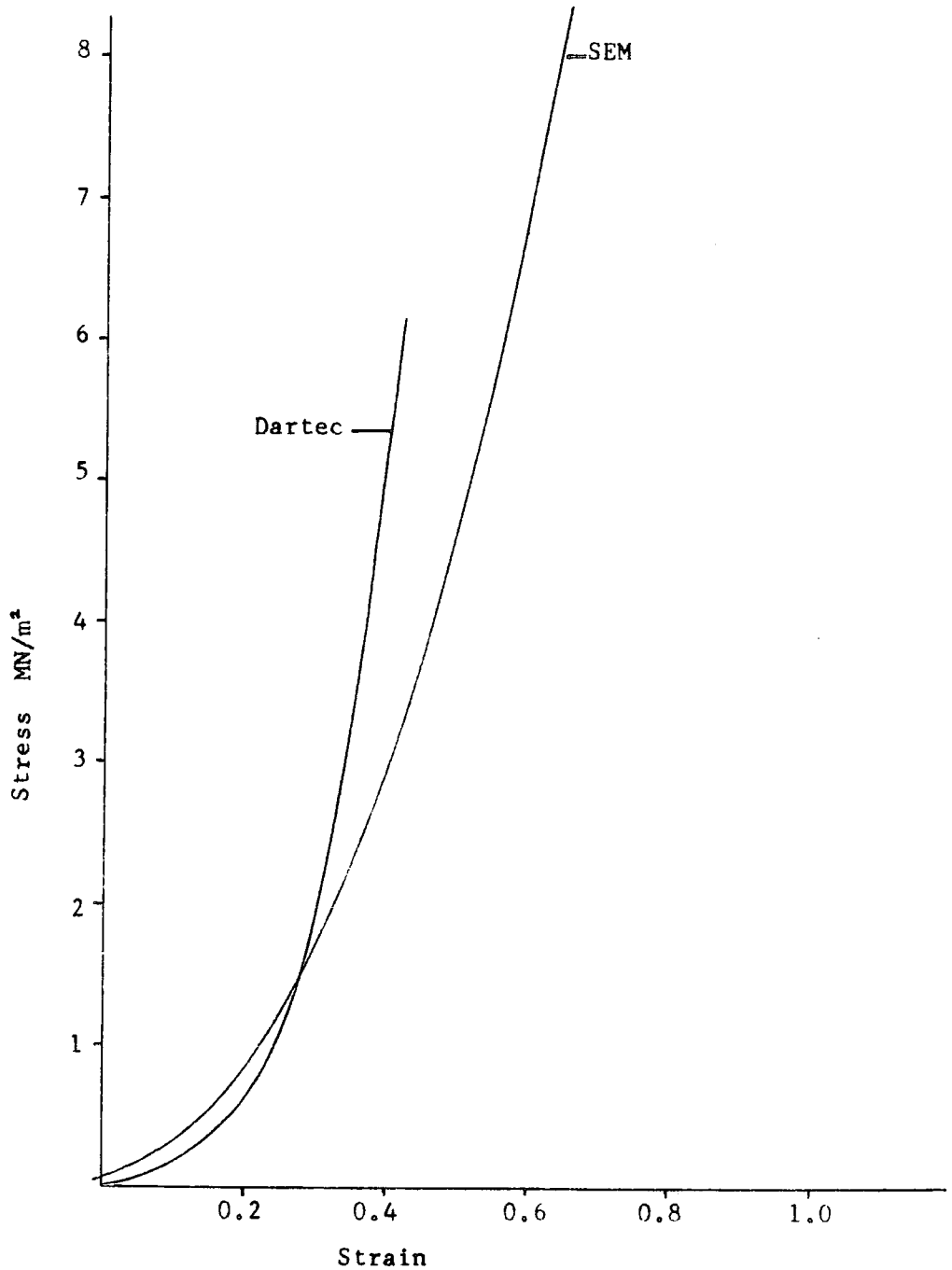


Fig7.24 Stress—strain response in SEM and Dartec.

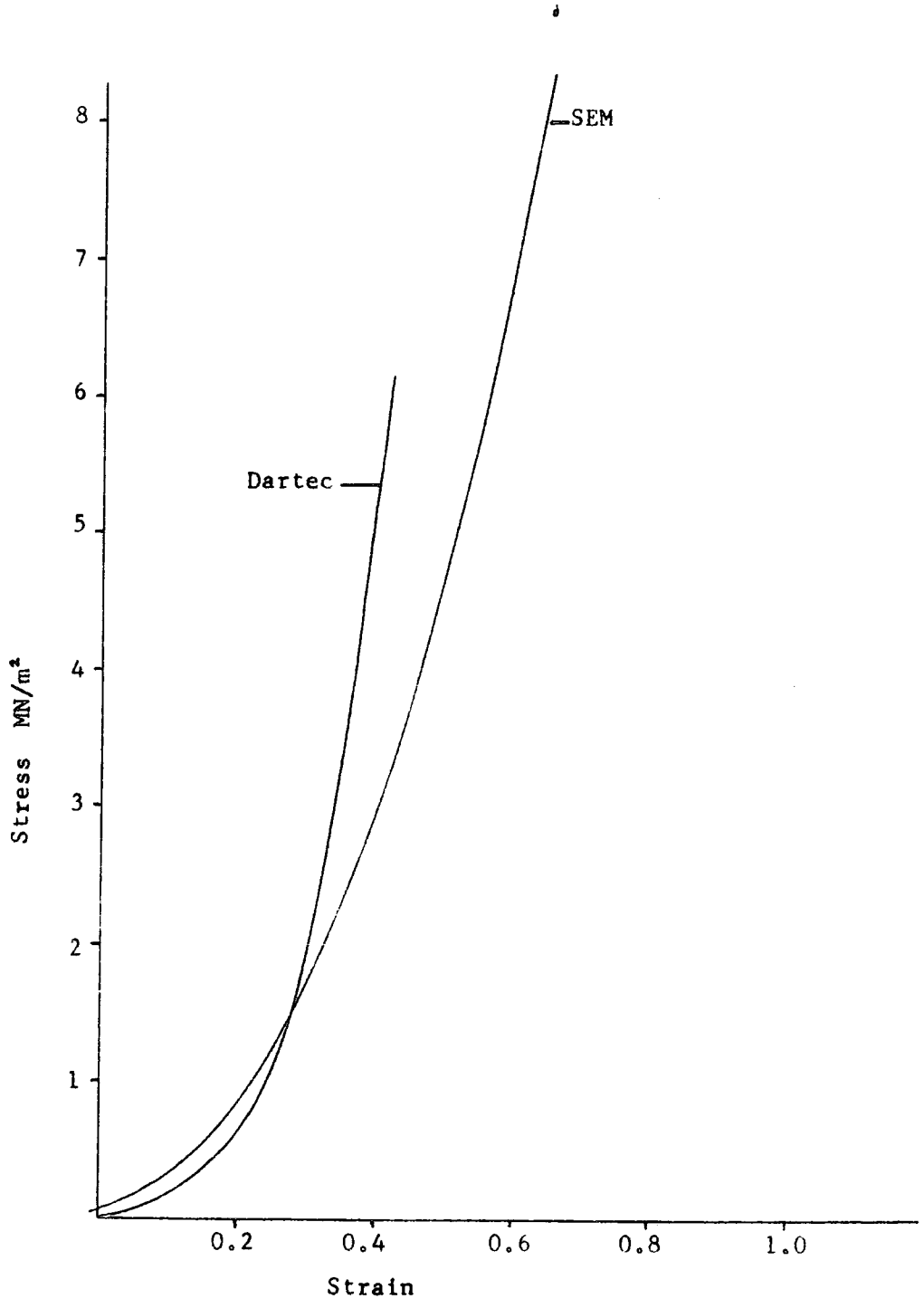


Fig7.24 Stress—strain response in SEM and Dartec.

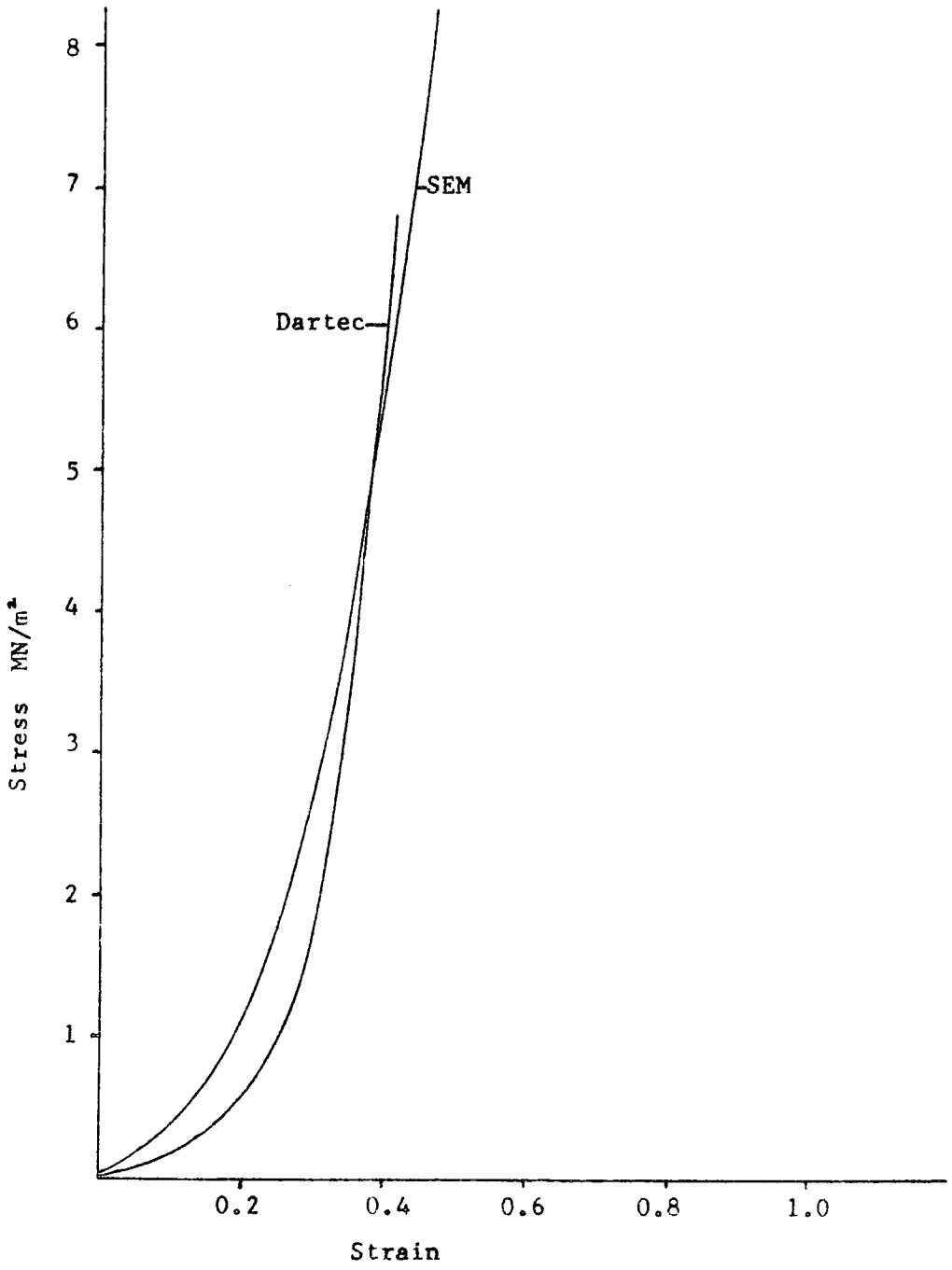


Fig7.25 Stress—strain response in SEM and Dartec.

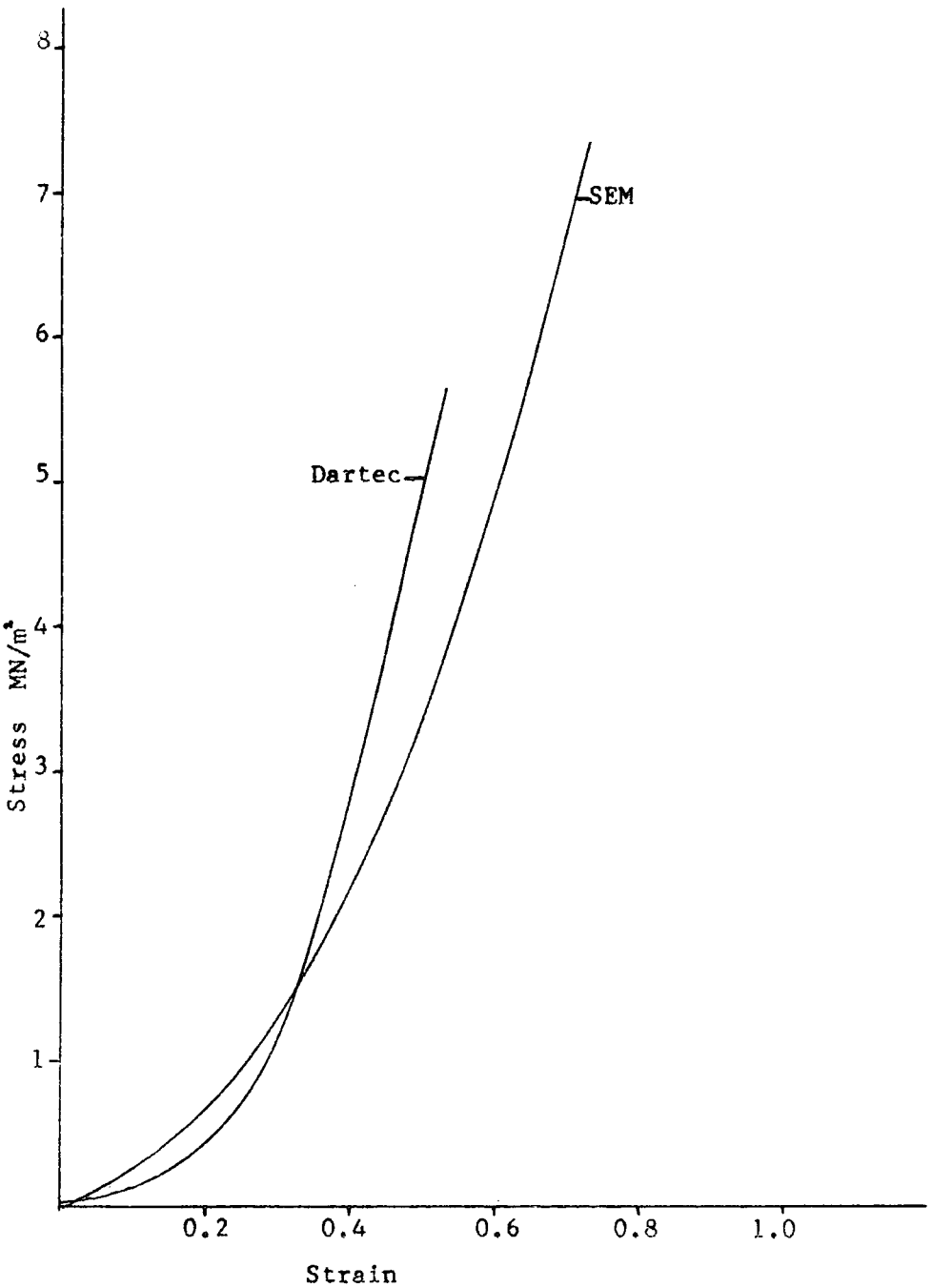


Fig7.26 Stress— strain response in SEM and Dartec.

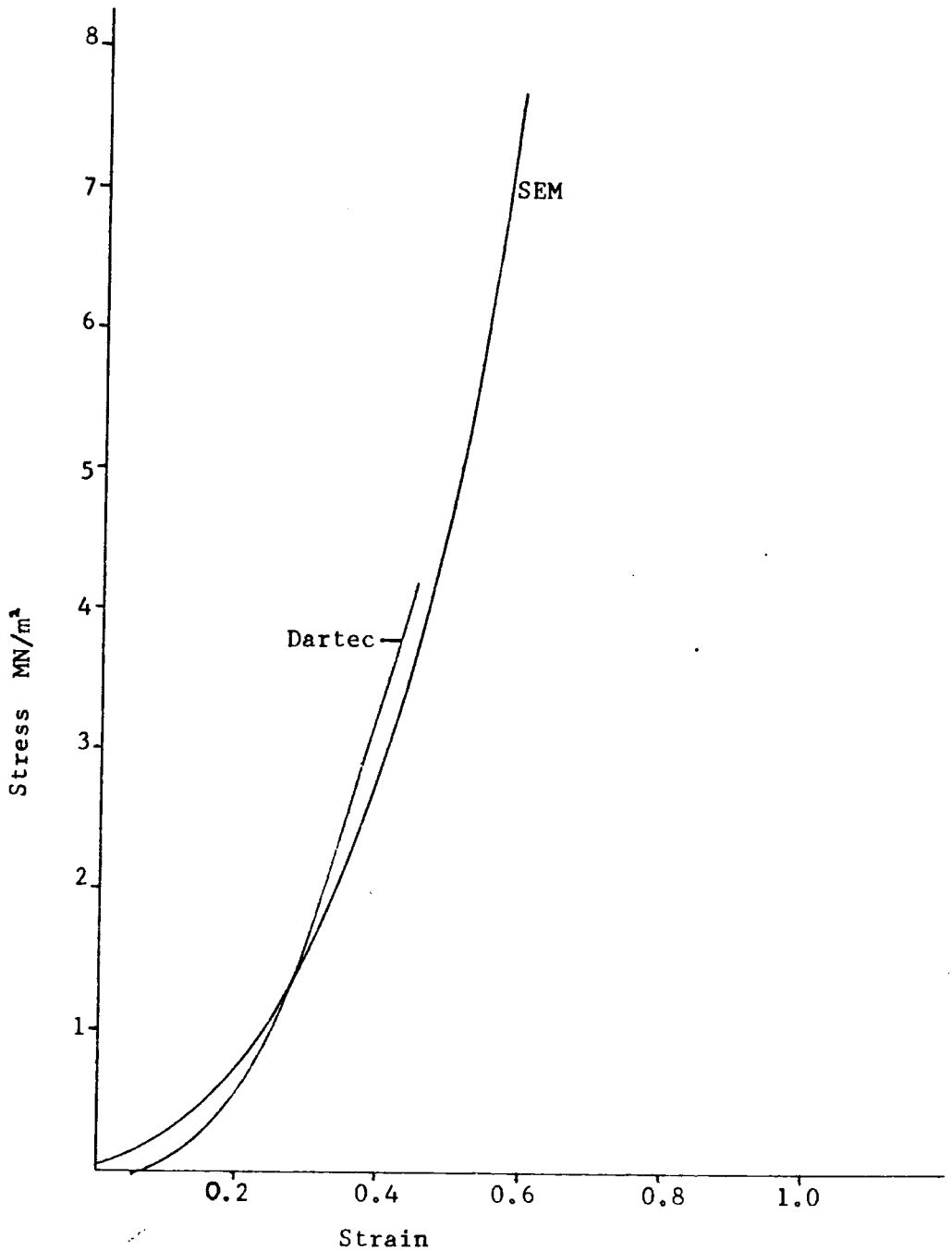


Fig7.27 Stress—strain response in SEM and Dartec.

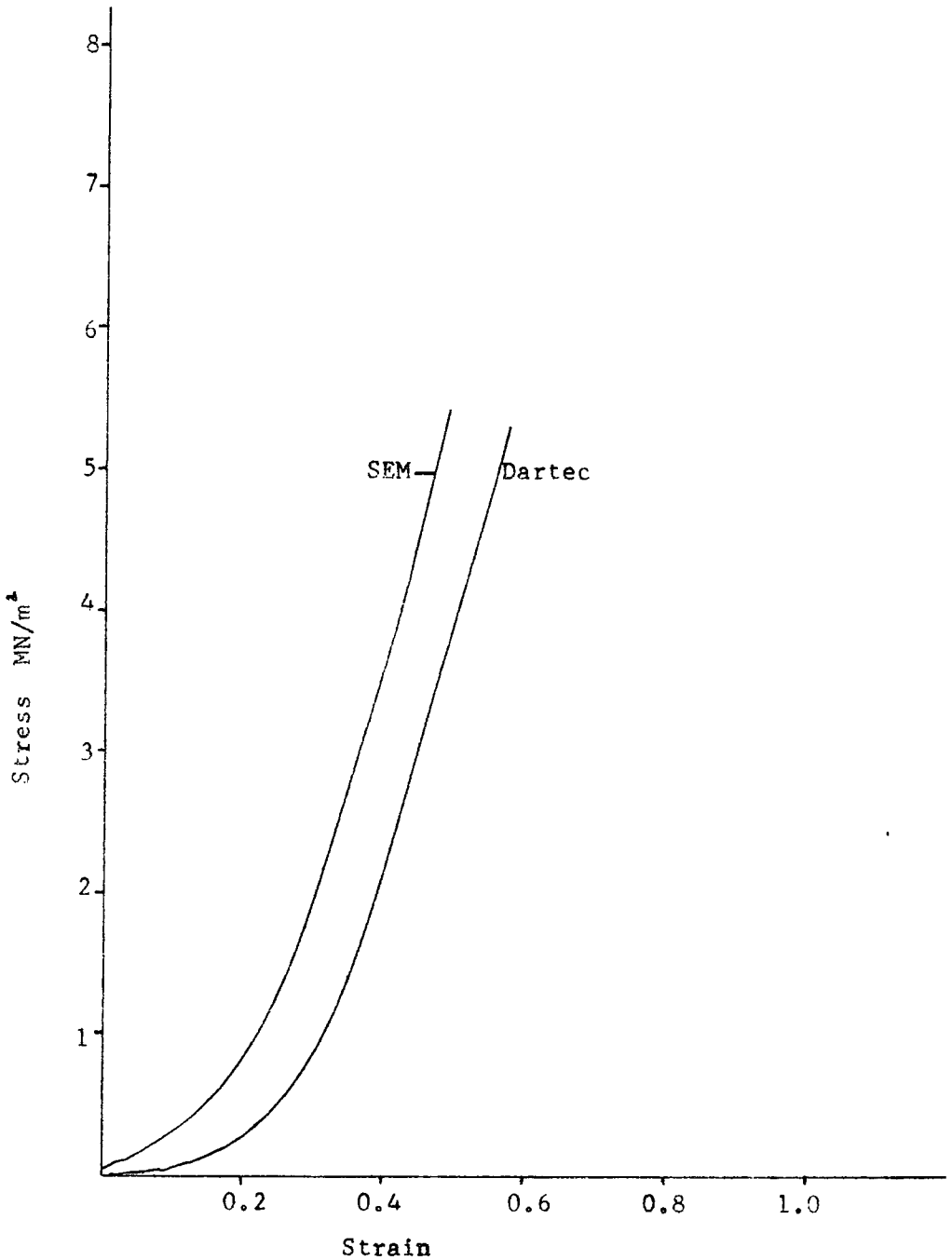


Fig7.28 Stress— strain response in SEM and Dartec.

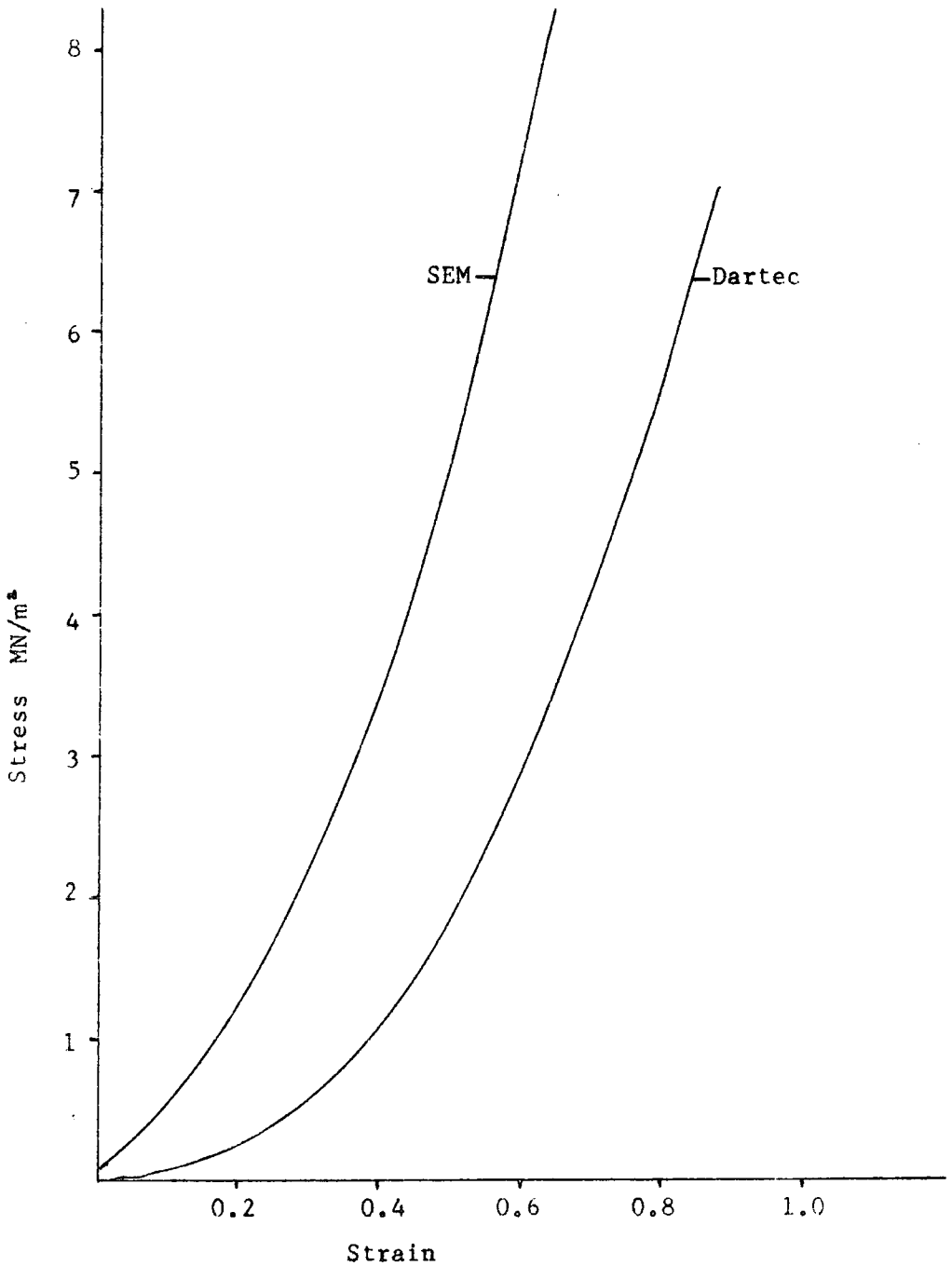


Fig7.29 Stress—strain response in SEM and Dartec.

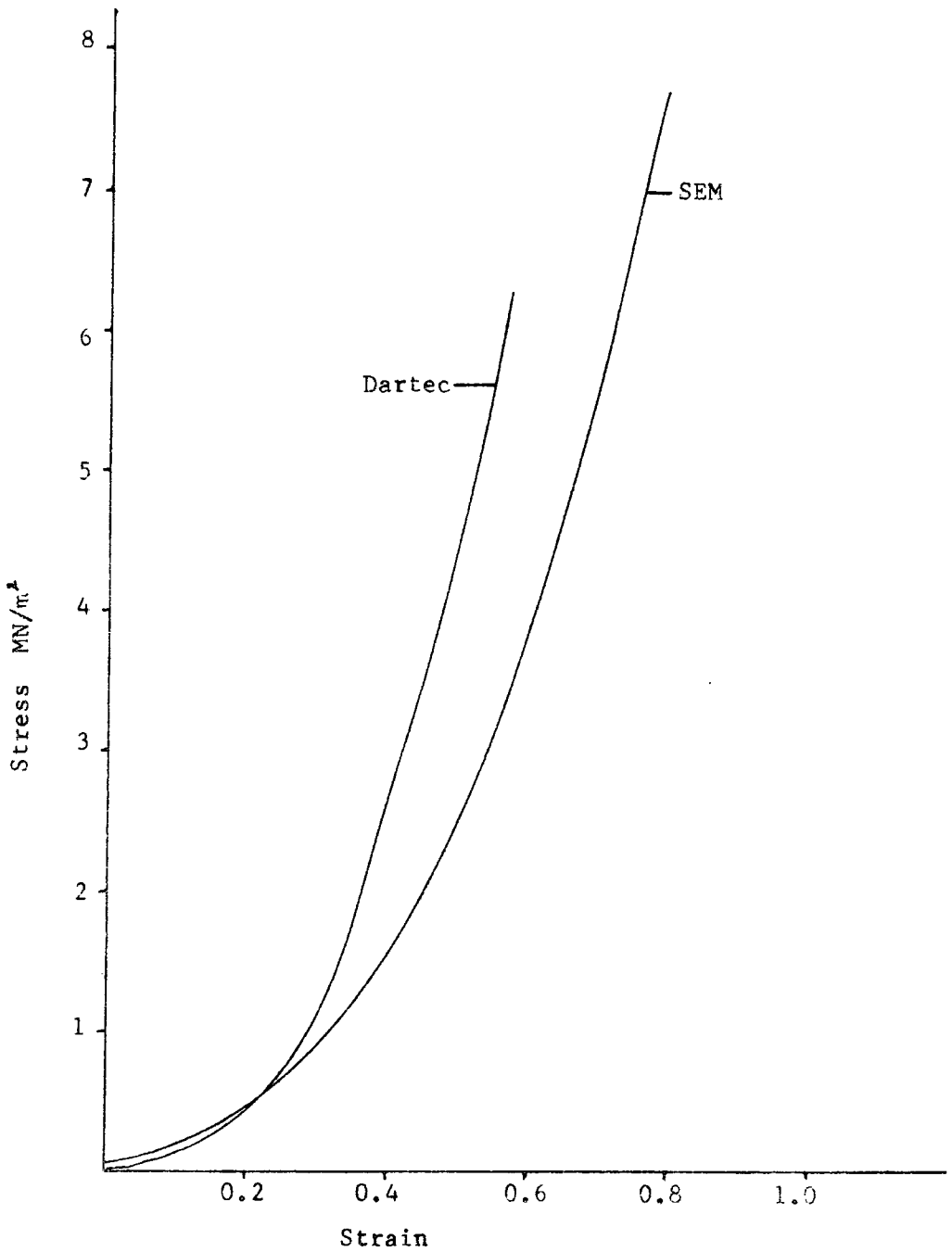


Fig7.30 Stress—strain response in SEM and Dartec.

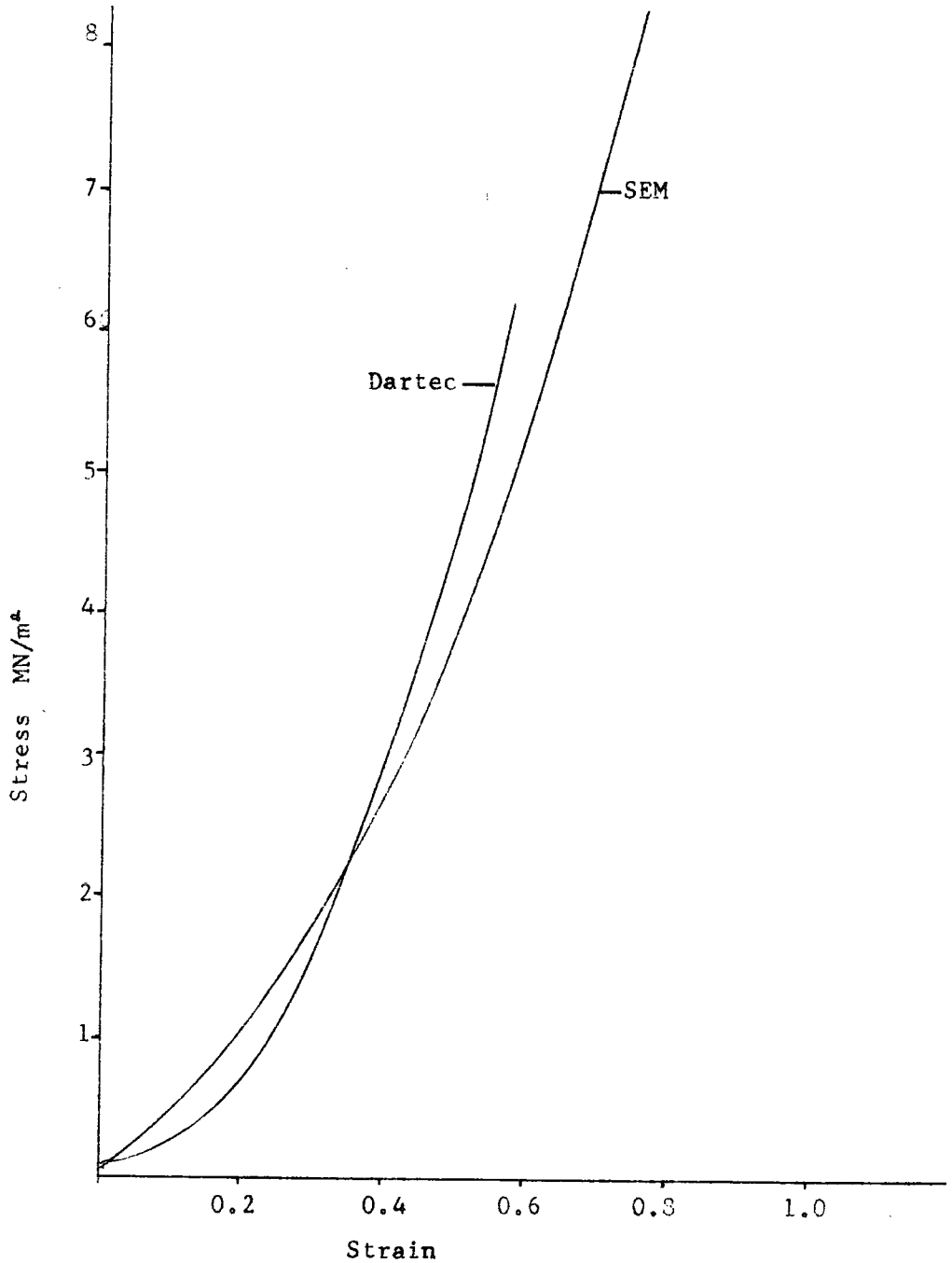


Fig7.31 Stress—strain response in SEM and Dartec.

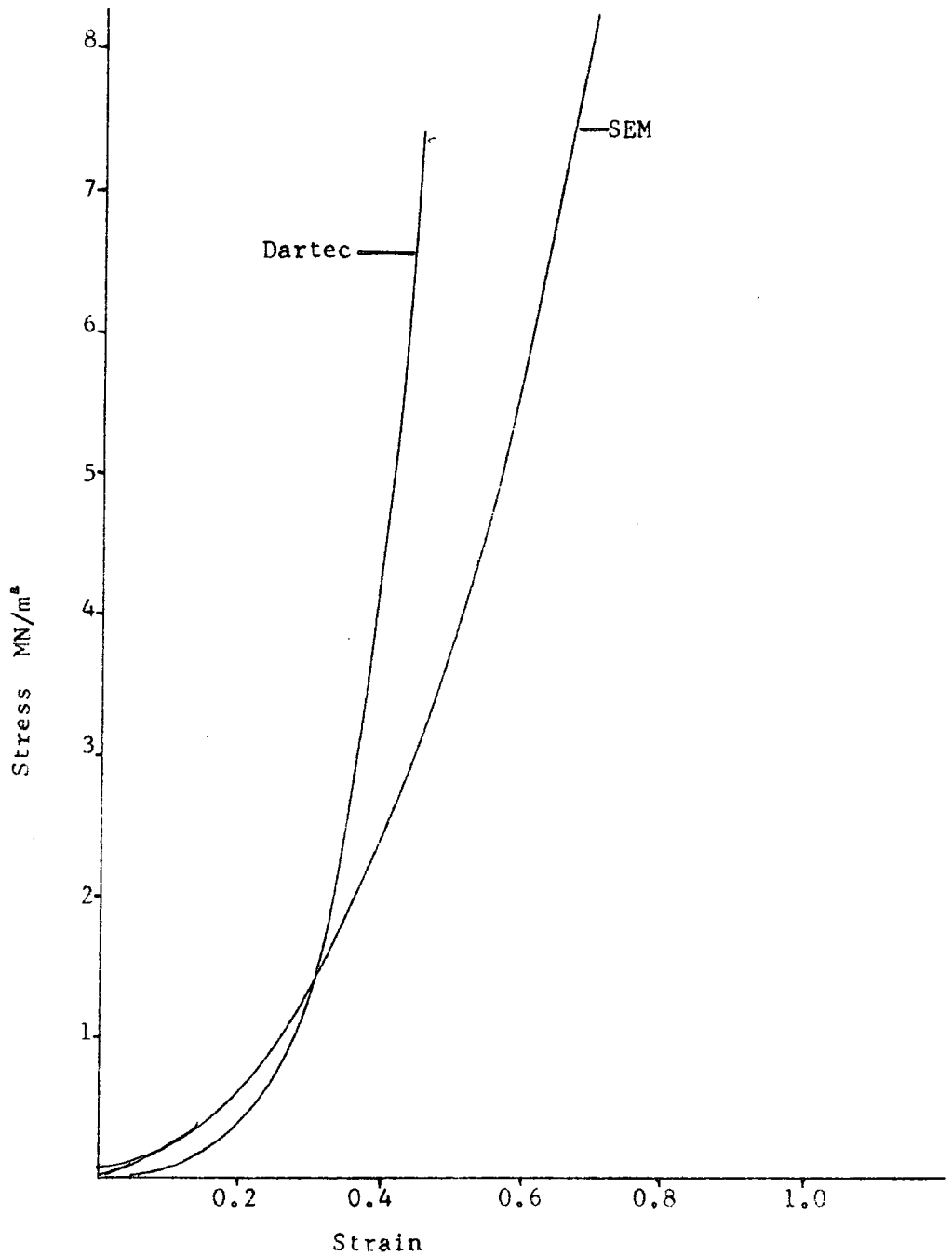


Fig7.32 Stress—strain response in SEM and Dartec.

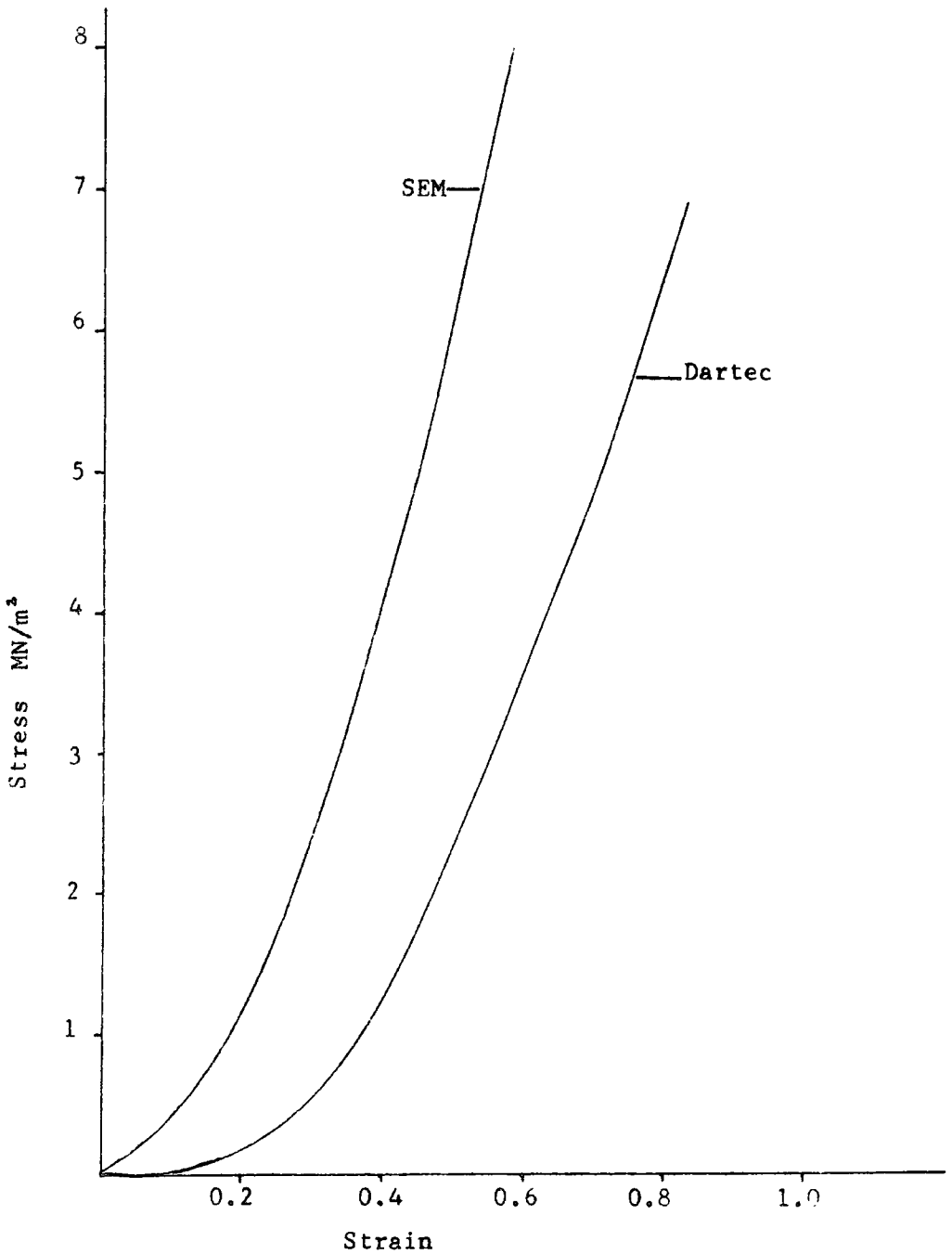


Fig7.33 Stress—strain response in SEM and Dartec.

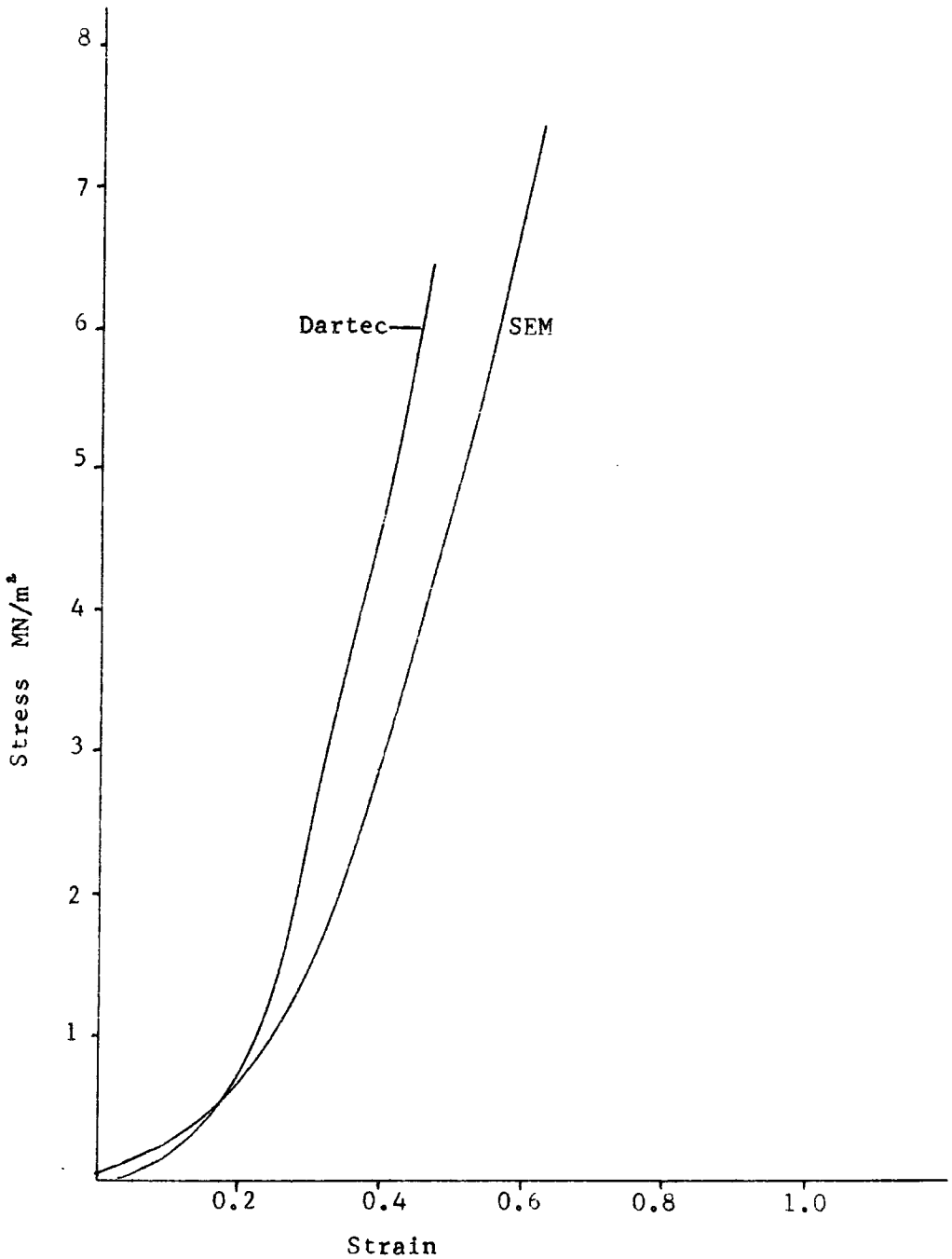


Fig7.34 Stress—strain response in SEM and Dartec.

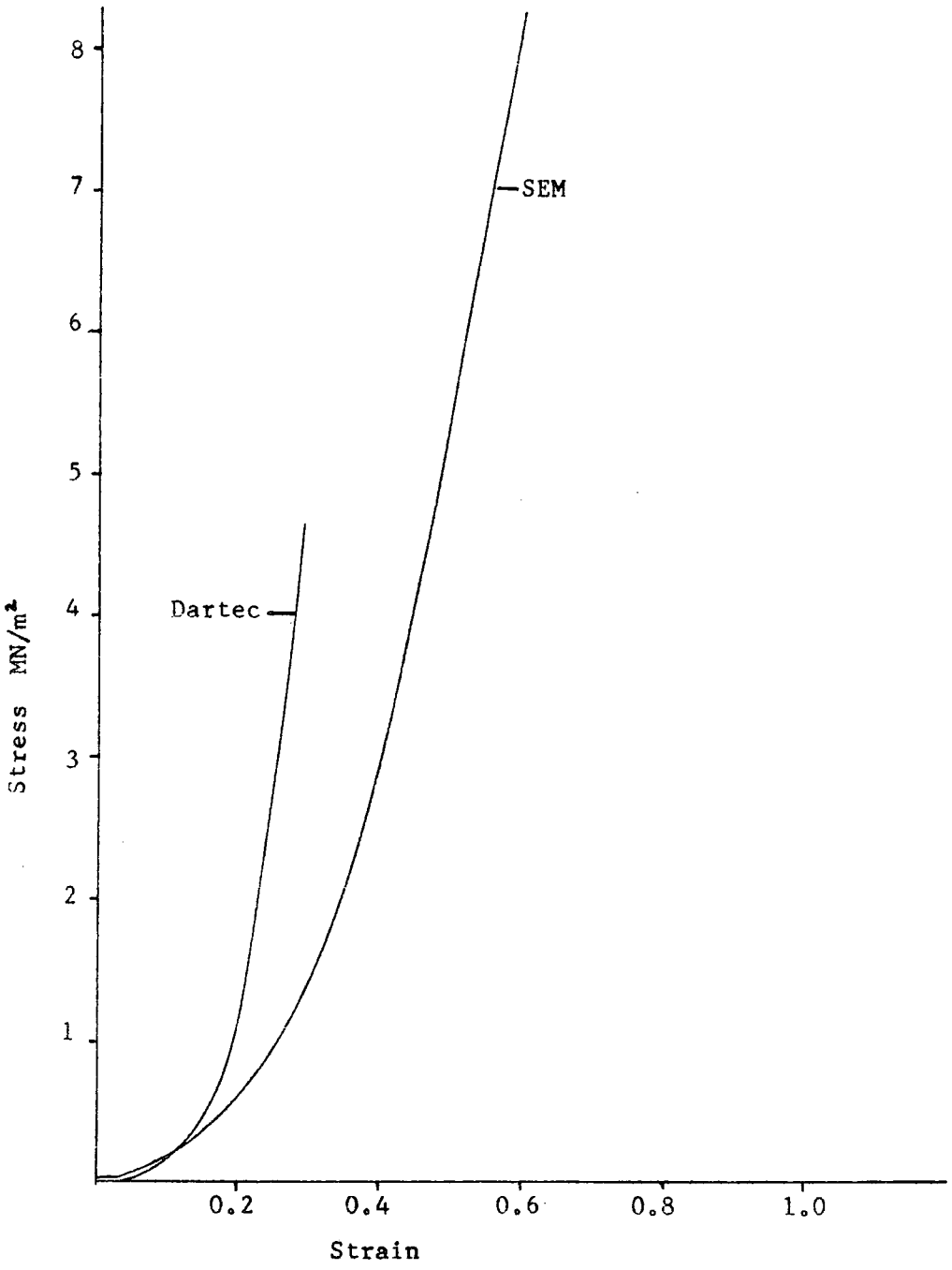


Fig7.35 Stress—strain response in SEM and Dartec.

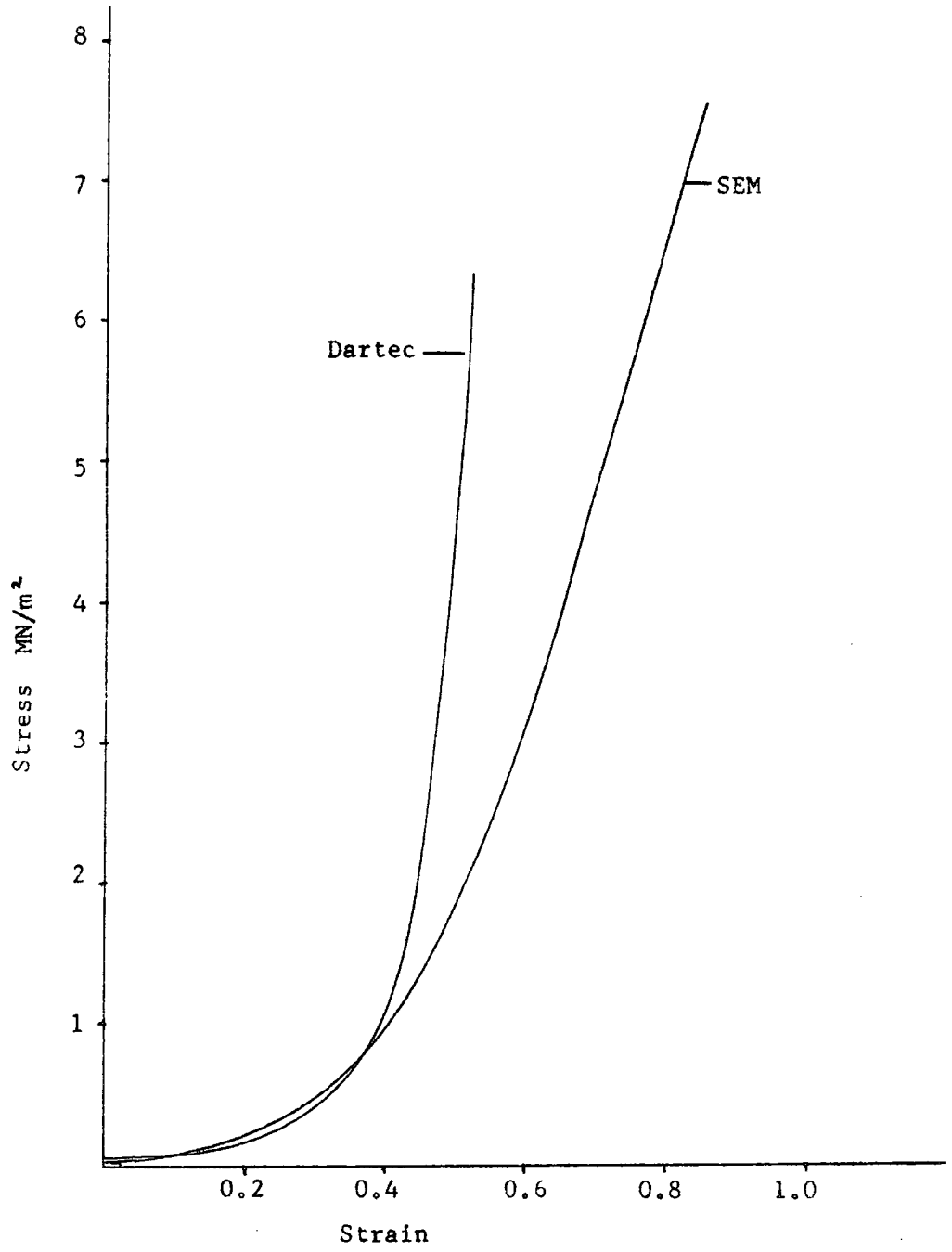


Fig7.36 Stress—strain response in SEM and Dartec.

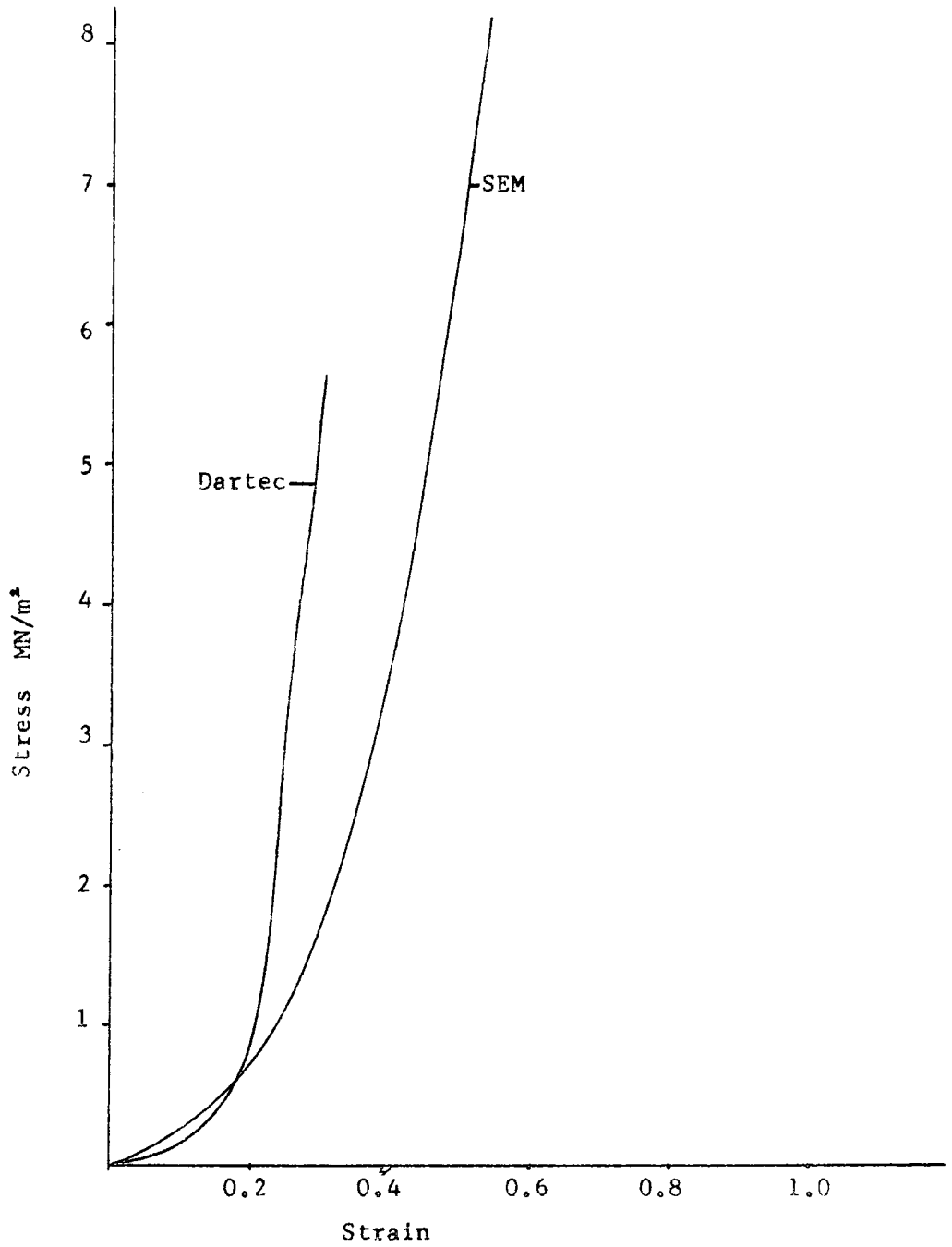


Fig7.37 Stress—strain response in SEM and Dartec.

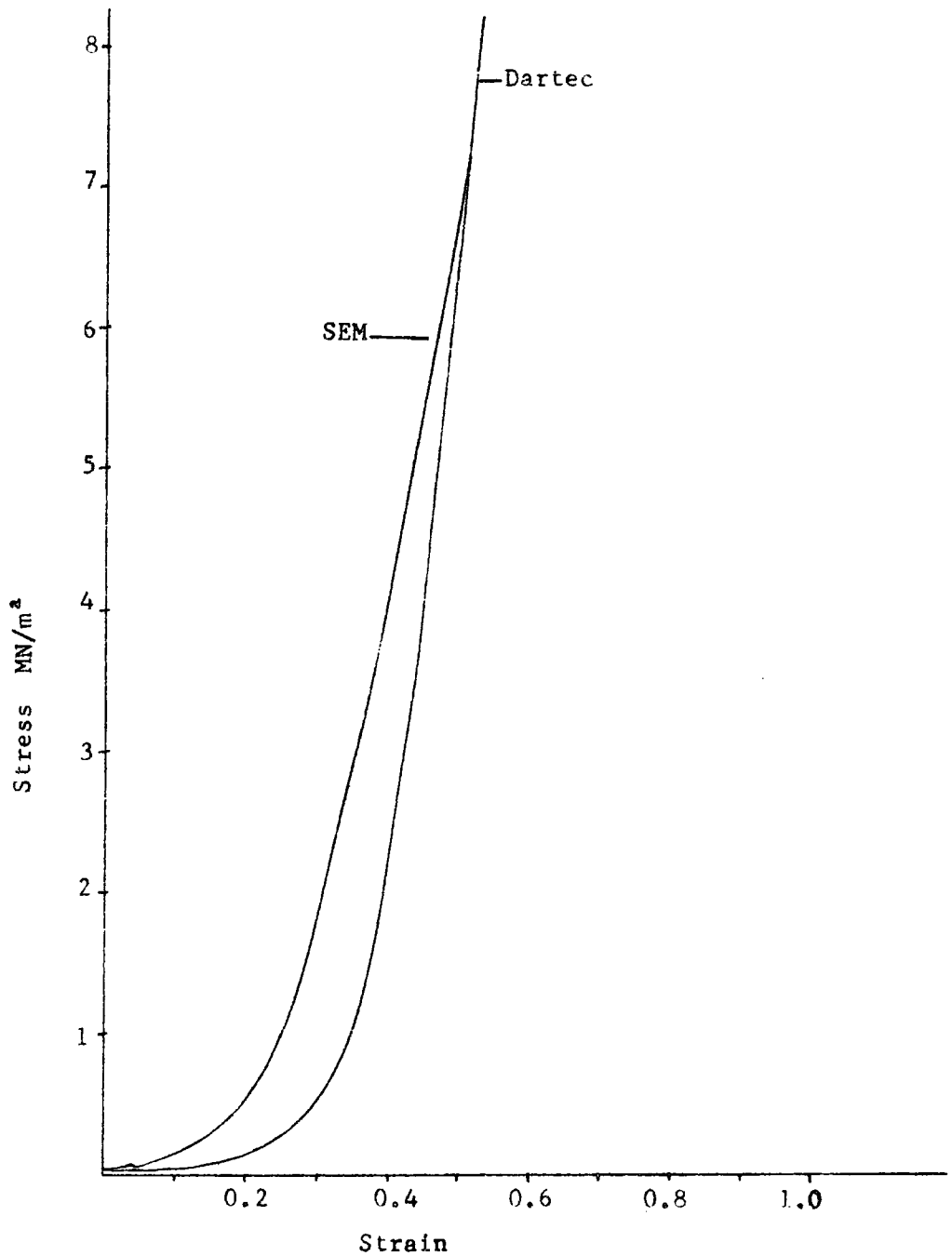


Fig7.38 Stress—strain response in SEM and Dartec.

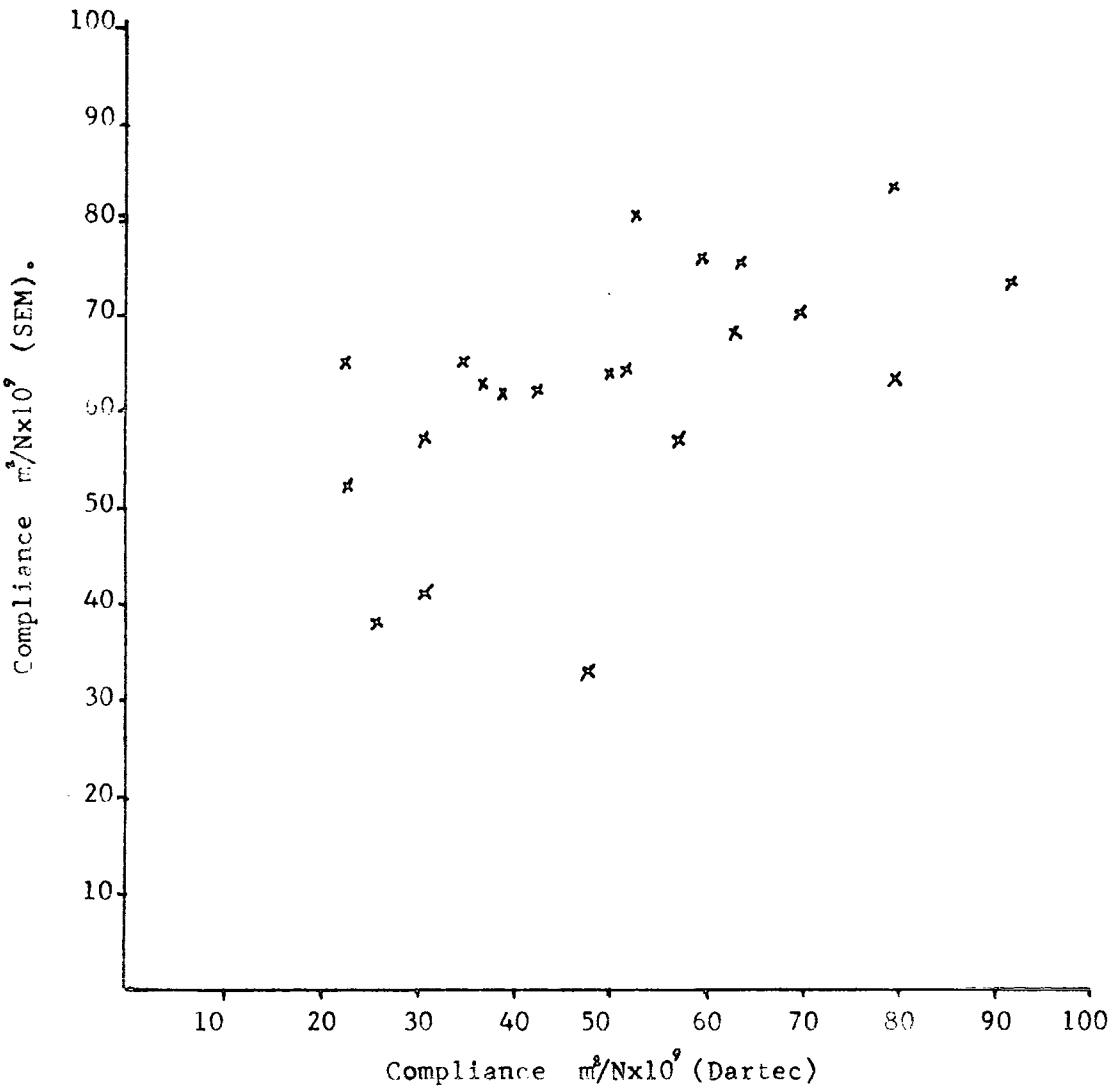


Fig7.39 Compliance in SEM versus compliance in Dartec.

7.4 Analysis of Data

7.4.1 Introduction

The definition of stress used in the analysis is

$$\sigma = P/A \qquad \text{(iv)}$$

where P = applied load

A = original cross sectional area of specimen

and compression is signified as positive.

The definition of strain used is the so called logarithmic strain, often used to describe large strains (Hill 1951)

$$\epsilon = -\int_{h_o}^{h} \frac{dh}{h} = \ln \frac{h_o}{h} \qquad \text{(v)}$$

where h_o = original thickness of specimen

h = current thickness of specimen

Compression is again signified as positive and it can be seen that as the thickness of the specimen approaches zero, $\epsilon \rightarrow \infty$.

The slope of a stress-strain curve is used to define the 'modulus' of the material. If the stress-strain curve is a straight line then the 'modulus' is constant for that specimen. If the curve is not straight then to enable comparison between specimens the 'modulus' can be defined as the slope of the tangent at a prescribed value of stress or strain. The inverse of the 'modulus' is designated here as the 'compliance'.

As mentioned in Chapter 4, each test rig used in this investigation exhibited a small, consistent load-deformation response and these are shown in Appendix B . For each specimen the measured deformation was corrected using the appropriate rig load—deformation curve before any analysis of the data was started.

7.4.2 Full Thickness Tests

The loads and displacements measured during the full thickness tests were converted to stress and strain using equations (iv) and (v) and a stress-strain curve was plotted for each specimen. Fig. 7.40 is an example of the response recorded for several specimens from one patella.

As can be seen the response is characterised by an initial

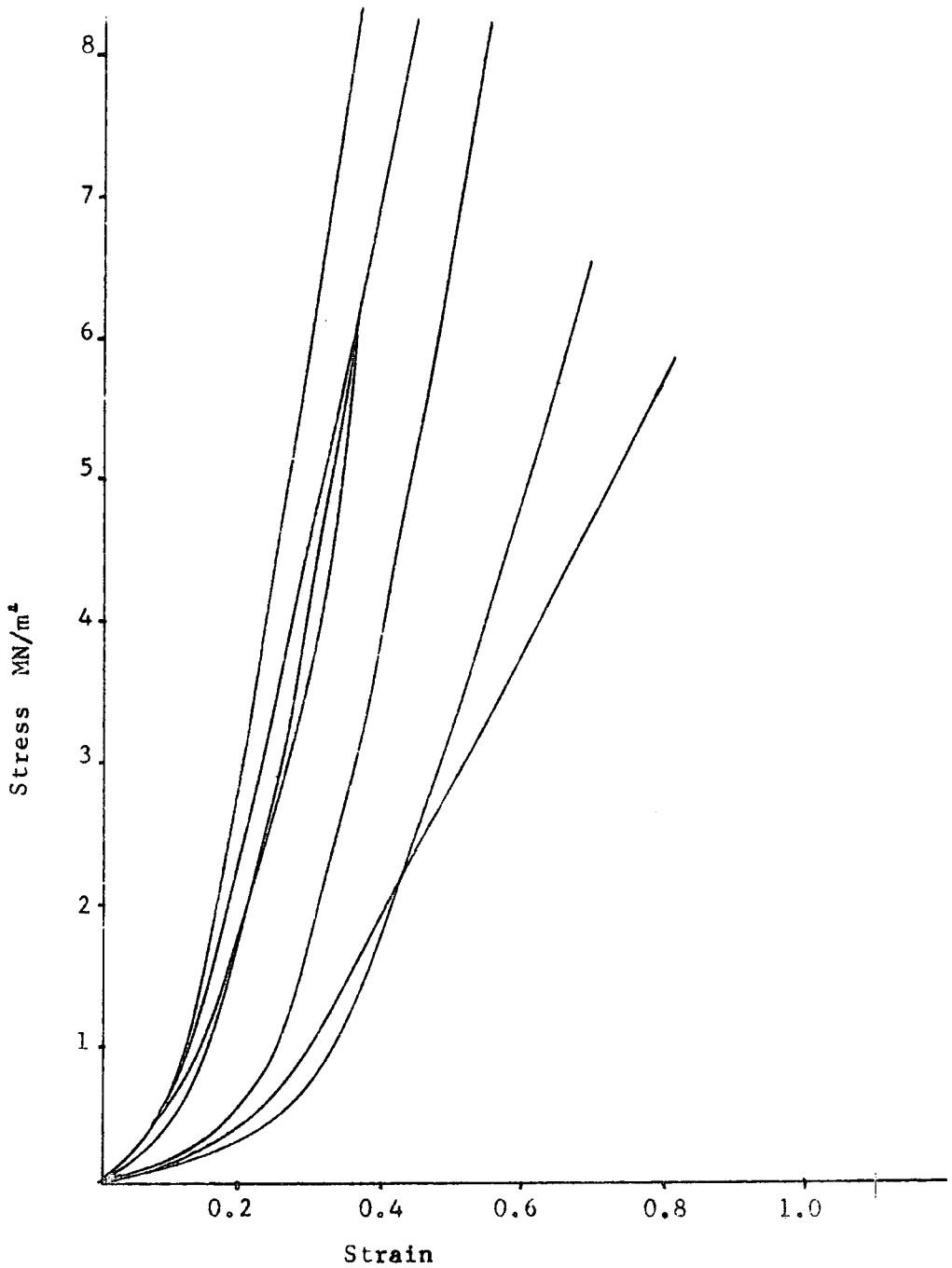


Fig7.40 Stress—strain response of seven specimens from one patella.

non-linear relationship between stress and strain followed by an almost linear relationship for the main part of the test. This lack of linearity in the response is in agreement with previous work using unconfined specimens (Johnson et al 1977).

As explained in Section 7.4.1, an overall 'modulus' can only be assigned to a specimen if the stress-strain relationship is constant. This is not the case for the specimens tested here and therefore to permit comparison between the response of a large number of specimens it was necessary to measure a characteristic of the response at a particular point during the test.

As will be shown in Section 7.4.3, the video analysis of specimens tested in the SEM indicate that in some cases there were sections through the thickness of the cartilage where there was virtually no deformation under the loads applied. Rather than measure a modulus or stiffness, which in these cases would be infinity, the stress-strain relationship of each specimen was therefore characterised by the compliance at a particular value of stress.

The stress that the compliance was measured at was 3 MN/m^2 as this value of stress was in the second, almost linear part of the response for each specimen and in most tests the level of stress reached was higher than 3 MN/m^2, therefore enabling the majority of specimens to be included in the analysis.

Once the stress-strain relationship of a specimen had been plotted the compliance at 3 MN/m^2 was measured and recorded.

7.4.3 SEM Layer Analysis

Using the video recordings made during the SEM tests, direct measurements of the deformations which occurred throughout the thickness of the specimen could be made. Although theoretically all the specimens tested in the SEM could be analysed for variation in compliance with depth, the number was limited by the quality of the video recording in each case. During this investigation, which was performed over a two year period, there was a noticeable decrease in the quality of video recording that could be made. The cause of this decrease in quality is

unknown. However, the effect was that analysis of video recordings of the later experiments became increasingly difficult and in some cases impossible. This problem and the possible solution to it are discussed in more detail in Chapter 8. For those specimens where the quality of the video recording made analysis possible the procedure was as follows -

By placing the control of the video recorder in the STOP position, it is possible to wind the tape on by hand. Each recording was wound on by this method until the compression stub just came into contact with the surface of the specimen. This zero displacement position was correlated with the zero load measurement recorded by the load cell described in Chapter 4. A grid was then placed on the screen of the video monitor and the co-ordinates of several, easily identifiable, features throughout the depth of the specimen were recorded. The video was then wound on until the compression stub had moved 5 mm on the monitor, equivalent to 0.1 mm compression of the specimen, and the co-ordinates of the same features were again recorded. This procedure was repeated for each 0.1 mm of displacement during the test.

Figs 7.41 to 7.43 show a series of still photographs taken of the video recording made during a test. By subtracting the co-ordinates of the adjacent features the deformation of the layer in between the features could be determined for each value of overall deformation.

The values of strain in each layer were calculated using equation (v) and these were correlated with the measured stress. Figs 4.44 and 4.45 show two examples of the stress-strain results obtained for different depths through the cartilage.

As with the other stress-strain responses, the graphs were characterised by the value of compliance at 3 MN/m^2 stress.

Fig. 4.45 is an example of the cases observed where no deformation occurred in a particular layer of the cartilage during the test. In these cases, by measuring compliance

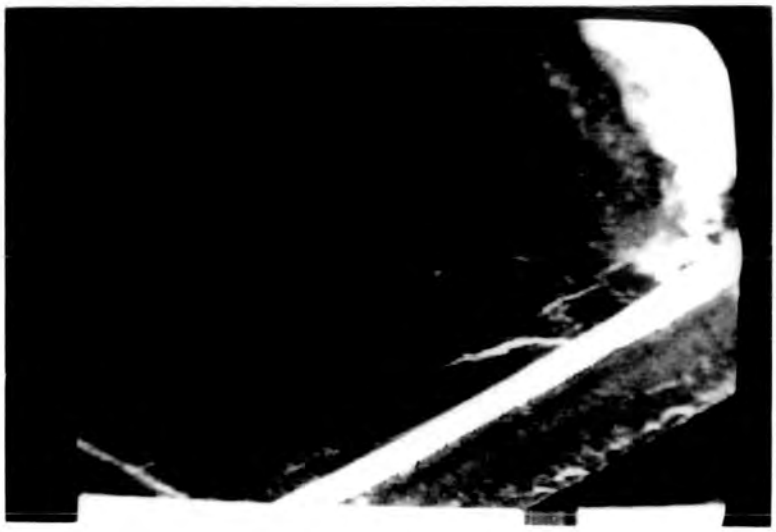


Fig.7.41

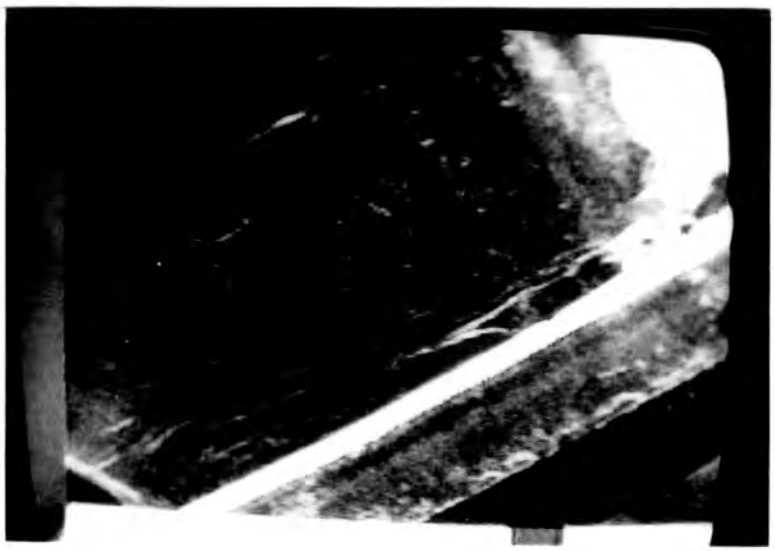


Fig.7.42

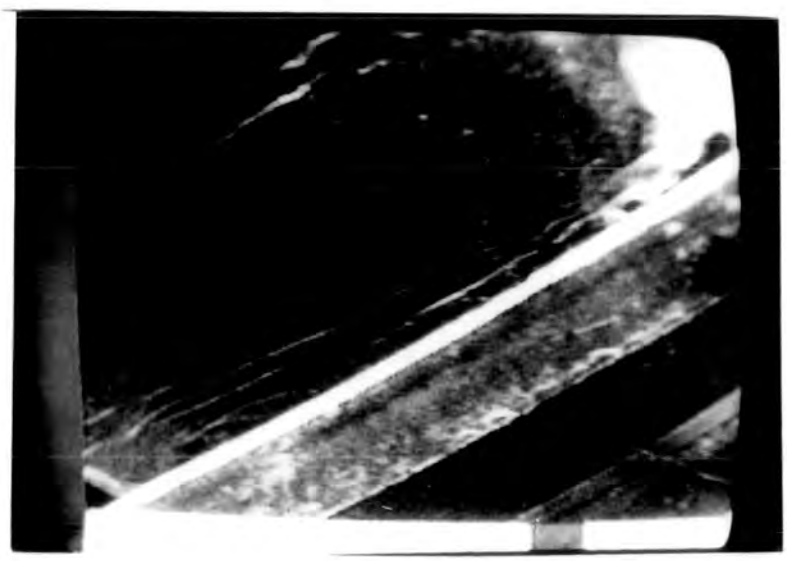


Fig.7.43
Sequential stills from video.

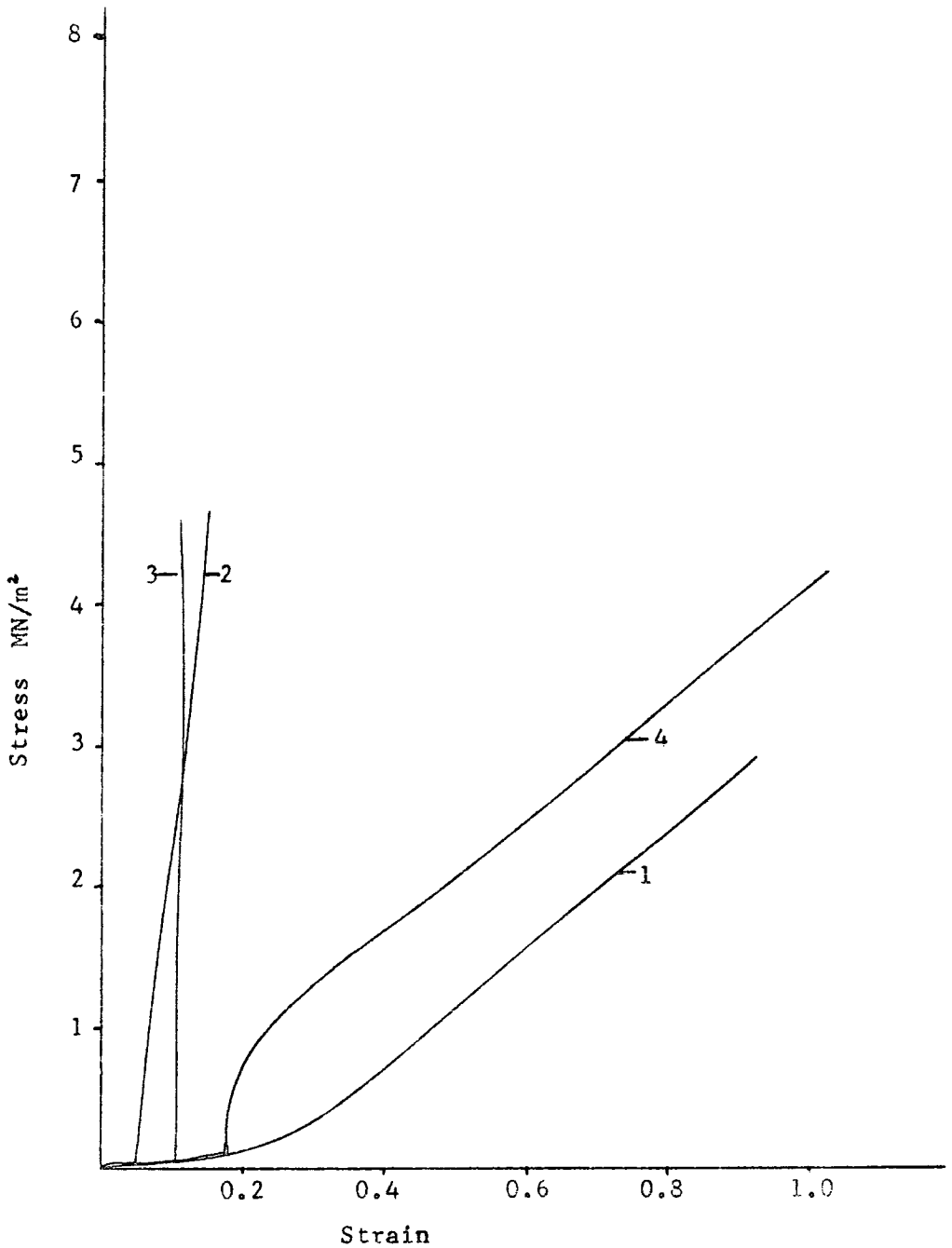


Fig7.44 Variation in stress—strain response with depth (SEM).

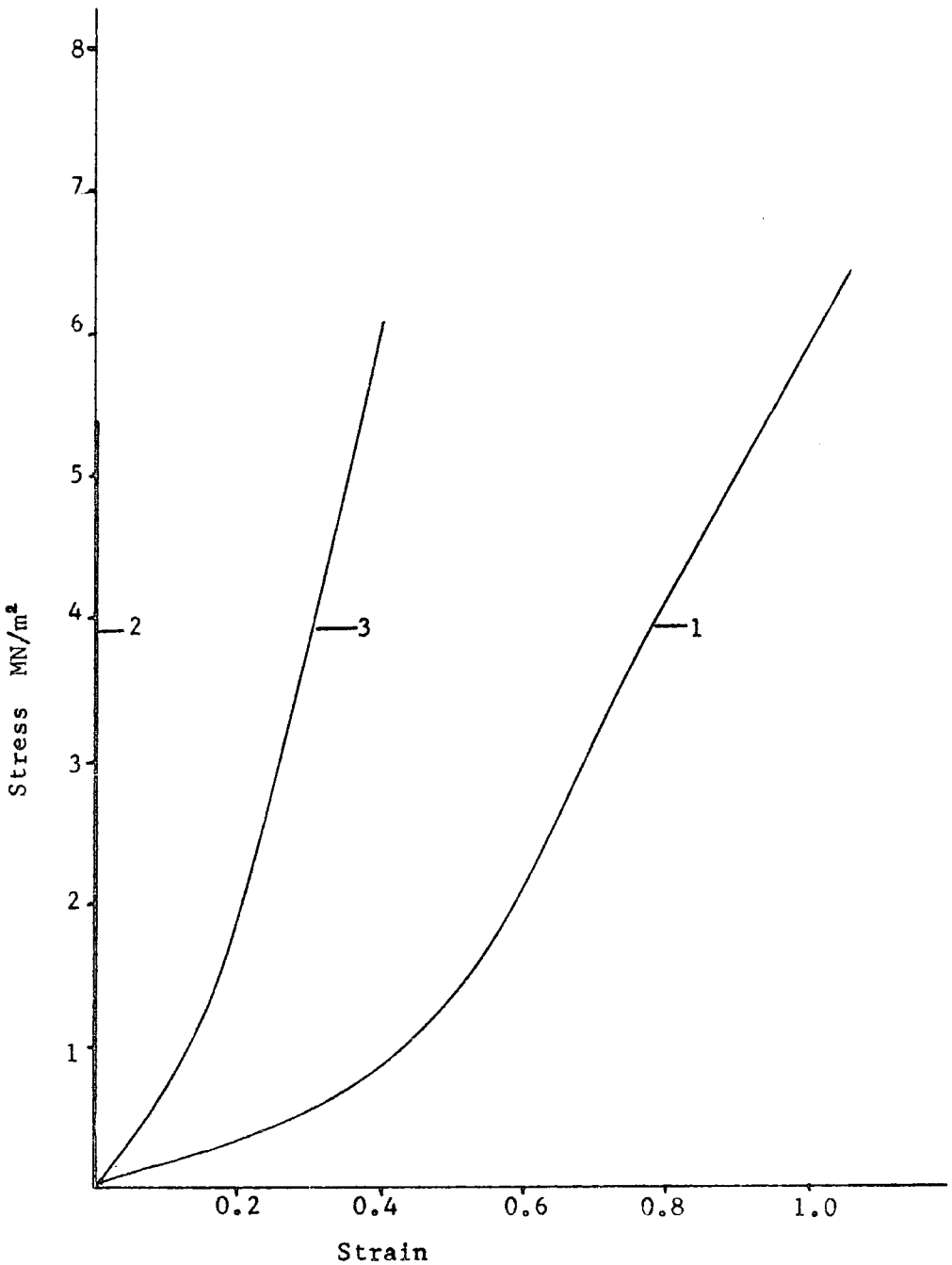


Fig7.45 Variation in stress— strain response with depth (SEM).

rather than stiffness the need to deal with infinite stiffness in the subsequent analysis is eliminated.

7.4.4 Dartec Slice Tests

As described in Section 7.2, the load-deformation response of each slice removed from the specimen is deduced by the difference in the load deformation response of the specimen with and without the particular slice. The load-deformation responses for one specimen as each slice was removed were therefore plotted together on one graph as shown in Figs 7.46 and 7.47. By measuring the difference in deformation at several loads during the test the load-deformation response of each slice was deduced and converted to stress and strain using equations (iv) and (v). As with the full thickness and SEM layer tests, the stress strain response for each slice was plotted and the compliance at 3 MN/m^2 measured and recorded. Fig. 7.48 shows an example of the stress-strain response recorded where slice 1 is nearest the articular surface.

7.4.5 Instron Slice Tests

As the slices tested in the Instron were all tested individually the analysis of the data was exactly as for the full thickness specimens. The load-deformation response was converted to stress-strain using equations (iv) and (v) and the compliance of the stress-strain curve was measured at 3 MN/m^2.

Fig. 7.49 shows an example of the stress strain response for slices from the same specimen with slice 1 again being the slice nearest the articular surface.

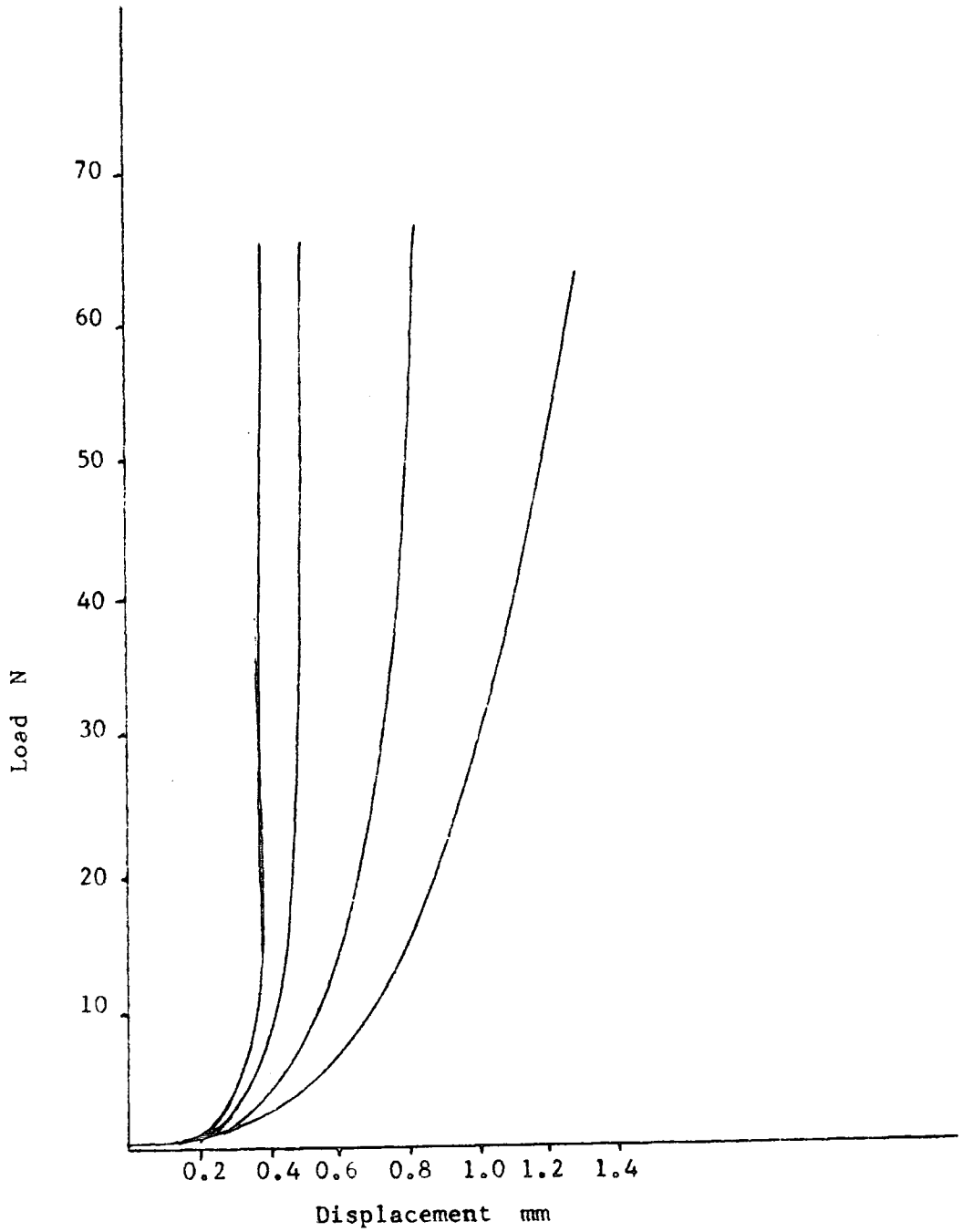


Fig7.46 Load—deformation response for one specimen with slices removed.

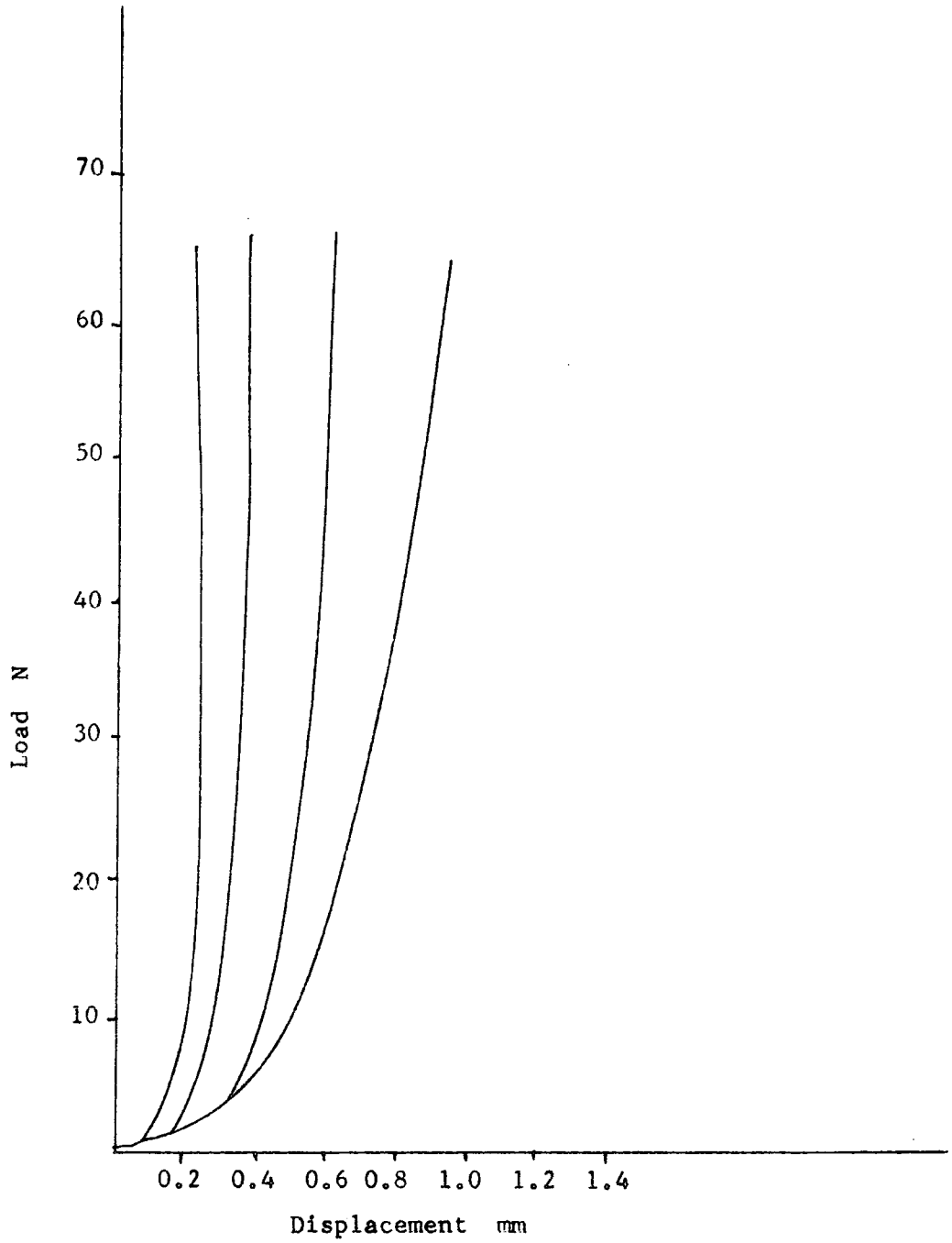


Fig7.47 Load—deformation response for one specimen with slices removed.

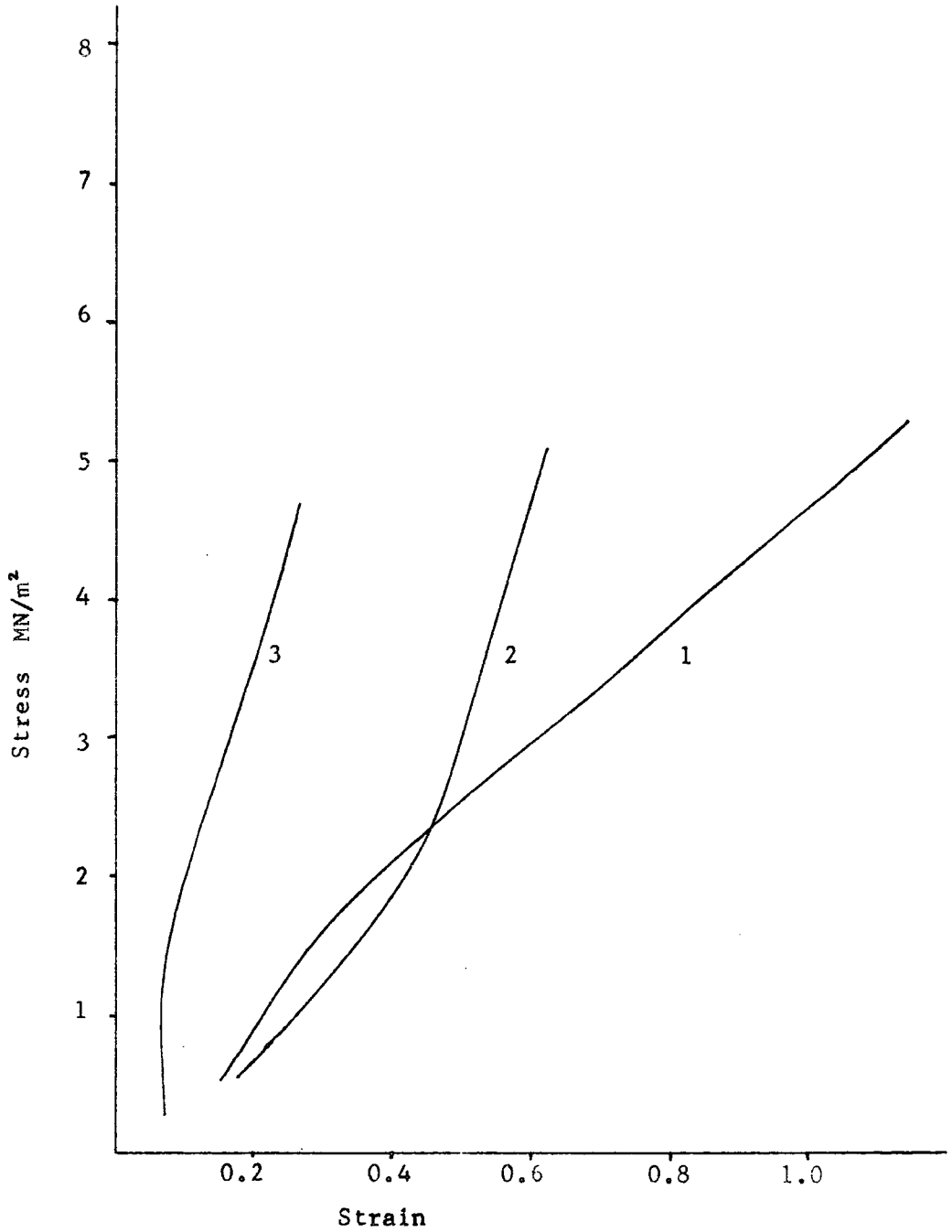


Fig7.48 Variation in stress—strain response with depth (Dartec).

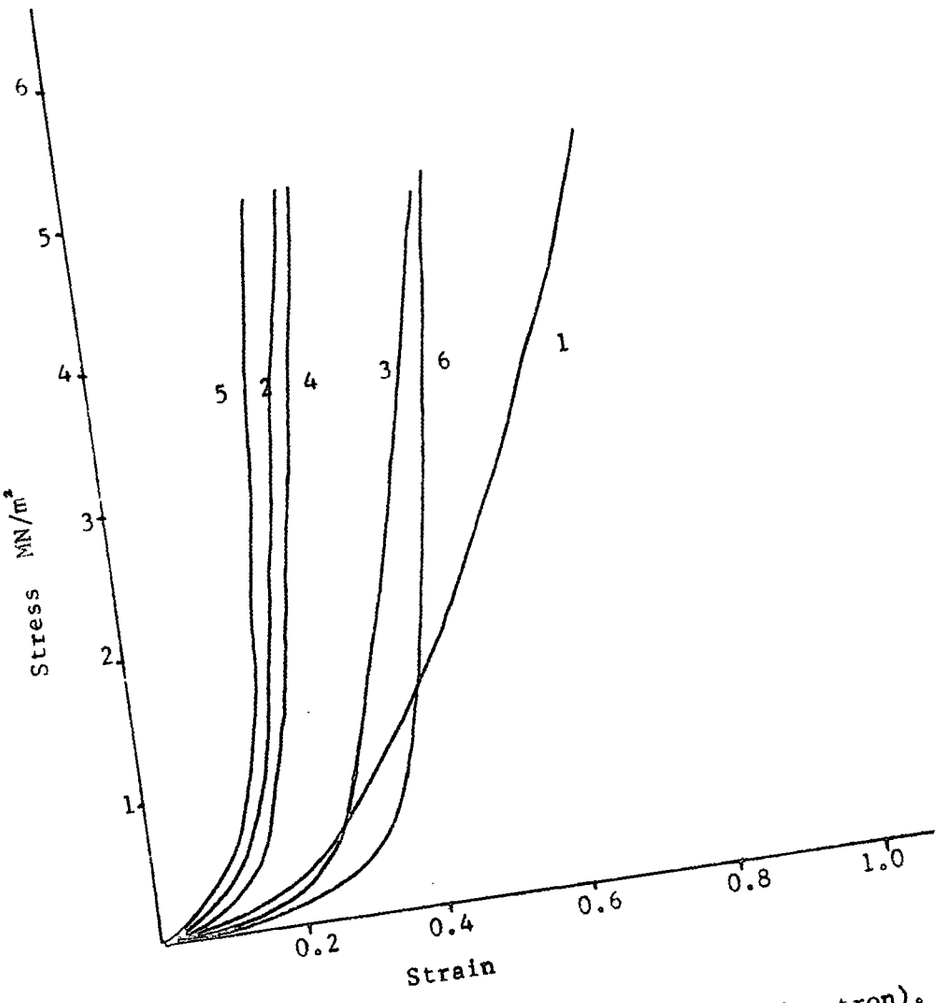


Fig7.49 Variation instress—strain response with depth (Instron).

7.5 Variation in Compliance with Depth

Figs 7.50, 7.51 and 7.52 show the values of compliance measured at different depths through the thickness of the specimens using the three experimental techniques described. The normalised depth was calculated by dividing the depth of the mid-point of each layer or slice by the full thickness of the specimen

A total of 49 specimens were tested; 20 in the SEM, 16 in the Dartec and 13 in the Instron.

40 of the specimens analysed showed a marked decrease in compliance with depth from the articular surface while 9 showed an increase in compliance with depth. Of the 40 specimens where compliance decreased with depth, 31 had a minimum value of compliance at a depth of between 25% and 75% of the specimen thickness. The 9 remaining specimens in this group showed a continual decrease in compliance with depth. No obvious reason could be found for the different compliance distribution of the 9 specimens whose compliance increased with depth, although it should be noted that 5 out of the 9 had been stored frozen which, as will be shown in Section 7.6, does seem to affect the full thickness response of the tissue. Fig. 7.53 shows the variation in compliance with depth of those specimens stored at + $4^{o}C$ and Fig. 7.54 is of those stored at - $20^{o}C$. Although no quantitative difference can be said to exist between the two groups, from a qualitative point of view the specimens stored frozen would appear, on average, to have higher values of compliance in the mid-zone than those stored unfrozen.

It can be seen from Figs 7.50, 7.51 and 7.52 that the extreme values for compliance come from the SEM analysis. This is to be expected as it is only when using this technique that the regions or layers to be analysed can be selected according to their behaviour.

With both the Dartec and Instron test procedures no such selection is possible and the results obtained must be the average for each slice, which may in fact contain regions of high and low compliance.

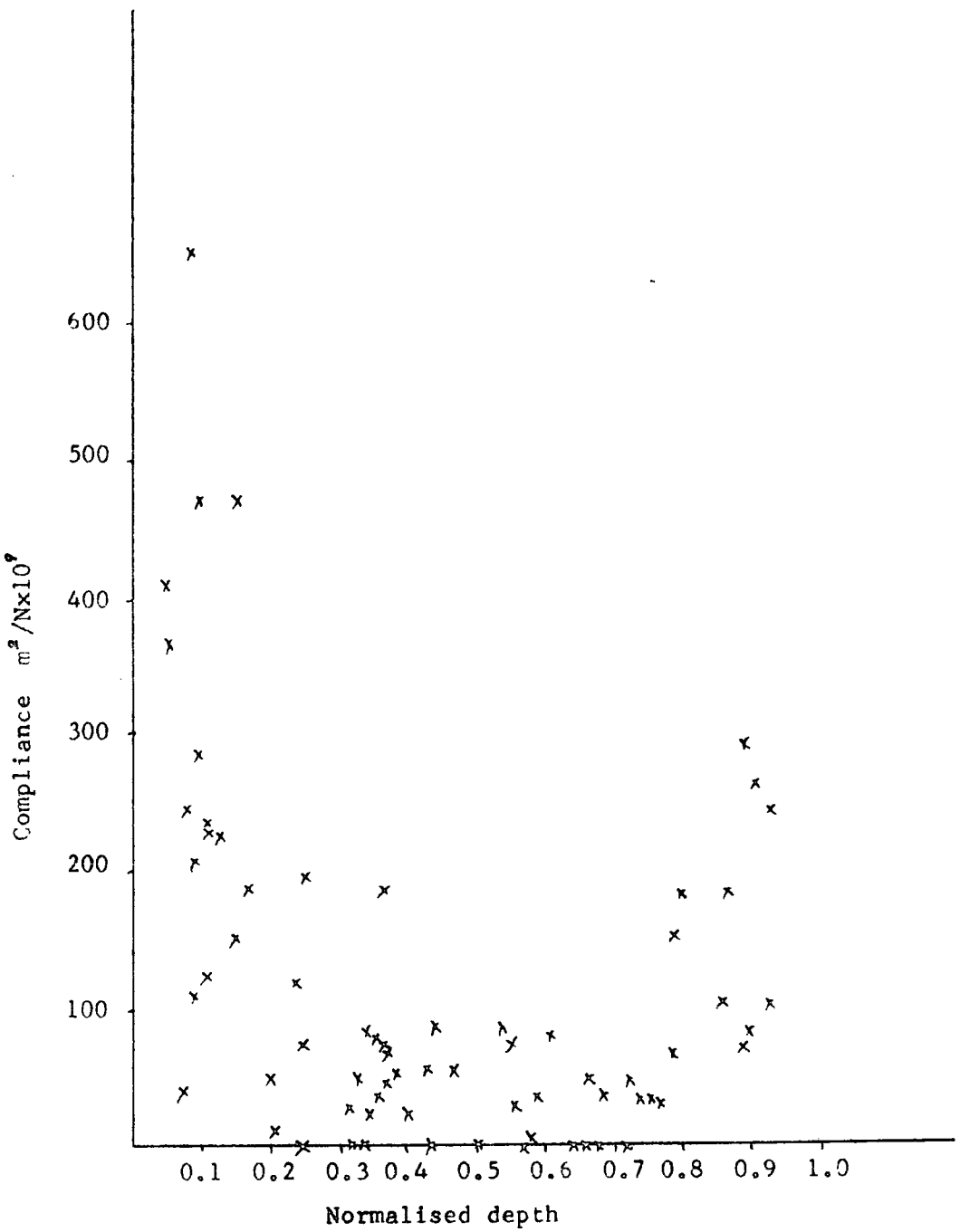


Fig7.50 Variation in compliance with depth (SEM).

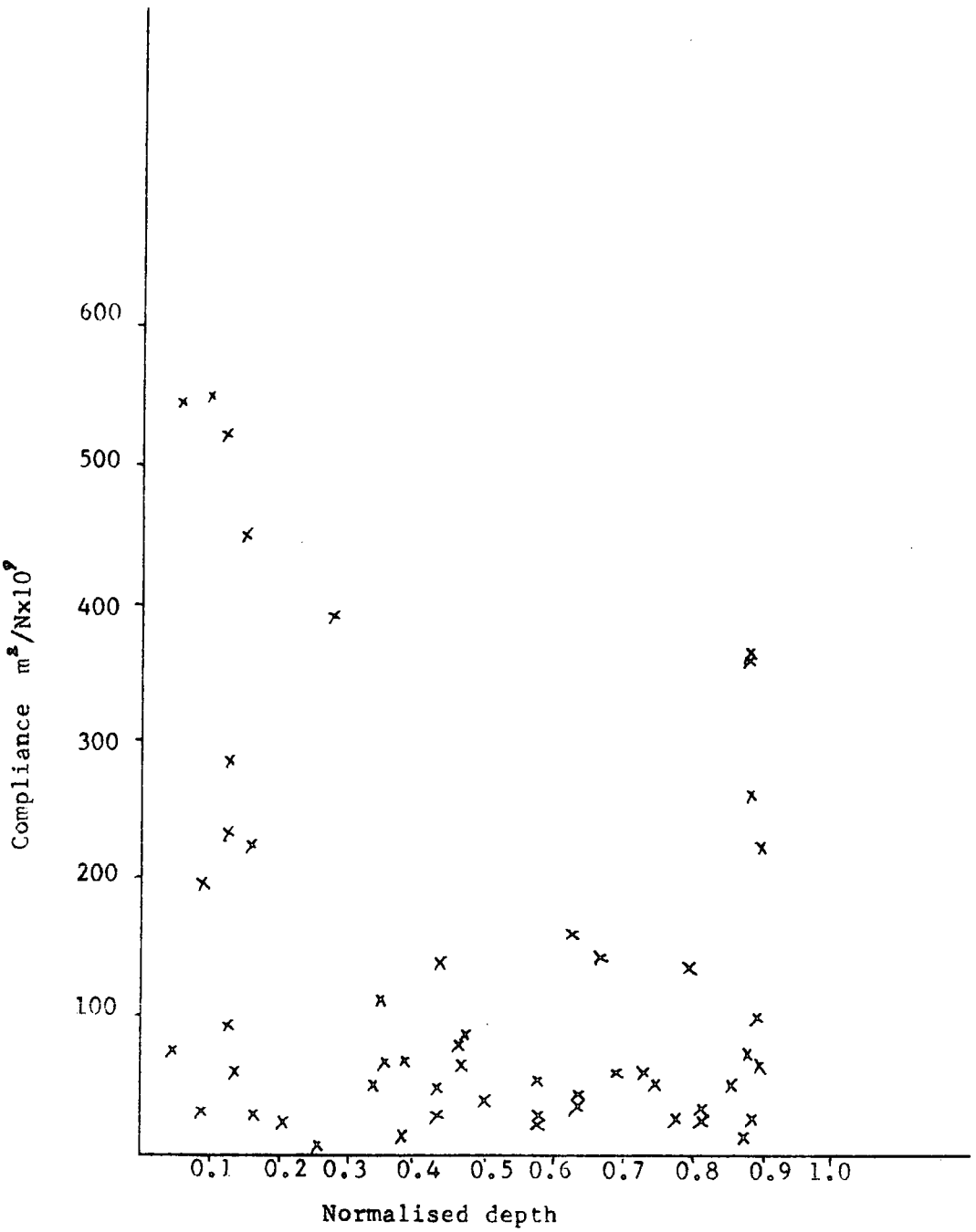


Fig7.51 Variation in compliance with depth (Dartec).

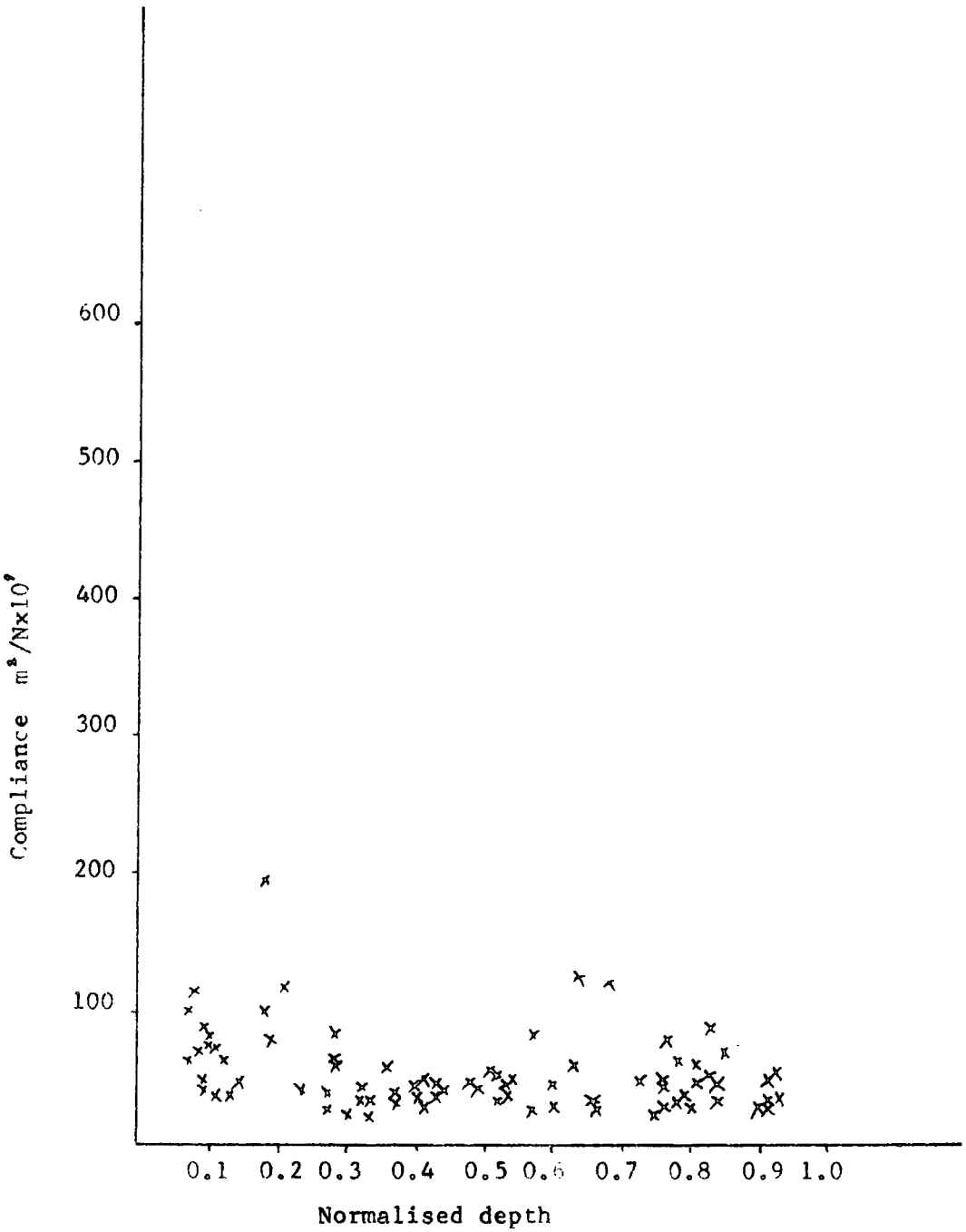


Fig7.52 Variation in compliance with depth (Instron).

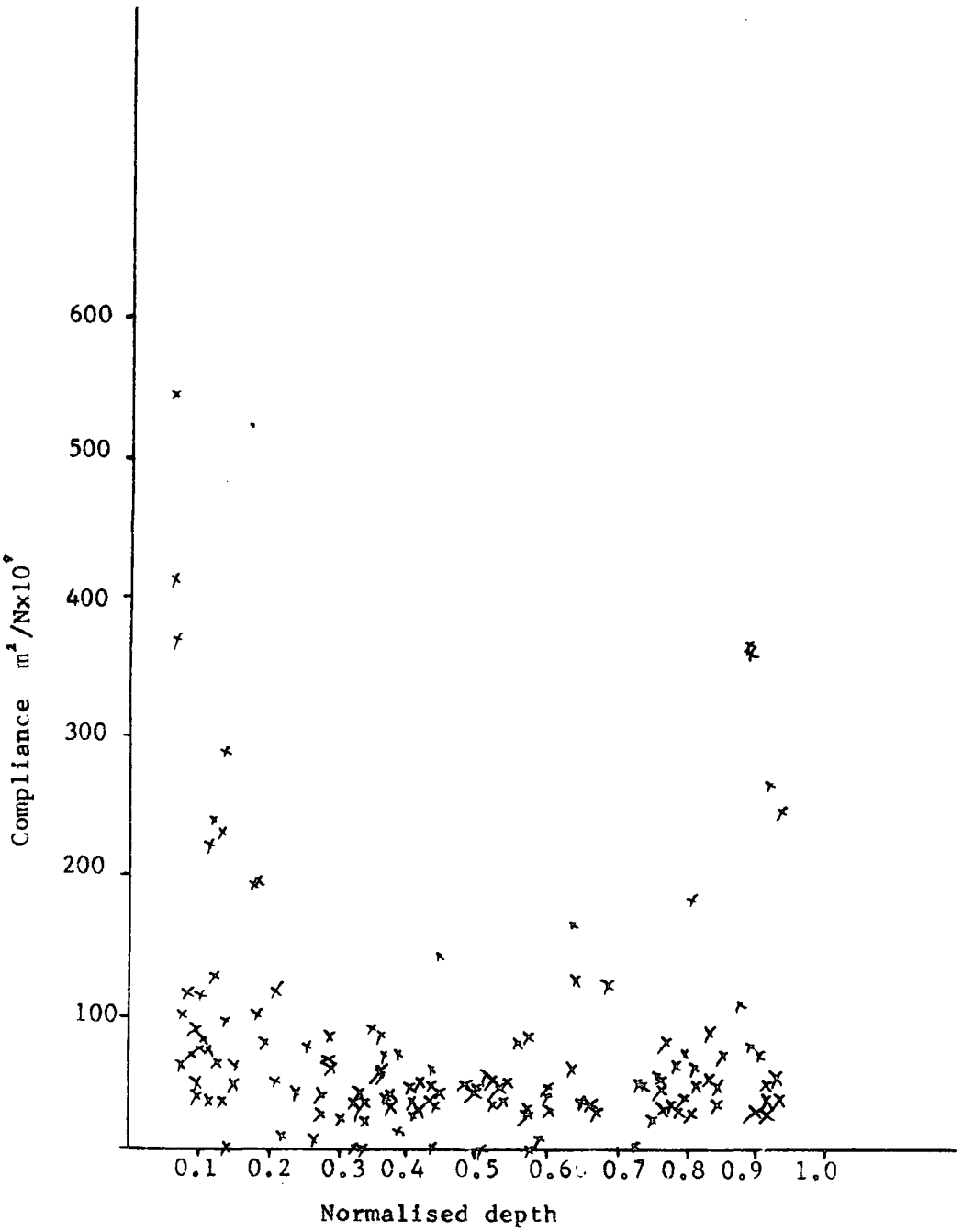


Fig7.53 Specimens stored at +4°C.

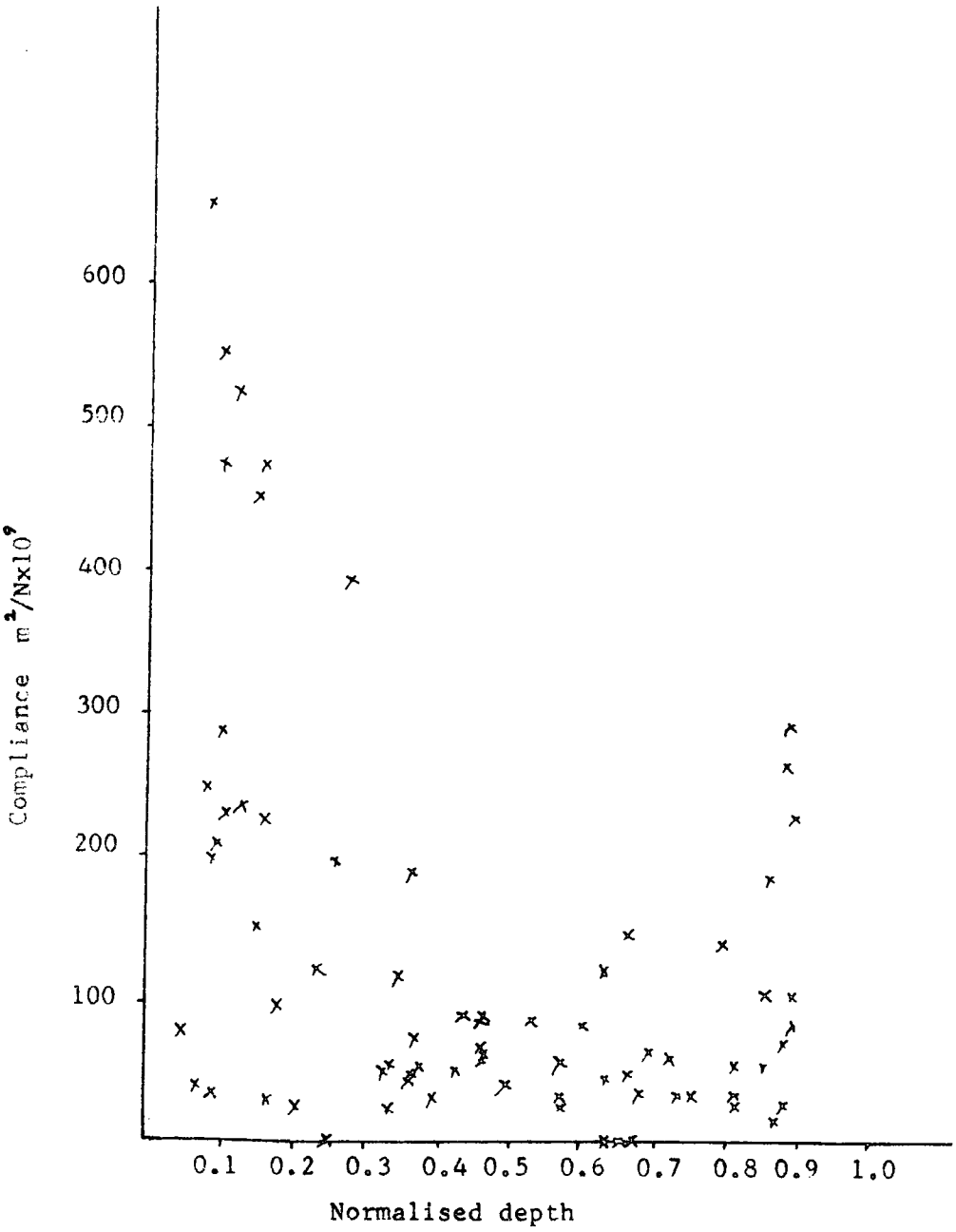


Fig7.54 Specimens stored at - 20°C

7.6 Variation in Full Thickness Compliance

110 full thickness specimens were tested in this investigation and the values of compliance measured ranged from 2.3×10^{-8} to 2.47×10^{-7} m^2/N with a mean value of 7.8×10^{-8} m^2/N.

Twelve of the patellae used in this study had eight or more specimens taken from different sites and Figs 7.55 and 7.56 show the variation in compliance with site observed in these patellae. No consistent pattern of compliance with site is evident from this study.

In Fig. 7.56 it can be seen that in eight cases out of the twelve the range of compliance is restricted to two or three of the arbitrary divisions of the total range of compliance, and only in four cases are there specimens from the same patella in both the extreme ranges. The results indicate therefore that it is likely that if an area of high compliance is present on a patella the remaining cartilage will also exhibit higher than average compliance.

Fig. 7.57 shows the variation in compliance with age for all the specimens. No obvious correlation between compliance and age is indicated by this graph although it can be seen that the range of compliance at any particular age increases with increasing age.

There are however two other variables, in addition to age, which may influence the compliance of a specimen. These are the method of storage and the state of biochemical normality of the specimen, as indicated by the water content of the tissue.

Figs 7.58 and 7.59 show the variation in compliance with age, with the specimens divided into those stored at - $20^{\circ}C$ and at + $4^{\circ}C$.

From a qualitative point of view these graphs would suggest that in fact in addition to the increase in range of compliance with age there is a general increase in compliance with age and that the relationship between compliance and age in each case might be of the form

$$\text{Compliance} = a \times \text{age}^{b} + c \qquad \text{(vi)}$$

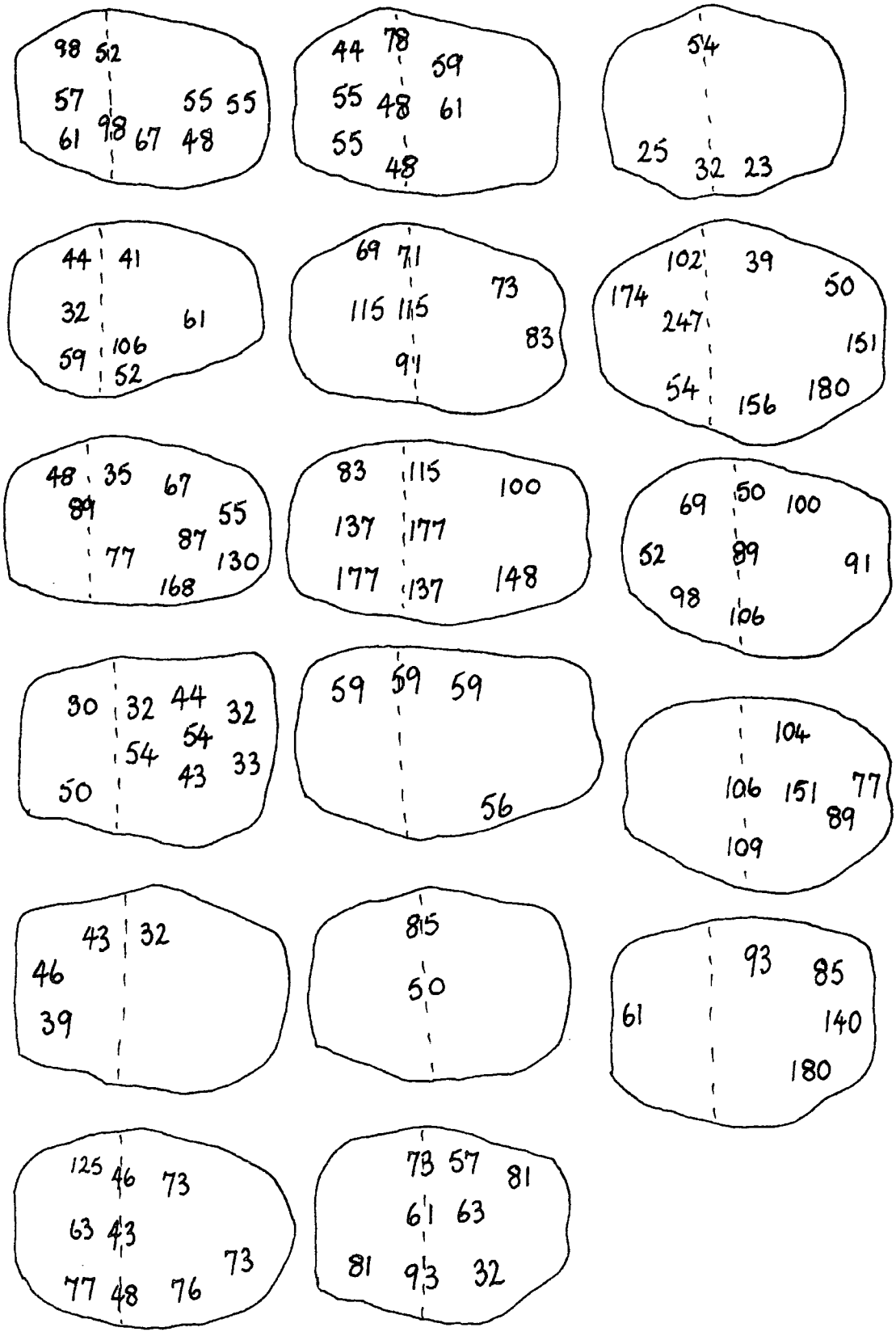


Fig7.55 Variation in compliance ($m^2/Nx10^9$) with site.

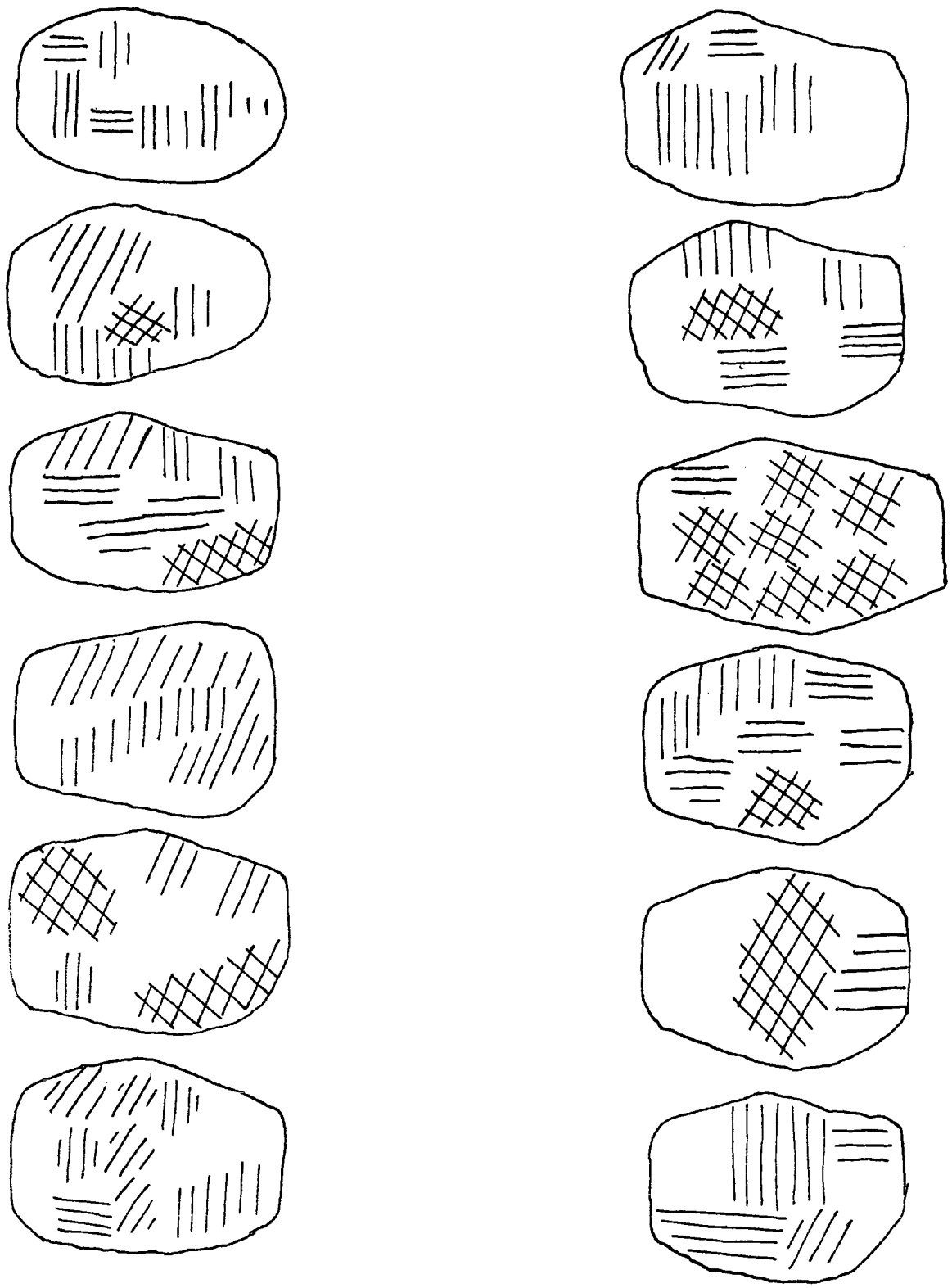


Fig 7.56 Variation in compliance with site.

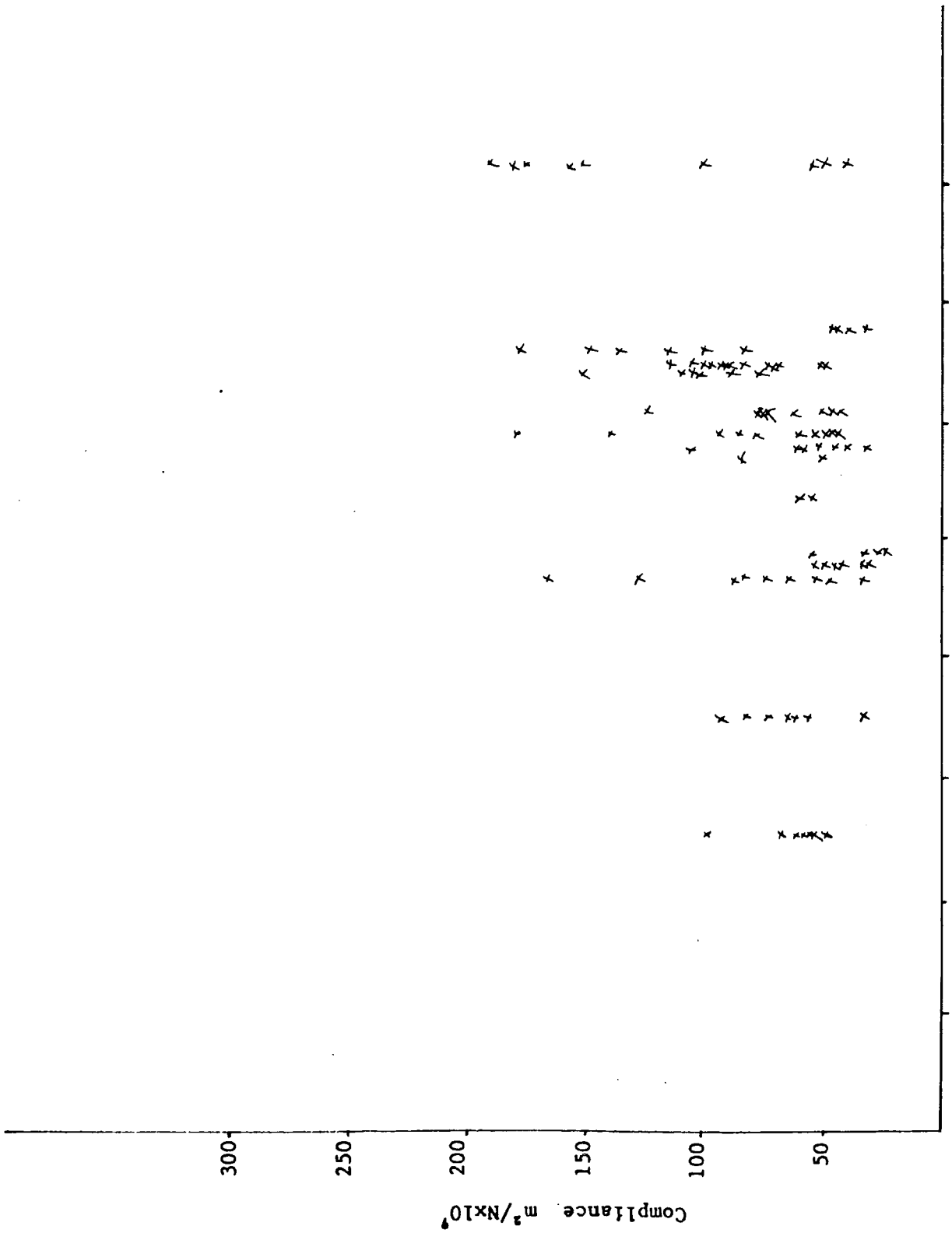


Fig7.57 Variation in compliance with age.

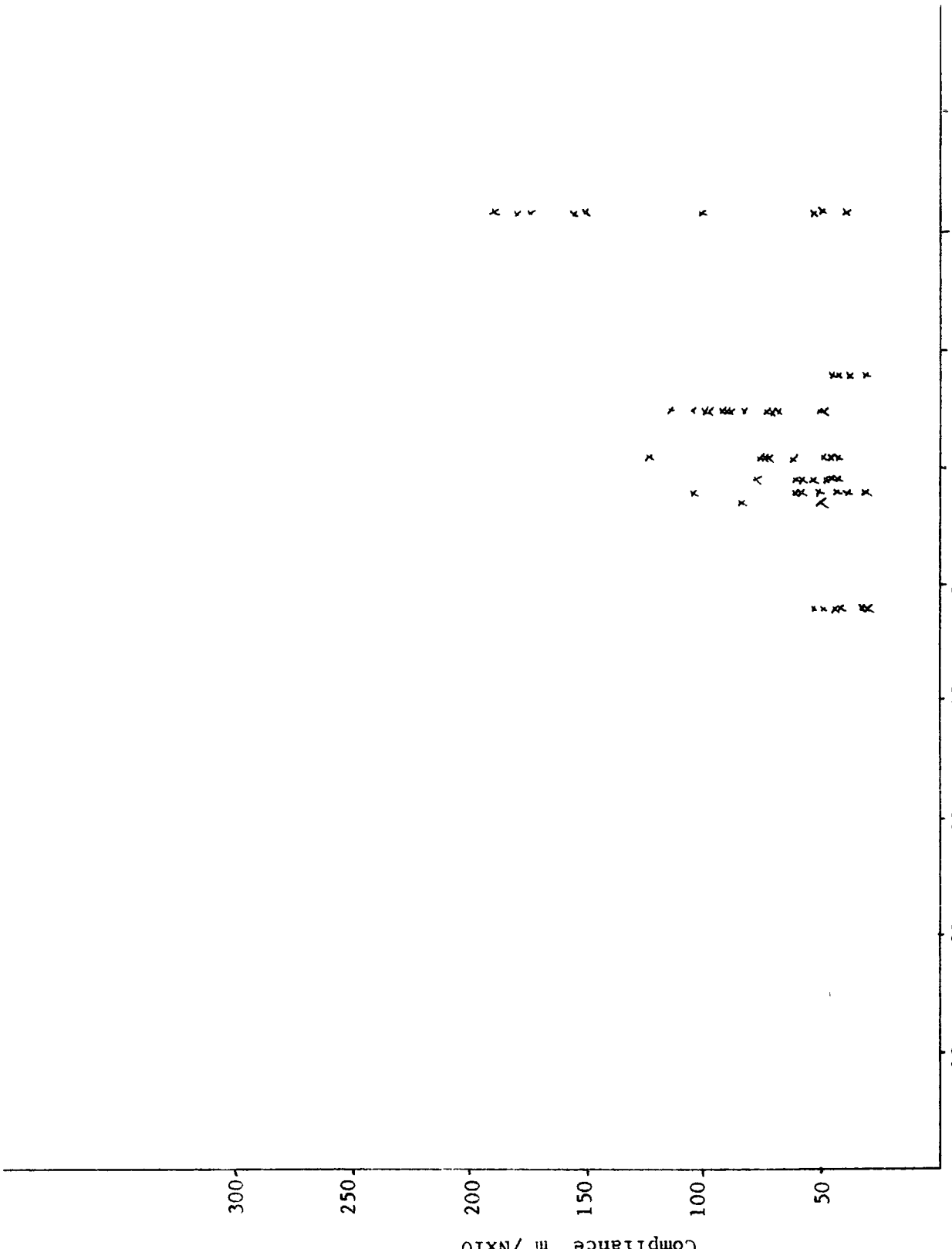


Fig7.58 Specimens stored at +4°C.

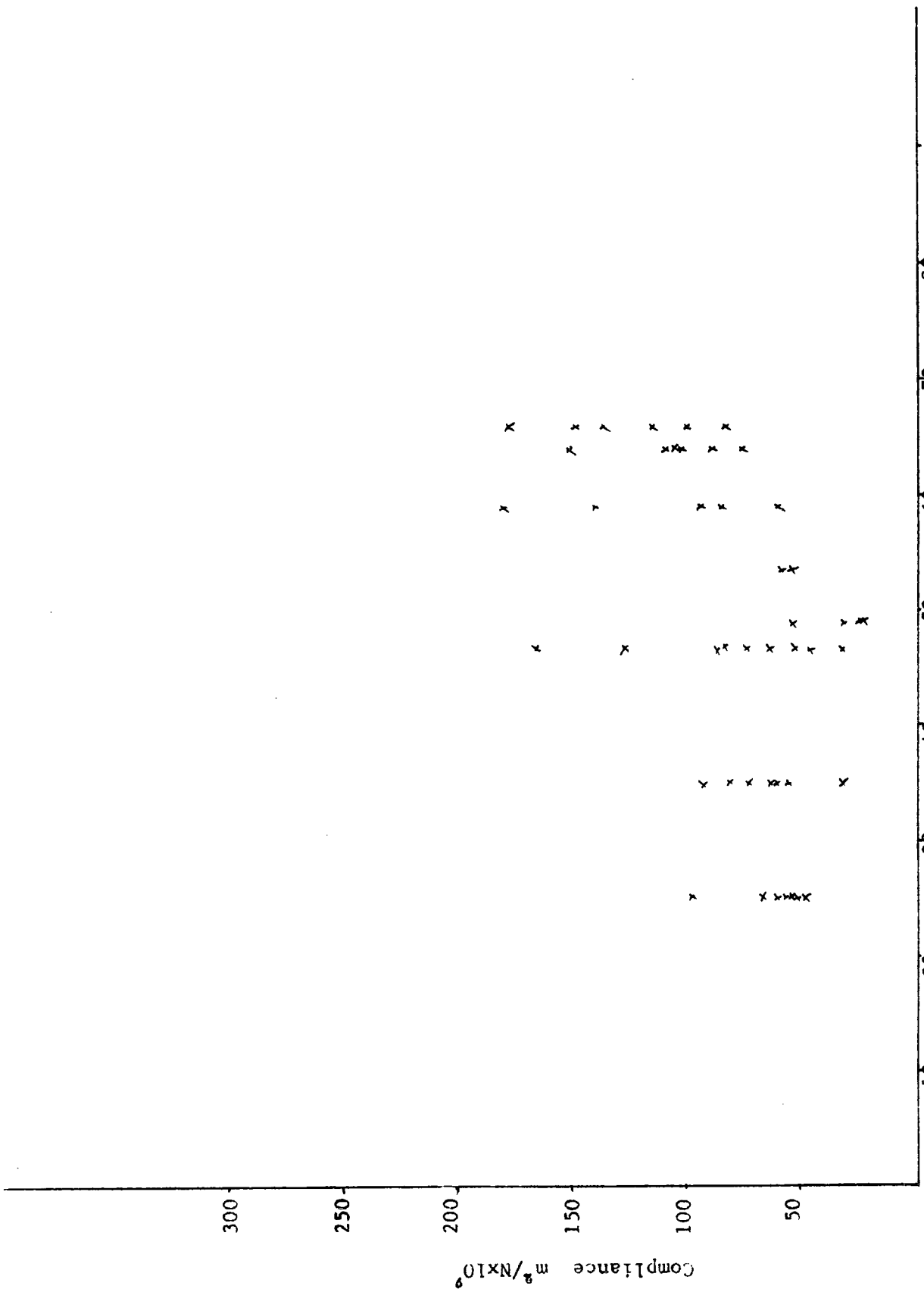


Fig7.59 Specimens stored at -20°C.

where a, b and c are constants.

To investigate this further the results of the specimens stored at + 4°C were considered in isolation from those specimens stored at - 20°C.

By curve fitting, the value of c was estimated as 20 m^2/MN. Rewriting equation (vi) this gives

$$y - 20 = a x^b \qquad \text{(vii)}$$

where y = compliance

x = age

Equation (vii) was rectified by log transformation to give

$$\log y' = \log a + b \log x \qquad \text{(viii)}$$

where $y' = y - 20$

The method of least squares was used to determine the 'best' straight line for the data.

$$y = 8.13 \times 10^{-4} x^{2.65} + 20 \qquad \text{(ix)}$$

($r = 0.526$ significant at the 1% level)

These results indicate therefore for tissue stored at + 4°C the compliance is highly dependent on the age, increasing with increasing age.

If we now consider the tissue stored at - 20°C it can be seen that the values of compliance measured are on average higher than those predicted by equation (ix), Fig. 7.60. This is seen even more clearly if just the mean values of compliance at each age are plotted against age as shown in Fig. 7.61. It appears that the compliance is on average 40 $m^2/N \times 10^9$ higher if the specimen is stored at - 20°C. Fig. 7.62 shows the mean measured values of compliance for specimens stored at + 4°C, the mean measured values of compliance minus 40 $m^2/N \times 10^9$ for specimens stored at - 20°C and the line predicted by equation (ix). It can be seen that there is now excellent correlation between predicted and measured values. The results therefore indicate that not only is compliance of articular cartilage highly dependent on age, it is also highly dependent on storage procedures, with tissue stored at - 20°C exhibiting higher compliance than tissue stored at + 4°C.

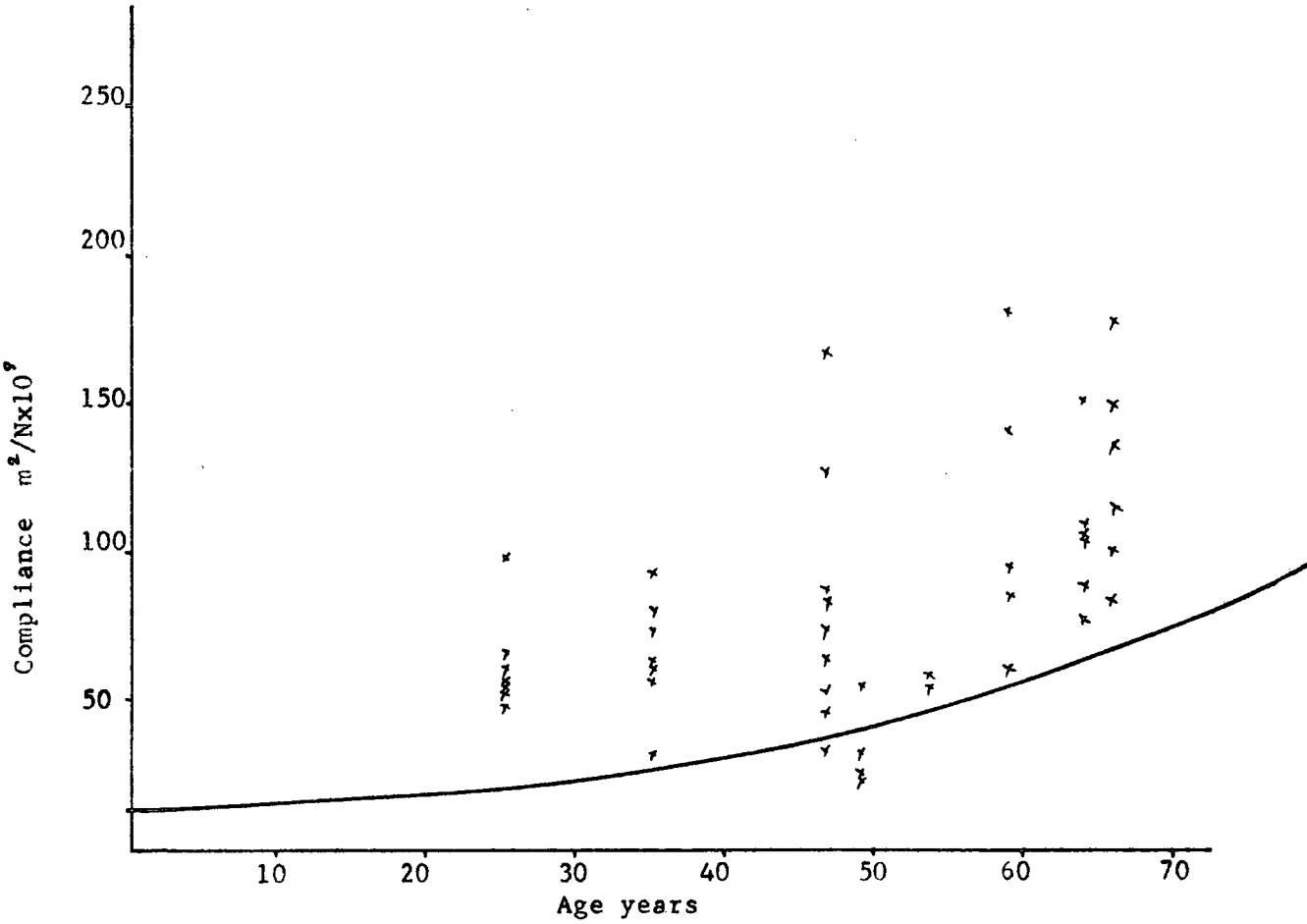


Fig7.60 Specimens stored at -20°C with equation (ix).

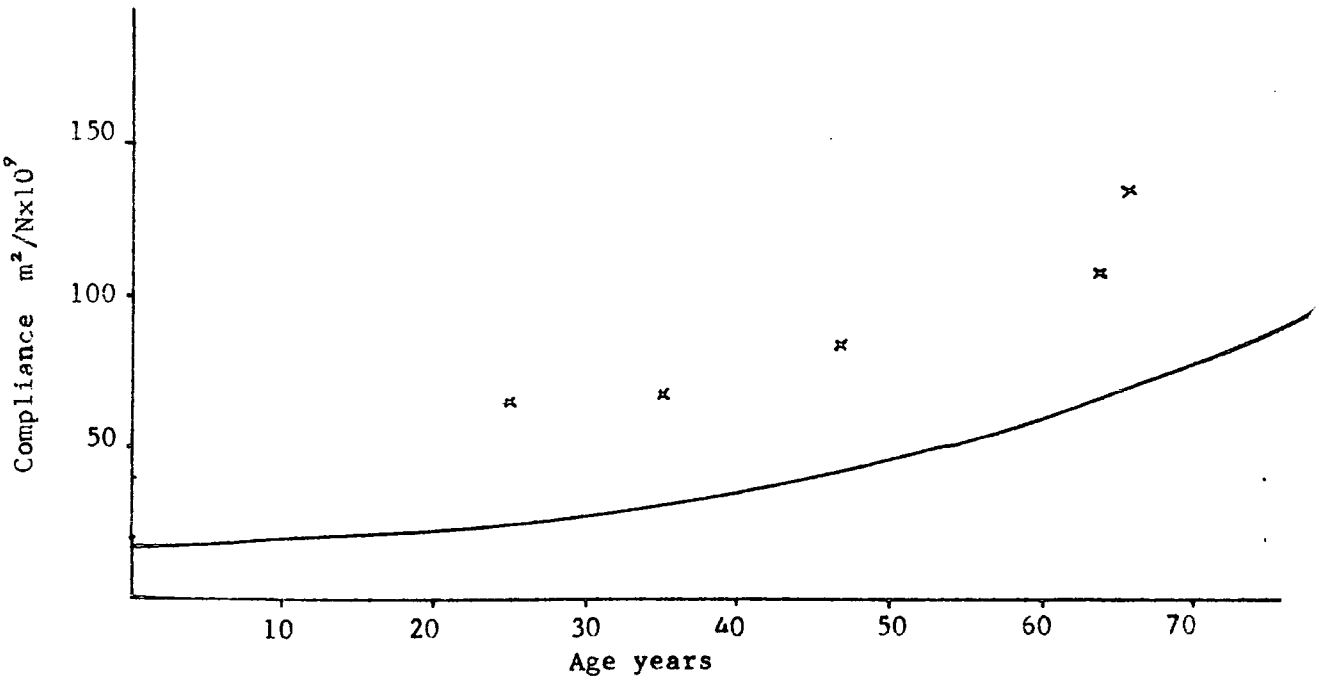


Fig7.61 Mean values of specimens stored at -20°C with eq. (ix).

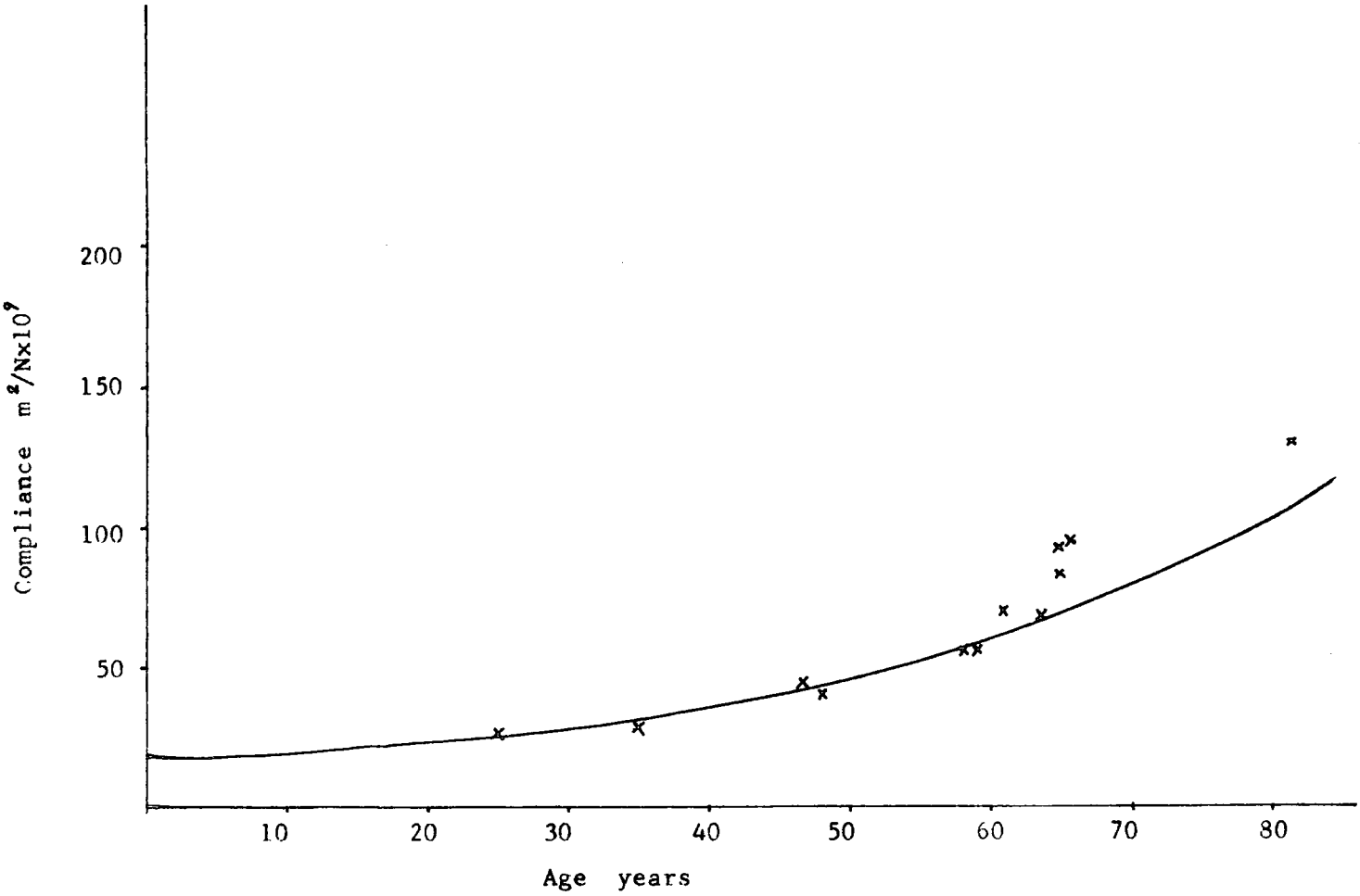


Fig7.62 Mean values of specimens stored at +4°C, adjusted mean values of specimens stored at -20°C and eq. (ix).

In addition to the effect of age and storage on compliance, the effect of biochemistry can be investigated using the results already discussed in Chapter 6. Figs 7.63 to 7.66 show the relationship between compliance and water content, Uronic Acid % dry weight and Uronic Acid % wet weight. The results are separated in specimens stored at - 20°C and + 4°C to elininate the effect of storage already described. As can be seen, there is no apparent correlation between compliance and any of these variables and statistical analysis confirms this lack of correlation.

If the results are separated in normal and abnormal as described in Chapter 6 the relationship between compliance and age are as shown in Figs 7.67 and 7.68, again the results being split into those stored frozen and those unfrozen. Because of the small number of specimens in each of the four groups, it is not possible to perform any useful statistical analysis of this data but from a qualitative point of view the relationship between compliance and age does not seem to be different for normal and abnormal specimens in either the frozen or non-frozen groups.

The results of this investigation do not indicate that the compliance is dependent on the biochemical composition of the tissue. However, it should be remembered that the Uronic Acid content of all the specimens is within the range for normal tissue and therefore none of the specimens tested can be classified as severely osteoarthrotic.

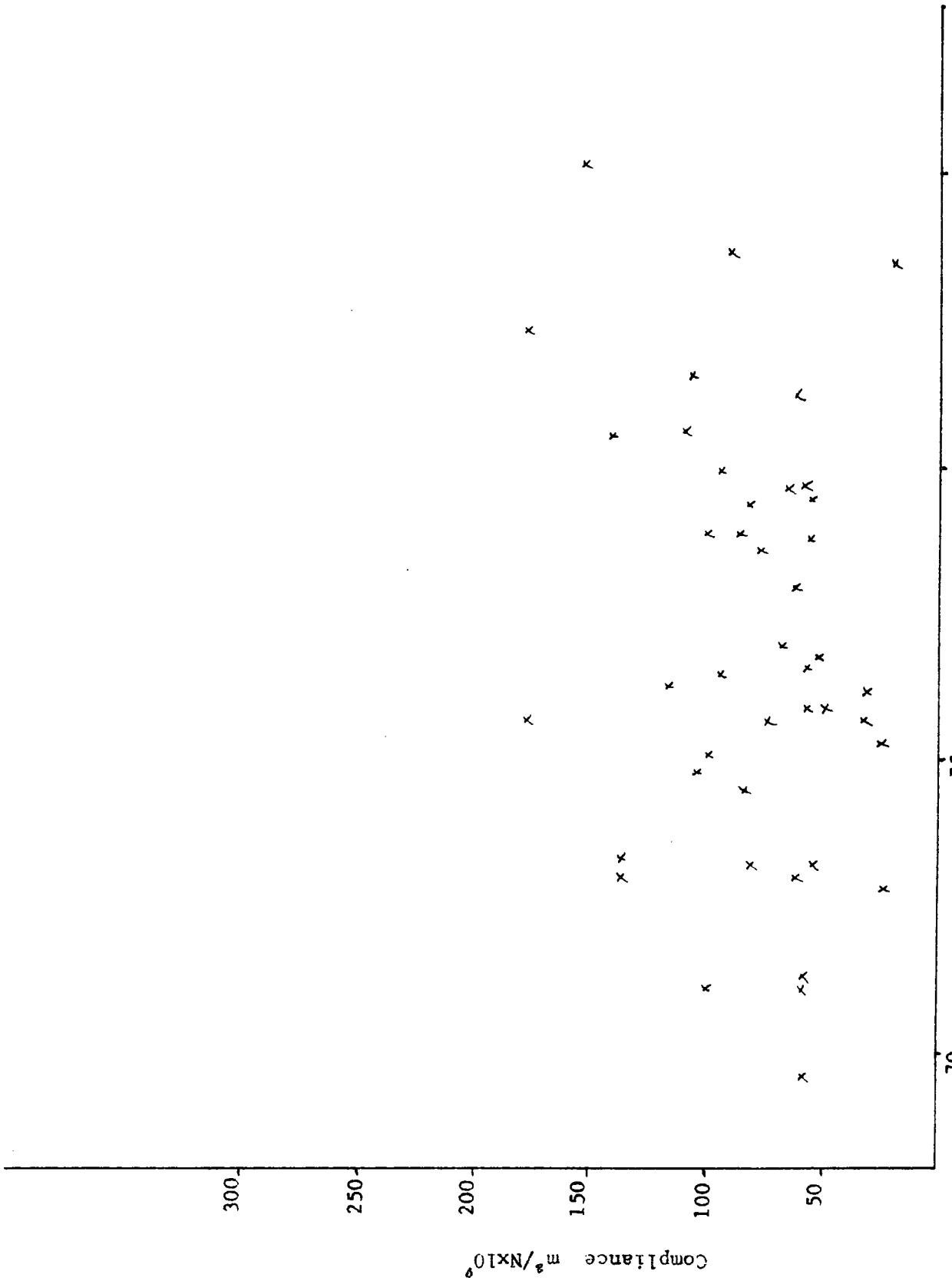


Fig7.63 Variation in compliance with water content (-20°C).

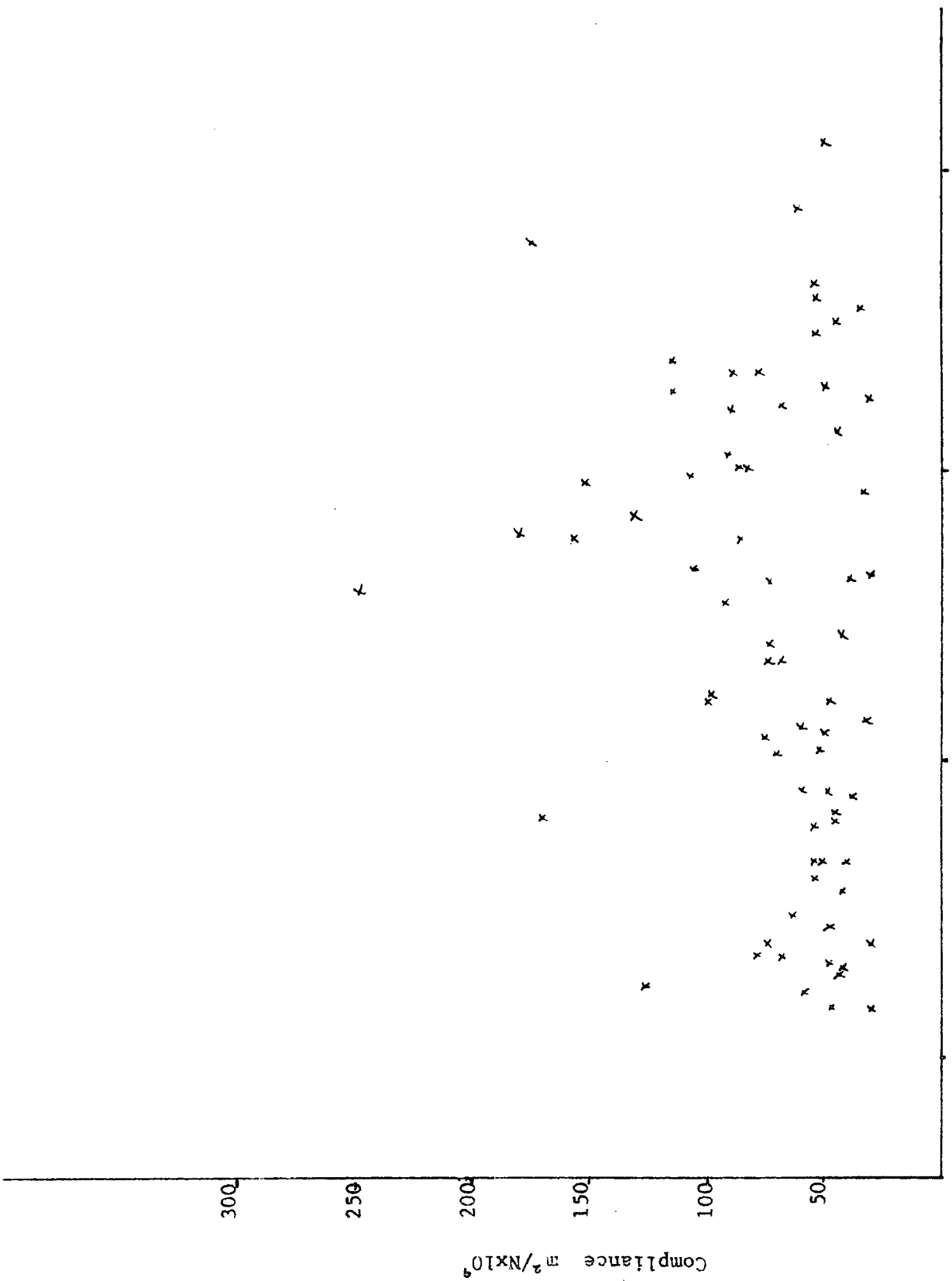


Fig7.64 Variation in compliance with water content (+4°C).

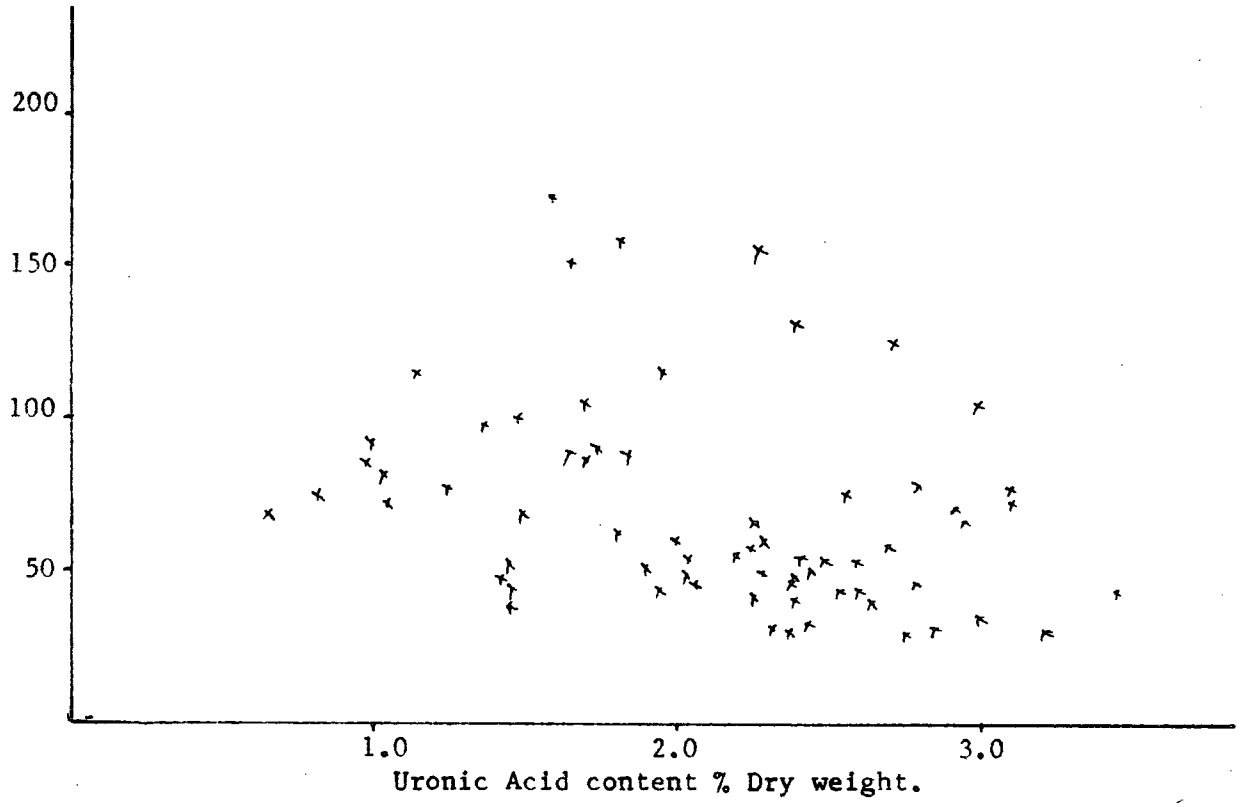


Fig7.65 Variation in compliance with Uronic Acid (+4°C).

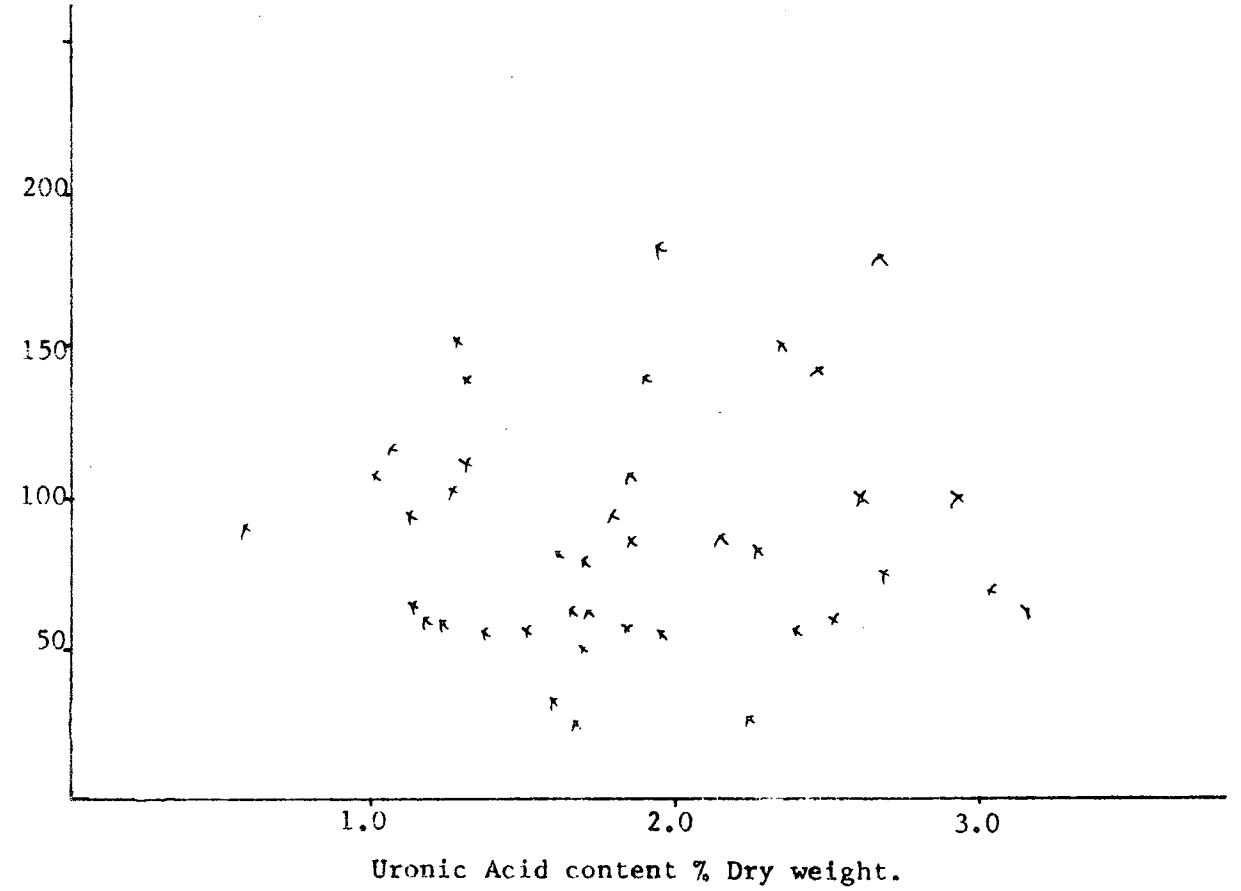


Fig7.66 Variation in compliance with Uronic Acid (-20°).

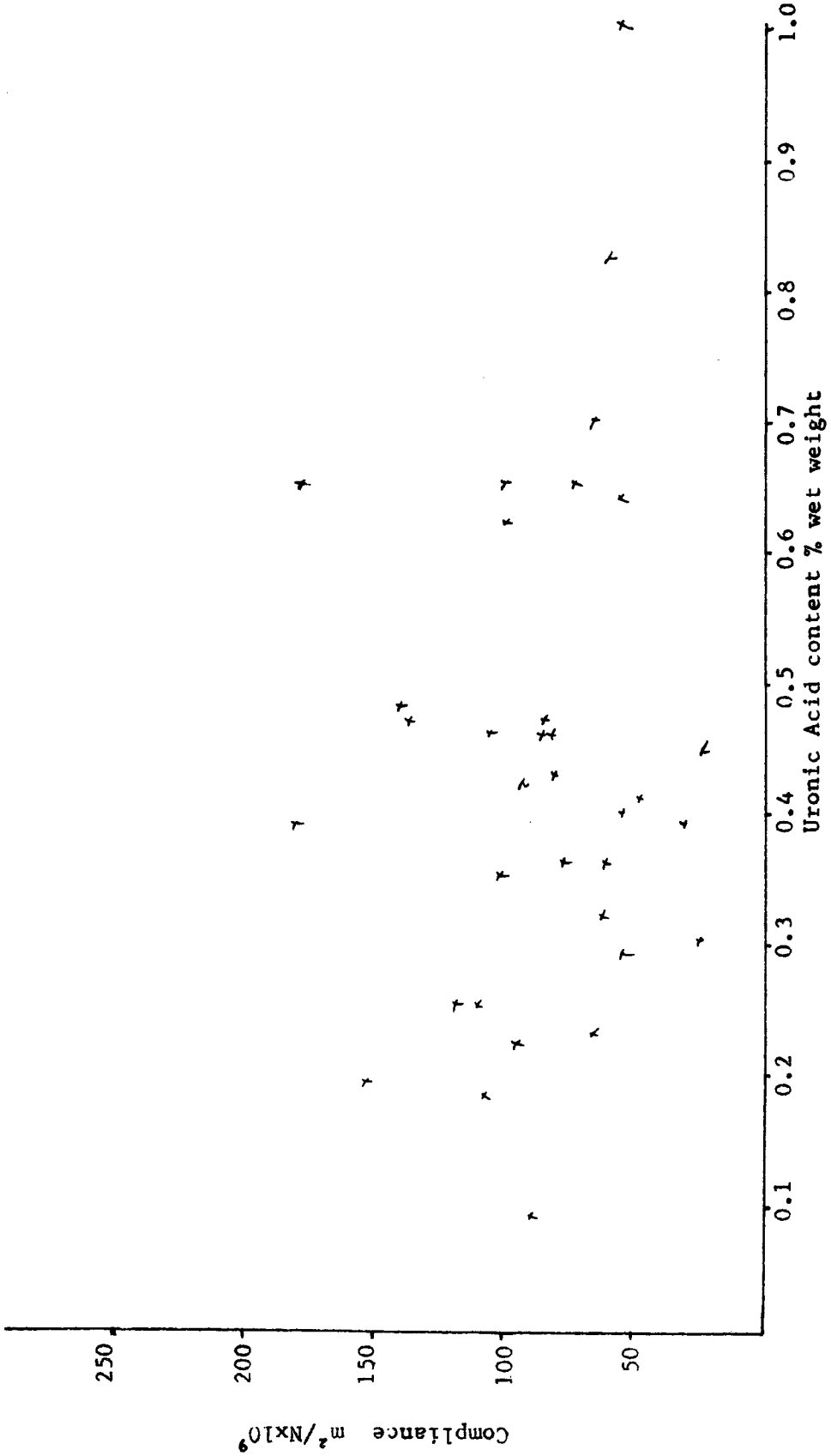


Fig7.67 Variation in compliance with Uronic Acid (-20°C).

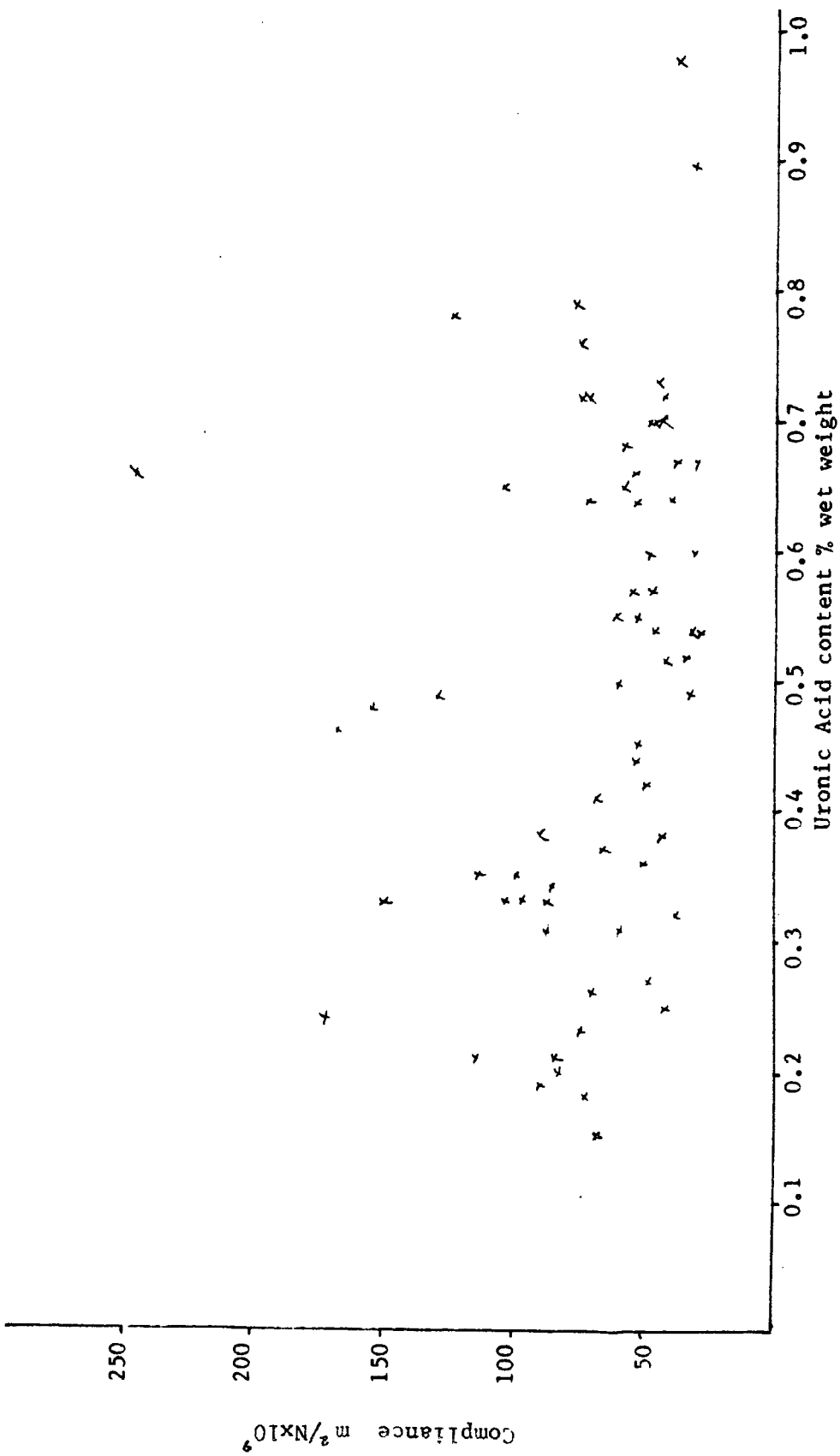
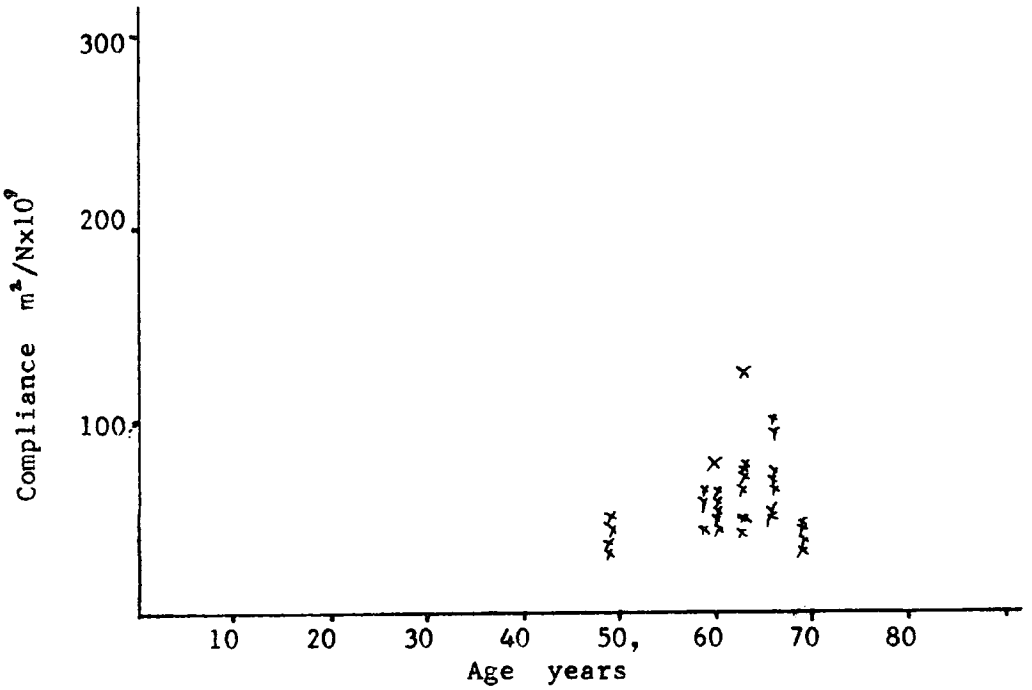
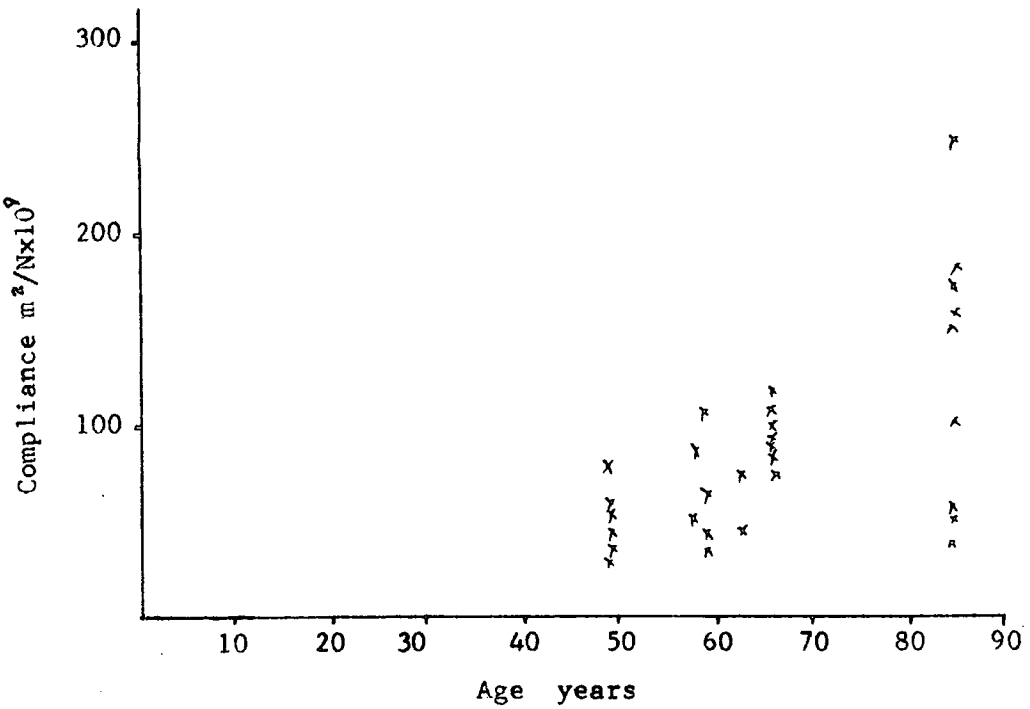


Fig7.68 Variation in compliance with Uronic Acid (+4°C).

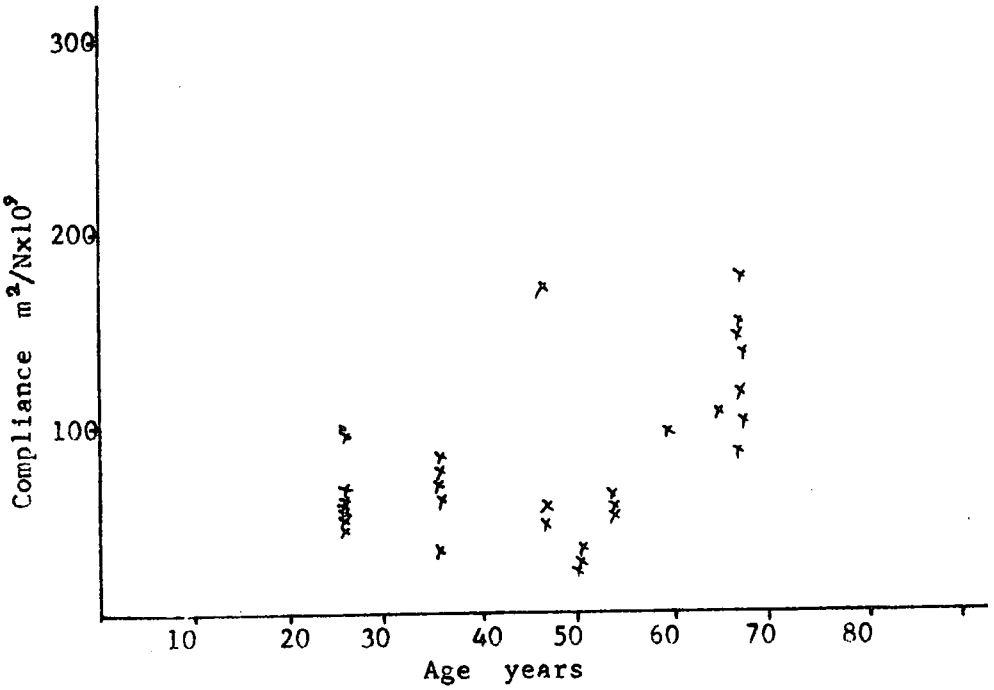


Normal specimens

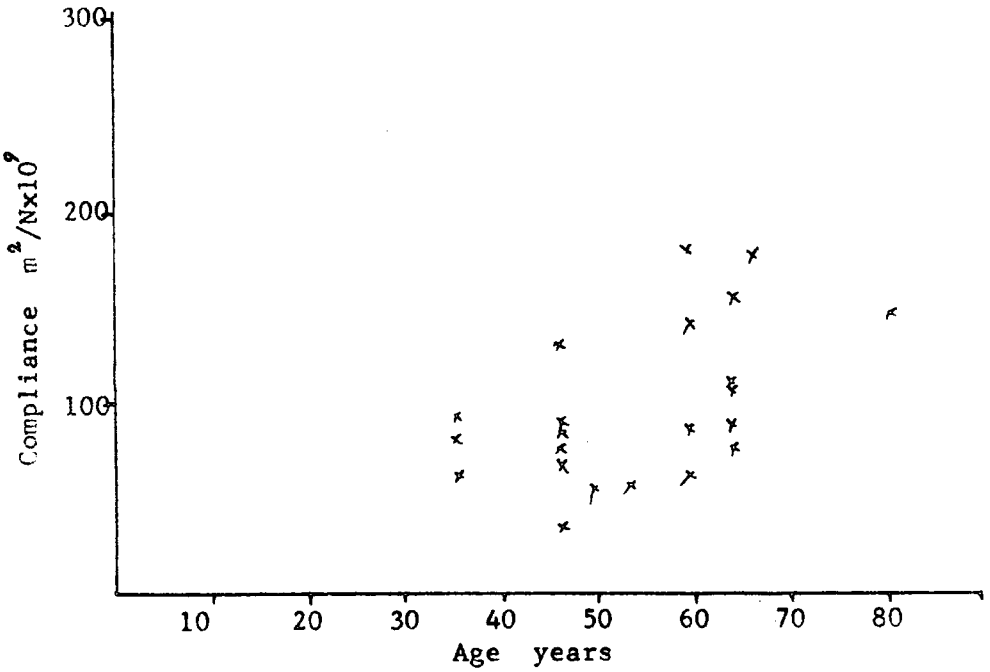


Abnormal specimens

Fig7.69 Variation in compliance with age (+4°C)



Normal specimens



Abnormal specimens

Fig7.70 Variation in compliance with age (-20°C)

7.7 Summary of Results

1. The compliance of articular cartilage is highest in the superficial zone, decreasing to a minimum in the mid-zone and increasing again slightly in the deep zone.

2. Compliance of articular cartilage is highly dependent on the age of the tissue, increasing with increasing age.

3. Compliance of articular cartilage is dependent on storage procedures; storage at - 20°C causes an increase in the compliance of specimens.

4. The response of articular cartilage to short term compression tests is independent of strain rate in the range 1 - 140% per second.

5. The response of articular cartilage to short term compression tests is repeatable if the specimen has been left to recover immersed in saline for 4 hours.

6. The response of articular cartilage to short term compression tests is the same whether the specimen is immersed in saline or in a vacuum.

7. There is no apparent systematic variation in compliance of articular cartilage with site on the patella.

8. There is no apparent correlation between compliance of articular cartilage and water content or Uronic Acid content of the specimen.

CHAPTER 8

CONCLUSIONS AND IMPLICATIONS

8.1 Introduction

The conclusions about relationships between variables were discussed in Chapter 7. In this chapter the relevance and implications of these conclusions to previous and future work in this field are discussed.

8.2 Storage Procedures

The results of this investigation strongly indicate that the mechanical response of articular cartilage to compressive loading is affected by the storage procedure used. As many workers in the past have tested tissue stored at - 20°C it must be assumed that their values for compliance will be higher or stiffness lower than if the specimen had not been frozen. It is suggested therefore that in any future study into the mechanical properties of articular cartilage fresh tissue should be used whenever possible. As storage of tissue is almost inevitably necessary during a study, it should be stored at above freezing point in a moist environment to prevent dehydration.

8.3 Ageing of Tissue

The compliance of normal articular cartilage is highly dependent on the age of the tissue but independent of either the water or Uronic Acid content. This would suggest that ageing factors such as shortening in chain length of the glycosaminoglycans and fatigue of collagen fibres may be more important in determining the overall mechanical response of normal tissue than the actual biochemical composition of the tissue. This seems a reasonable supposition as the mechanical properties of the tissue must be dependent on the interaction of the constituents and any alteration in this interaction is likely to affect the mechanical response. Once the interaction between the constituents is disrupted to such an extent that there is a large alteration in the biochemical composition, as in severe osteoarthrosis, then the mechanical properties may depend on the biochemistry of the tissue, as has been shown by previous workers.

This influence of age must be taken into consideration in any future investigation of mechanical properties of articular cartilage and may give a clue to the aetiology of osteoarthrosis as will be discussed in Section 8.4.

8.4 Relevance of Results to the Aetiology of Osteoarthrosis

The results of this investigation indicate that during normal activities deformation of articular cartilage is restricted to the surface zone of the cartilage. This supports the suggestion that one of the main functions of articular cartilage is to increase the contact area in a joint, thus reducing the stress in the underlying bone.

However, if unusually high loads are applied to articular cartilage deformation will occur in the mid-zone and the collagen fibres present there will be subjected to tensile strain. It has been shown that not only do the collagen fibres in the mid-zone exhibit lower ultimate tensile stress levels than those in the surface zone but that collagen is fatigue prone and there is a decrease in tensile fatigue resistance with age (Kempson 1973, Weightman 1975, 1976).

Based on the observations made during this investigation and the above statements as to the tensile and fatigue properties of collagen, the following hypothesis as to the aetiology of osteoarthrosis is proposed.

During the lifetime of an individual the articular cartilage in the load bearing joints may be subjected to occasional extremely high loading such as occurs when jumping from a height or periods of above average loading, such as during running. In either of these cases the collagen fibres in the mid-zone will be subjected to tensile strain.

If the occasional loading is high enough or the period of above average loading long enough tensile failure of collagen fibres will occur in the mid-zone. The likelihood of this happening will increase with age due to the decrease in the fatigue resistance of collagen. This disruption of the fibre network will result in an increase in water content in the mid-zone, due

to swelling of the glycosaminoglycans (which is the first biochemical alteration observed in experimentally induced osteoarthroses (McDevitt and Muir 1974, 1975, 1976, McDevitt et al 1977) and an increase in the normally low compliance of the mid-zone.

Once the compliance of the mid-zone rises the tensile strains in the surface zone during normal loading will be increased, due to increased deformation of the mid-zone. This is shown diagramatically in Figs 8.1 and 8.2.

The increased tensile strains in the surface layer will result in tensile failure of the collagen fibres and ultimately total disruption of the fibrous network will become evident. It is therefore suggested that progressive fibrillation which ultimately leads to osteoarthrosis is in fact initiated by tensile failure of collagen fibres in the mid-zone. Non-progressive fibrillation which occurs mainly in non-load bearing areas of the joint is probably due to 'wear and tear' of the articular surface and, as the mid-zone structure is not affected, is unlikely to lead to complete disruption of the cartilage.

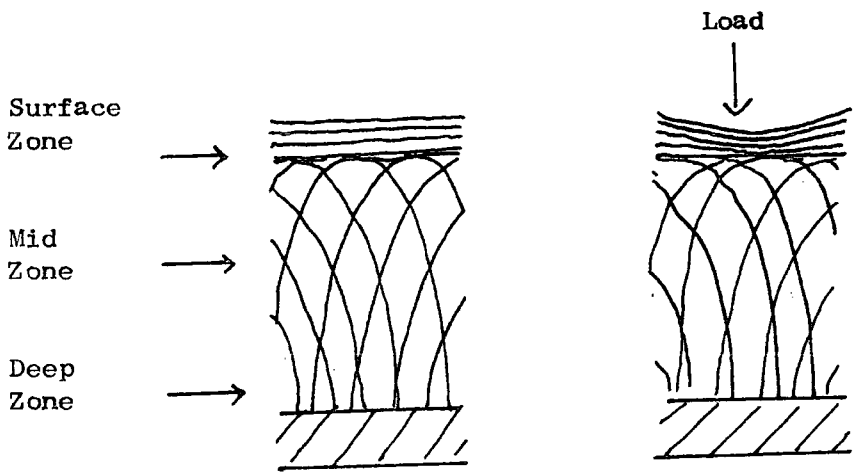


Fig8.1 Loading of normal cartilage.

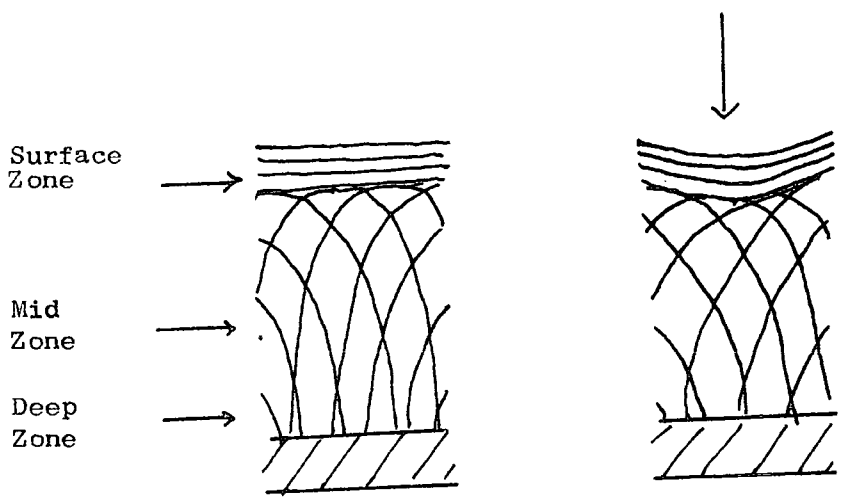


Fig8.2 Loading of abnormal cartilage.

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APPENDIX A

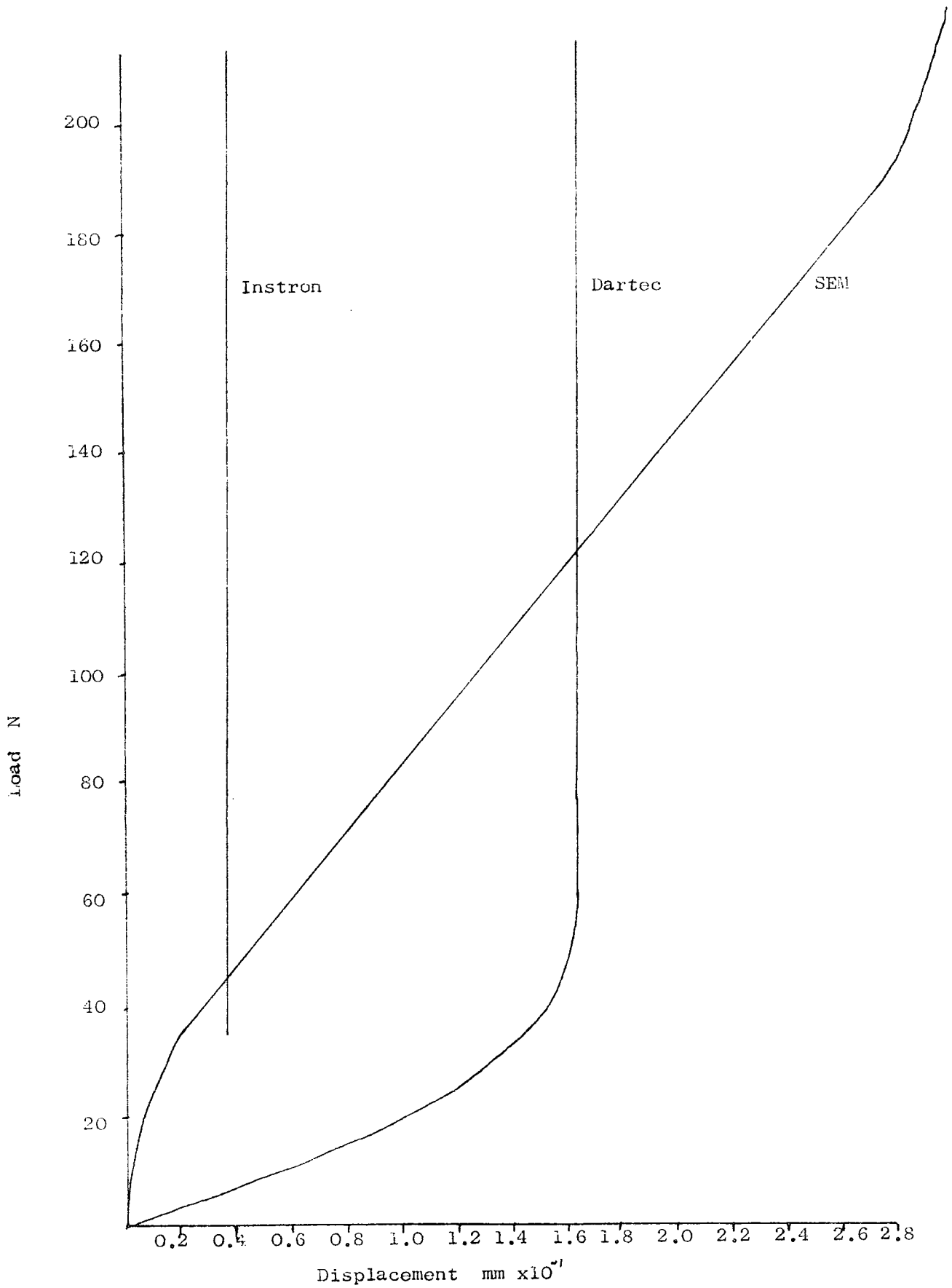
Specimen	Age (Years)	Water Content (% w/wt)	Uronic Acid (% d/wt)	Uronic Acid (% w/wt)	Storage	Compliance ($m^2/Nx10^9$)
A1	25	78.9	2.93	0.62	FR	78
A2	25	76.8	4.36	1.01	FR	52
A3	25	73.0	3.15	0.85	FR	61
A4	25	75.9	4.37	1.05	FR	57
A5	25	75.1	2.61	0.65	FR	98
A7	25	77.0	3.03	0.7	FR	67
A8	25	75.8	1.68	0.41	FR	48
A9	25	78.8	1.35	0.29	FR	55
A10	25	73.2	2.4	0.64	FR	55
C1	58	73.4	2.41	0.64	NOT FR	41
C2	58	74.6	2.69	0.68	NOT FR	59
C3	58	82.5	1.45	0.25	NOT FR	44
C8	58	81.3	2.85	0.54	NOT FR	32
C10	58	84.5	2.01	0.31	NOT FR	61
C11	58	80.5	1.7	0.33	NOT FR	106
C12	58	73.4	3.9	1.04	NOT FR	52
D1	46	81.1	1.95	0.37	FR	67
D2	46	82.8	3.02	0.52	FR	35
D3	46	72.3	2.04	0.57	FR	48
D4	46	81.1	1.63	0.31	FR	89
D7	46	81.7	1.24	0.23	FR	77
D8	46	74.0	2.2	0.57	FR	55
D9	46	80.1	1.7	0.34	FR	87
D10	46	74.1	1.81	0.47	FR	168
D11	46	79.3	2.39	0.49	FR	130
G1	48	80.7	1.95	0.38	NOT FR	44
G2	48	83.2	3.9	0.66	NOT FR	54
G4	48	72.9	2.6	0.7	NOT FR	43
G6	48	82.4	2.5	0.44	NOT FR	54
G7	48	70.9	2.32	0.6	NOT FR	32
G9	48	78.3	2.38	0.52	NOT FR	30
G10	48	75.6	2.45	0.6	NOT FR	50
G11	48	75.8	2.76	0.67	NOT FR	32
G12	48	79.7	2.44	0.49	NOT FRR	33
H1	68	71.6	3.46	0.98	NOT FR	43

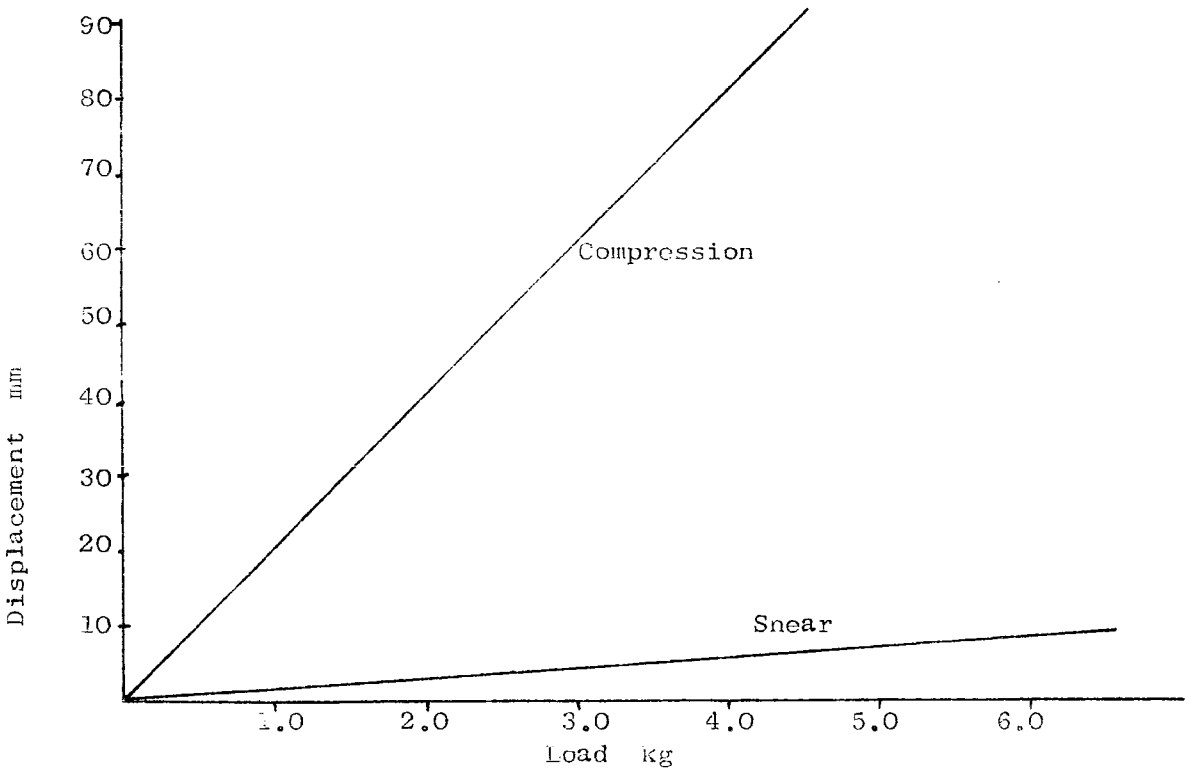
Specimen	Age (Years)	Water Content (% w/wt)	Uronic Acid (% d/wt)	Uronic Acid (% w/wt)	Storage	Compliance ($m^2/Nx10^9$)
H4	68	74.5	2.63	0.67	NOT FR	39
H5	68	74.2	2.07	0.54	NOT FR	46
H6	68	72.0	3.22	0.9	NOT FR	32
L1	61	71.3	2.72	0.78	NOT FR	125
L2	61	72.5	1.8	0.5	NOT FR	63
L3	61	75.5	3.11	0.76	NOT FR	77
L4	61	76.1	2.4	0.57	NOT FR	48
L5	61	77.3	2.26	0.52	NOT FR	43
L6	61	74.1	2.8	0.73	NOT FR	46
L7	61	78.2	2.92	0.64	NOT FR	73
L9	61	72.0	2.57	0.72	NOT FR	75
L10	61	76.8	3.12	0.72	NOT FR	73
Q1	59	70.9	2.39	0.7	NOT FR	48
Q2	59	71.7	1.43	0.7	NOT FR	48
Q3	59	73.4	2.43	0.64	NOT FR	55
Q4	59	73.1	2.04	0.55	NOT FR	55
Q5	59	71.2	2.25	0.65	NOT FR	59
Q6	59	75.7	2.28	0.55	NOT FR	61
Q8	59	71.5	2.53	0.72	NOT FR	44
Q9	59	71.8	2.8	0.79	NOT FR	78
R1	65	75.2	1.04	0.26	NOT FR	71
R2	65	81.4	1.14	0.21	NOT FR	115
R3	65	77.8	1.72	0.38	NOT FR	91
R4	65	77.1	0.81	0.18	NOT FR	73
R6	65	80.1	1.01	0.2	NOT FR	83
R7	65	76.8	0.63	0.15	NOT FR	69
R8	65	81.9	1.94	0.35	NOT FR	115
S1	66	76.3	1.04	0.25	FR	115
S3	66	73.0	1.31	0.47	FR	137
S4	66	74.5	1.84	0.47	FR	83
S5	66	75.7	2.66	0.65	FR	177
S6	66	73.3	1.89	0.5	FR	137
S7	66	65 7	2.34	0.78	FR	148
S9	66	71.1	1.23	0.35	FR	100
T1	53	71.3	1.17	0.33	FR	59

Specimen	Age (Years)	Water Content (% w/wt)	Uronic Acid (% d/wt)	Uronic Acid (% w/wt)	Storage	Compliance ($m^2/Nx10^9$)
T2	53	71.1	1.21	0.35	FR	59
T5	53	79.7	1.83	0.37	FR	56
T6	53	69.5	2.51	0.77	FR	59
U1	57	78.9	0.91	0.21	NOT FR	85
U2	57	85.6	1.9	0.27	NOT FR	50
V1	35	75.7	2.68	0.65	FR	73
V2	35	78.0	1.64	0.36	FR	61
V3	35	80.0	1.12	0.22	FR	93
V4	35	76.6.8	1.5	0.35	FR	57
V5	35	79.7	1.14	0.23	FR	63
V6	35	79.4	2.26	0.46	FR	81
V7	35	75.7	1.59	0.39	FR	32
V9	35	73.2	1.6	0.43	FR	81
W1	49	79.5	1.96	0.4	FR	54
W4	49	72.8	1.67	0.45	FR	23
W6	49	75.3	2.24	0.3	FR	25
X1	81	79.8	1.64	0.33	NOT FR	151
X2	81	81.5	2.28	0.42	NOT FR	50
X4	81	78.9	2.23	0.48	NOT FR	156
X5	81	78.2	1.45	0.32	NOT FR	39
X9	81	77.0	2.86	0.66	NOT FR	247
X10	81	83.0	2.62	0.45	NOT FR	54
X12	81	84.9	1.58	0.24	NOT FR	174
Y1	65	81.7	1.83	0.33	NOT FR	89
Y2	65	76.1	1.47	0.35	NOT FR	100
Y4	65	76.2	1.37	0.33	NOT FR	98
Y5	65	75.3	1.45	0.36	NOT FR	52
Y6	65	72.8	1.49	0.41	NOT FR	69
Y7	65	78.4	3.0	0.65	NOT FR	106
Y9	65	80.3	0.98	0.19	NOT FR	91
Z2	64	81.6	1.0	0.18	FR	106
Z3	64	80.7	1.29	0.25	FR	109
Z5	64	74.8	1.84	0.46	FR	104
Z6	64	78.6	1.68	0.36	FR	77
Z7	64	85.3	1.26	0.19	FR	151

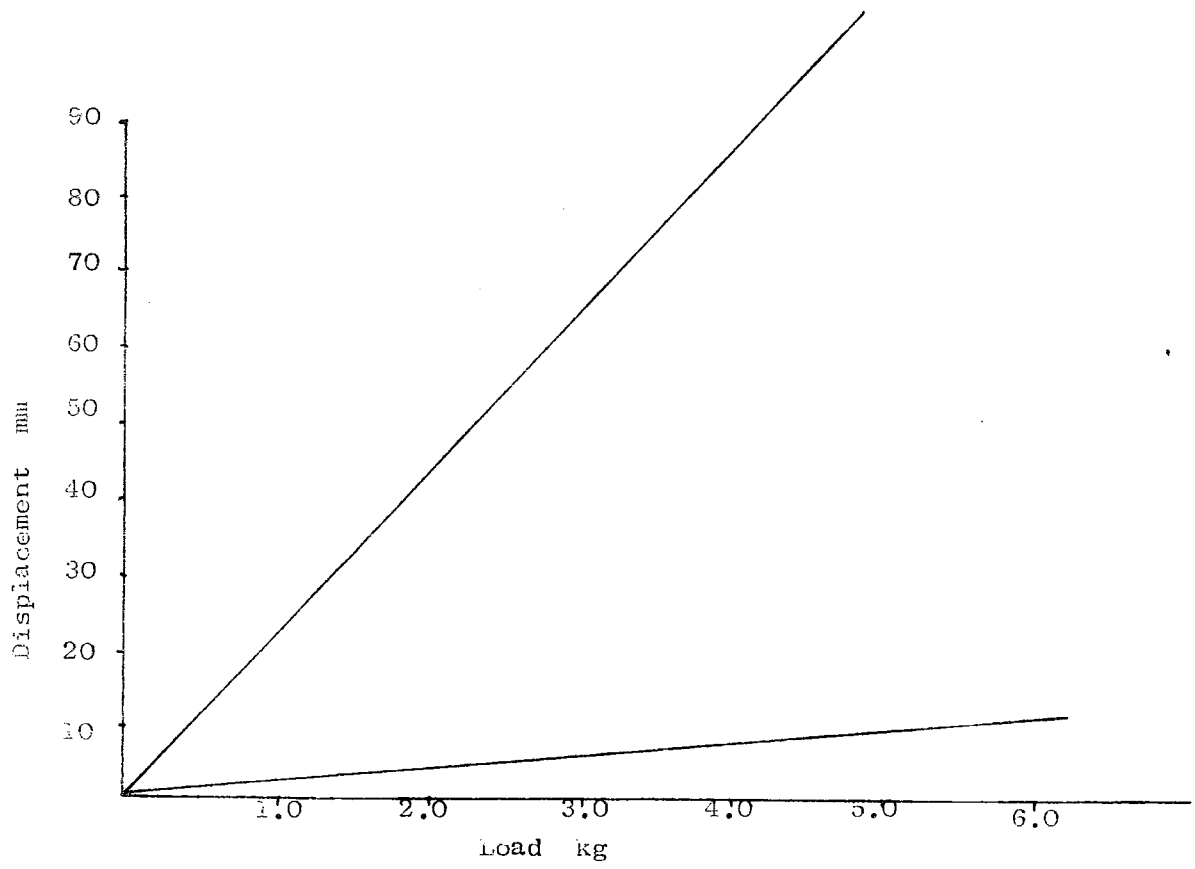
Specimen	Age (Years)	Water Content (% w/wt)	Uronic Acid (% d/wt)	Uronic Acid (% w/wt)	Storage	Compliance ($m^2/Nx10^9$)
Z8	64	83.8	0.57	0.09	FR	89
AA1	59	76.5	1.79	0.42	FR	93
AA3	59	78.9	2.16	0.46	FR	85
AA4	59	80.6	2.46	0.48	FR	140
AA5	59	80.0	1.94	0.39	FR	180
AA8	59	81.3	1.71	0.32	FR	61

APPENDIX B


200
180
160
140
120
100
80
60
40
20
Load N
Instron
Dartec
SEM
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Displacement mm x10⁻¹



Guages 1 and 2



Guages 3 and 4

APPENDIX C

DARTEC RAMP TEST

1. Align load cell and top jaw.
2. Place rig in machine.
3. Position cross-head so that, when bottom jack is in lowest position, the two specimen holders are just separated (1mm).
4. Raise bottom jack.
5. Insert specimen, perspex cylinder and saline.
6. Lower bottom jack so that upper stub is just above the specimen.
7. Position external extensometer using screw and dial.
8. Switch from stroke to external control.
9. Switch to run.
10. Select -ve ramp.
11. Select hold.
12. Position bottom jack so that upper stub is just above the specimen, by moving extensometer.
13. Select +ve ramp. Record load/disp.
14. Select hold.
15. Select -ve ramp.
16. Raise bottom jack so that upper stub is several mm above specimen by moving extensometer.
17. Switch to Stop/Zero.
18. Switch to Stroke Control
19. Raise bottom jack
20. Remove specimen