MECHANICAL PROPERTIES OF HUMAN ARTICULAR CARTILAGE

by

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ABSTRACT

Articular cartilage is the deformable tissue which covers the bone extremities of synovial joints. It appears to have two important functions:

Firstly, it limits the stresses applied to the underlying or subchondral bone, due to its ability to deform, and secondly, cartilage provides the smooth articulating surfaces necessary for a joint to behave as an efficient bearing.

The stiffness of cartilage will obviously influence the first of these two functions, and may well play a part in joint lubrication. Progressive softening of cartilage probably indicates early degenerative changes, which in time may lead to mechanical failure of the cartilage, and thence to osteoarthrosis.

Most published work concerning the mechanical properties of articular cartilage has dealt mainly with the patella, and most comparative studies have been performed between degenerate and visibly normal cartilage. Thus there is a lack of information in the published literature, concerning the mechanical properties of cartilage on surfaces other than the patella, and it has not been established whether a typical pattern of cartilage stiffness variation exists in the normal, healthy state.

The object of this investigation was initially to determine whether a pattern of stiffness variation exists for cartilage on the human femoral head. If a pattern was observed, then its relationship to other variables such as age, sex and the severity of localised degeneration, was to be determined. The relationship between the stiffness and chemical components of cartilage was to be investigated, as was the relationship between the stiffness and permeability to water.

Indentation tests were performed normal to the articular surface using two cylindrical indenters of the same diameter but with different end profiles; one being a plane end and the other a hemispherical end.

Sixteen femoral heads were subjected to detailed indentation surveys. Layered maps and histograms of the variation of both indentation and creep modulus, at two seconds after load application, were drawn for each femoral head.

The results revealed that a reproducible pattern of stiffness exists for cartilage on the femoral head. The stiffest cartilage is usually located in a band which extends over the superior aspect and around to the anterior and posterior facets. The softest cartilage is invariably located at or near to the margin of the fovea, and near to a localised area of degeneration.

An overall marked softening of the entire visually normal cartilage surface was observed in some femoral heads. This softening was progressive, and closely related to the severity of localised areas of degeneration, even though they were not tested. No significant dependence on age or sex was recorded.

A strong correlation was however recorded between the creep modulus and total mucopoly saccharide content of the tested areas. In addition a strong negative correlation was recorded between the creep modulus and permeability to water.

In the course of the investigation considerable attention was paid to experimental controls and associated experiments, in order to ensure maximum accuracy of the results.

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CHAPTER 1

INTRODUCTION

(1) Qualitative description of articular cartilage

Articular cartilage is the resilient tissue which covers the bone extremities of synovial joints.[1] In the weight bearing joints of humans, the thickness of cartilage[2] varies approximately between 1mm and 5mm., but in the hip joint it is commonly between 1mm and 3mm thick. During the early decades of life, it is bluish-white in colour, and to a limited extent, translucent. With increasing age however, the colour becomes more yellowish, and there is an increase in opacity. In the normal, healthy state, the texture of cartilage resembles that of a medium stiff rubber, and the articular surface is smooth and slippery to the touch.

Structurally, cartilage consists mainly of collagen fibres embedded in a gel of mucopolysaccharides (chondroitin sulphate and keratan sulphate), and water. This gel is commonly referred to as the matrix. The physico-chemical studies of Maroudas (1968) have shown that the matrix consists of a network of linear polyanions, with fixed negative changes, and water. As such, it may be considered to be a typical ion-exchange material. The water is present partly in the form of solvation shells, and partly as free water with mobile cations and anions in solution. Maroudas has also shown that the transport of free water through cartilage occurs as a result of both permeability under load, and diffusion, in which case it is governed by the conditions of Donnan equilibrium, [McCutchen (1962), Linn and Sokoloff (1965) and Maroudas (1968)].

---

1. For a detailed description of synovial joints, the reader is referred to a standard textbook, such as those of Barnett, C.H., Davies, D.V., and MacConaill, M.A. (1961) or Collins, D.H., (1949) which are listed in the Bibliography, pp. 121 - 126.

2. Unless otherwise stated, cartilage is to be interpreted as articular cartilage.

Interspersed throughout the matrix, and between the collagen fibres, are the chondrocytes, or cartilage cells.

The arrangement of chondrocytes is highly ordered, and unique to this tissue. This structural organisation will be described more fully, later in the text.

2. The mechanical functions of articular cartilage

Essentially, cartilage has two mechanical functions: Firstly it limits the stresses applied to the bone extremities to safe working values, because of its ability to deform under load and thereby distribute the load; Secondly, in conjunction with synovial fluid, it provides joints with extremely efficient bearing surfaces. The mode of joint lubrication is not yet fully understood, and indeed, it has been shown by Ropes et al. (1947), and Charnley (1959), that synovial fluid is only a moderately good lubricant on surfaces other than cartilage.

(3) The process of cartilage degeneration

It is immediately apparent from Para. 2 of this Chapter that healthy cartilage is vital to the efficient functioning of synovial joints. Any degenerative changes which might impair this efficiency, are therefore of immediate importance.

From a survey of five hundred and thirty six hips taken from human cadavers, Byers et al. (1969) has defined a sequence of visible degenerative changes of cartilage in the hip, and in particular on the femoral head, which is the subject of this investigation.

These changes have been classified according to both the site affected, and to the appearance of the degenerative tissue. The classification according to appearance is illustrated by the sequence of photographs shown in



Fig. 1a. Normal (N)



Fig. 1b. Surface Flaking Grade 1.

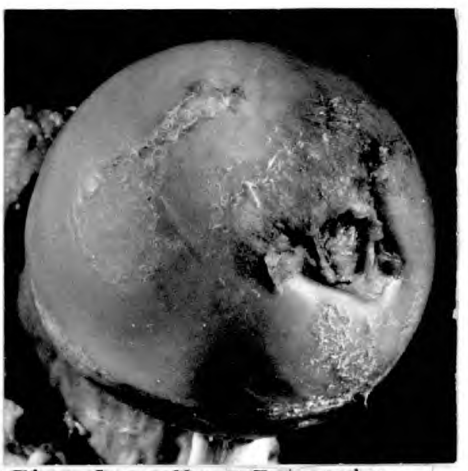


Fig. 1c. More Extensive Surface Flaking Grade 1 - 2



Fig. 1d. Fibrillation and Partial Loss of Tissue Thickness Grade 2



Fig. 1e. Ulceration with Full Depth Tissue Loss Grade 3

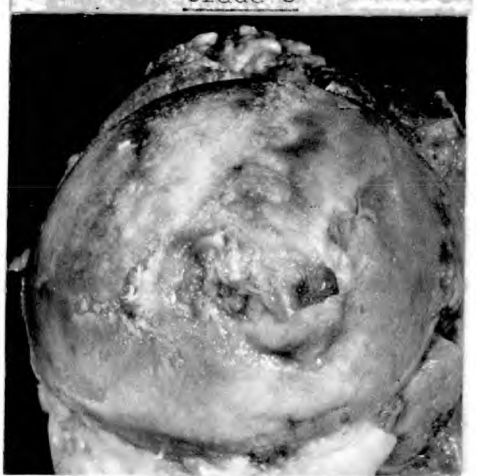


Fig. 1f. Osteoarthrosis Grade 4.

Fig. 1. Classification of Femoral Heads According to the Severity of Cartilage Degeneration

Figs. 1a to 1e, (facing p. 3), which show the degenerative changes in order of increasing severity. Each femoral head is graded, and the diagrams given in Fig. 2 (facing p. 4) indicate the different views of Fig. 1. Fig. 1a shows a normal femoral head on which the cartilage surface is smooth and unbroken, (the dark area in each photograph is the root of the severed ligamentum teres). Figs. 1b and 1c show the earliest visible signs of cartilage degeneration, in which the articular surface has a slightly roughened or fibrillated appearance, in localised areas. Fig. 1d shows gross fibrillation, again over a localised area of the cartilage surface. Partial loss of the tissue thickness has also occurred. Finally, Fig. 1e shows an area of ulcerated cartilage. Full thickness loss of the tissue has occurred, and the sub-chondral bone is covered by granulation tissue. Each of the stages of degeneration shown in Fig. 1 is commonly seen on cadaveric femoral heads after approximately the fifth decade of life. It is important to emphasize however, that a relatively large area of cartilage, mainly situated on the superior surface and posterior and anterior facets of a femoral head, is visibly normal, despite the other areas of degeneration.

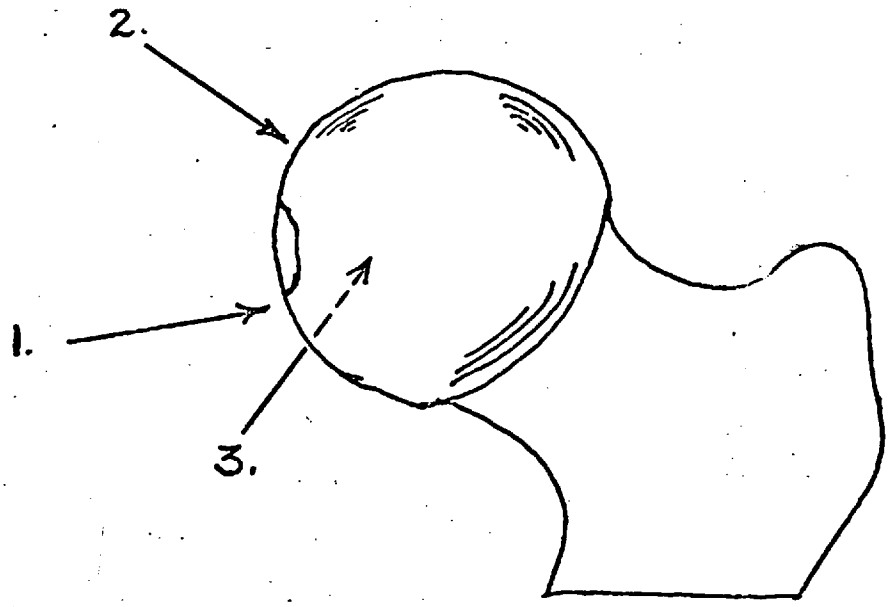
A further stage of degeneration, which is observed in comparatively few hips, is shown in Fig. 1f. Typical of this condition is a large area of exposed and eburnated bone (not covered by granulation tissue), with similar changes on the opposing acetabular surface.

This advanced stage of degeneration is characteristic of thedisease known as osteoarthrosis.[1]

Whereas a joint displaying the degenerative changes seen in Figs. 1a to 1e usually functions normally or apparently so, a joint suffering from osteoarthrosis is stiff and painful. The sociological problem and

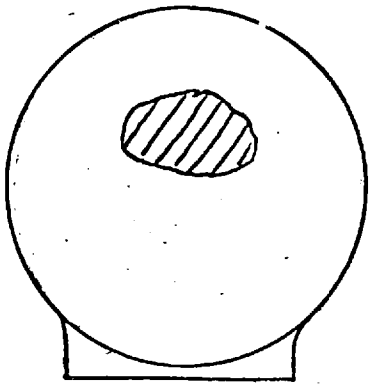
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1. Osteoarthrosis, osteoarthritis, degenerative joint disease, arthritis deformans are all synonymous.



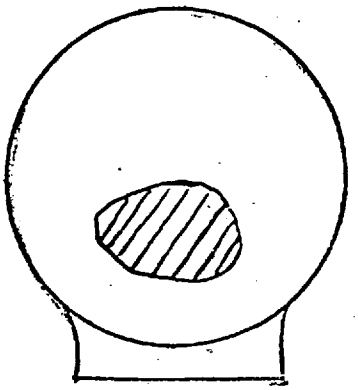
DIAGRAMMATICAL VIEW OF A RIGHT FEMORAL HEAD: ANATOMICAL POSITION.

1.



FIGS. 1a AND 1d.

2.



FIGS. 1b, 1e AND 1f

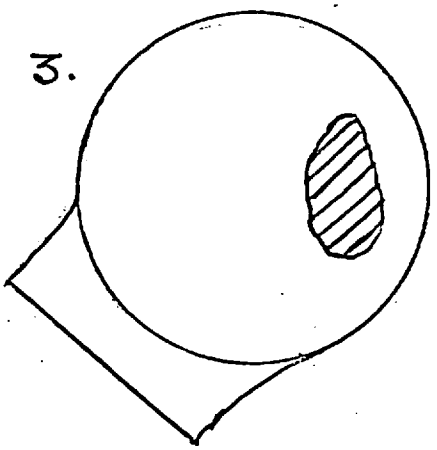


FIG. 1c.

FIG. 2. SHOWING THE VIEWS OF A RIGHT FEMORAL HEAD IN FIG. 1.

personal suffering caused by this disease are very serious.

The aetiology of osteoarthrosis has not been fully defined as yet. The progression of visible degeneration in localised areas according to both Byers and Collins, shown in Figs. 1a to 1f, was accepted in this investigation, and it was hoped to seek concomi tant changes in the visibly normal cartilage. If such changes were shown to exist, then it was intended to assess their role in the development of osteoarthrosis.

The published results of most previous investigations concerning the mechanical properties of articular cartilage will now be presented and discussed.

(4) Early investigations

A survey of the literature revealed that some of the earliest experiments concerning the mechanical properties of cartilage were performed on rib cartilage. Rauber (1876), and Triepel (1902) determined an elastic modulus for rib cartilage both in tension and compression, assuming it to obey Hooke's law. The range of modulus values which they obtained was 87.5 to 104.6 Kg/cm^2 by Rauber, and 83.6 to 154 Kg/cm^2 by Triepel. It is interesting that these values are in close agreement both with each other, and with the result of 78.6 Kg/cm^2 quoted by Sokoloff (1966), in his more refined experiments.

Two of the earliest observations that the deformation of articular cartilage varied according to its location were made by Fürbringer (1888), and Lubosch (1910). Both of these investigators suggested that the cartilage on articular heads, for example the femoral head, or the head of the humerus, possessed a different elasticity from that in the corresponding articular socket. These observations must be treated with caution however, since the experimental methods employed, were rather crude compared to present day techniques.

Interest in the functional importance of articular cartilage was shown by Braune and Fischer (1891), and Fick (1911). Braune and Fischer demonstrated the deformability of cartilage experimentally, particularly on the knee joint, and in addition they emphasized the importance of cartilage in maintaining joint congruity. Fick assumed that articular cartilage possessed the same elasticity as rib cartilage, and drew attention to the 'elastic spring arrangement' between the bone ends.

Thus, the combined facts that articular cartilage is deformable in compression, and that the deformability varies, were established. The function of cartilage as a 'cushion' between the bone extremities had been realised.

A significant development which influenced the work of several later investigators, was the construction of an apparatus by Schäde (1912), which enabled him to perform indentation tests on a variety of tissues, and to measure the deformations produced. Schäde's elastometer, as it may be referred to, consisted of a vertical indenter with a small load platform at the top. The indenter spindle was guided so that its motion was vertical only, and attached to it was the arm of a long pointer, which recorded indentation of the tissue on a rotating drum. [Very little attempt was made to eliminate dry friction, which is a problem when measuring extremely small deformations.]

Schäde recorded incomplete recovery of the deformation after removal of an applied load. He suggested, on the strength of this observation, that the elasticity of a tissue could be represented by the ratio of the persistent deformation to the total deformation. The more complete was the recovery, the greater was the elasticity of the material. It will be shown later, that from an applied load within the physiological range, incomplete

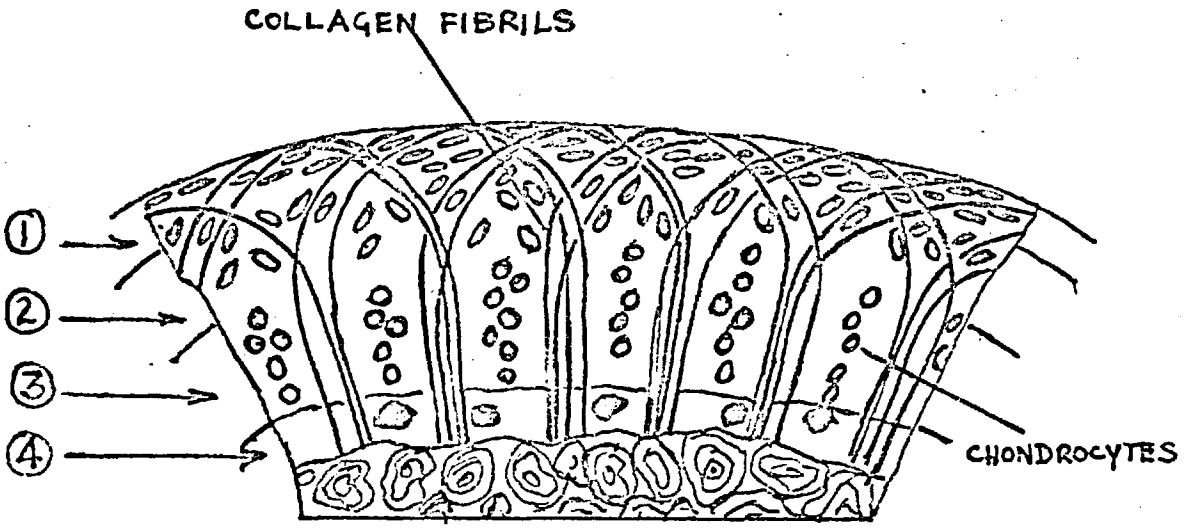


FIG.3.2 DIMENSIONAL DIAGRAM OF THE STRUCTURE OF ARTICULAR CARTILAGE. SHOWING PARTICULARLY THE COLLAGEN ARCADES (3D).

KEY:

1. SUPERFICIAL ZONE : CHONDROCYTES ARE FLATTENED AND ELONGATED PREDOMINANTLY PARALLEL TO THE ARTICULAR SURFACE. COLLAGEN FIBRILS ARE ALSO ORIENTATED PARALLEL TO THE ARTICULAR SURFACE.

2. TRANSITIONAL ZONE : CHONDROCYTES ARE ELLIPTICAL IN SHAPE AND ORIENTATED OBLIQUELY TO THE ARTICULAR SURFACE. COLLAGEN FIBRILS ARE ALSO MAINLY ORIENTATED OBLIQUELY.

3. PROLIFERATIVE ZONE : CHONDROCYTES ARE APPROXIMATELY SPHERICAL IN SHAPE AND ARRANGED IN GROUPS OR COLUMNS, ORIENTATED PREDOMINANTLY RADIALLY OR NORMAL TO THE ARTICULAR SURFACE COLLAGEN FIBRES TEND TO GROUP TOGETHER TO FORM FIBRES, ORIENTATED RADIALLY.

4. CALCIFIED ZONE: CARTILAGE AND SUB-CHONDRAL BONE ARE CONTINUOUS IN THIS ZONE. LARGE, IRREGULAR, APPARENTLY DEAD CELLS ARE CHARACTERISTIC OF THIS ZONE.

recovery of cartilage is an experimental artefact, but several workers nevertheless subsequently accepted the criterion suggested by Schäde.

The detailed structure of cartilage and its relation to the mechanical environment within synovial joints, constituted a significant part of the outstanding work of Benninghoff (1924, 1925, 1925 and 1939). As a result of his investigations, Benninghoff established that articular cartilage consists of cells distributed throughout a matrix of collagen fibres, and a formless ground substance. The collagen fibres, it was suggested, are arranged in bundles, which form a three dimensional arcade system. The fibres in the deepest layers of cartilage are orientated predominantly normally to the articular surface, but with increasing proximity to the surface they become more oblique, until finally they are orientated predominantly parallel to the surface at, or near to, the latter. It was due mainly to the work of Benninghoff, that the idea of sub-dividing cartilage into four zones throughout its total thickness, according to the shape and distribution of the chondrocytes, was initiated. A diagram of the currently accepted structure, (after Benninghoff), together with definitions of the four zones is shown in Fig. 3 (facing p. 6).

It must be emphasized, that the existence of continuous arcades of collagen fibres, has not been positively proven. Bullough and Goodfellow (1968) using a method of polarised light microscopy, and Weiss et al.(1968), and Maroudas and Bullough (1968) using electron microscopy, have all confirmed the predominant orientations suggested by Benninghoff, but continuous arcades have not been demonstrated, MacConaill (1951), and McCall (1969), (personal communication, see Bibliography).

Finally Benninghoff suggested that the collagen fibre arrangement, particularly near to the articular surface, was such that the fibres con-

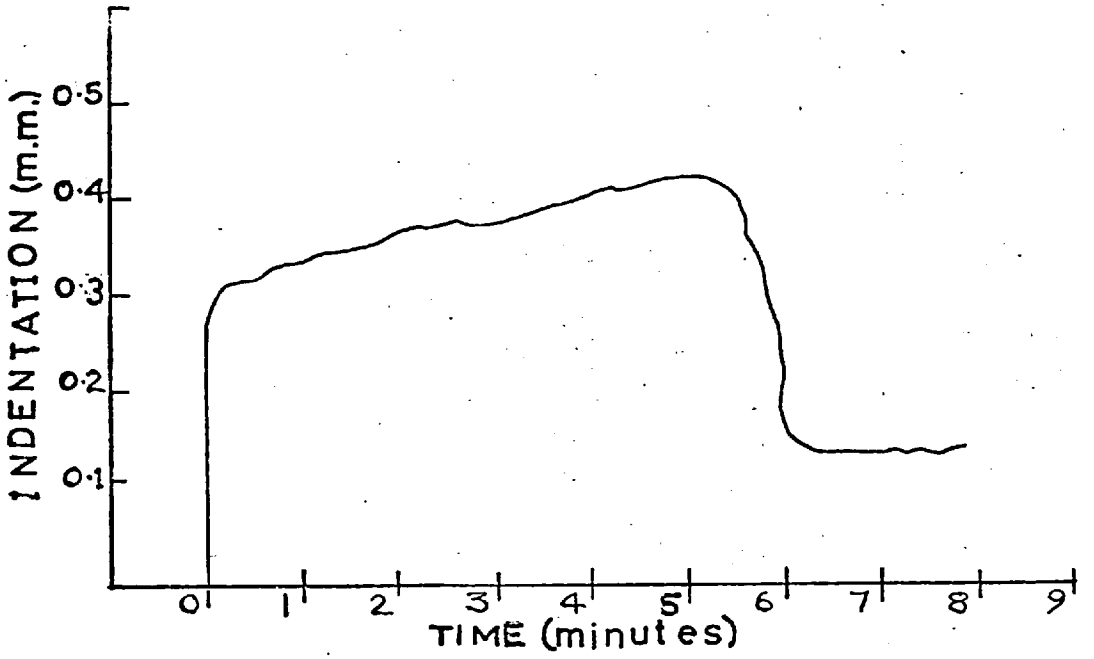


FIG. 4a. INDENTATION-TIME CURVE OBTAINED BY BÄR

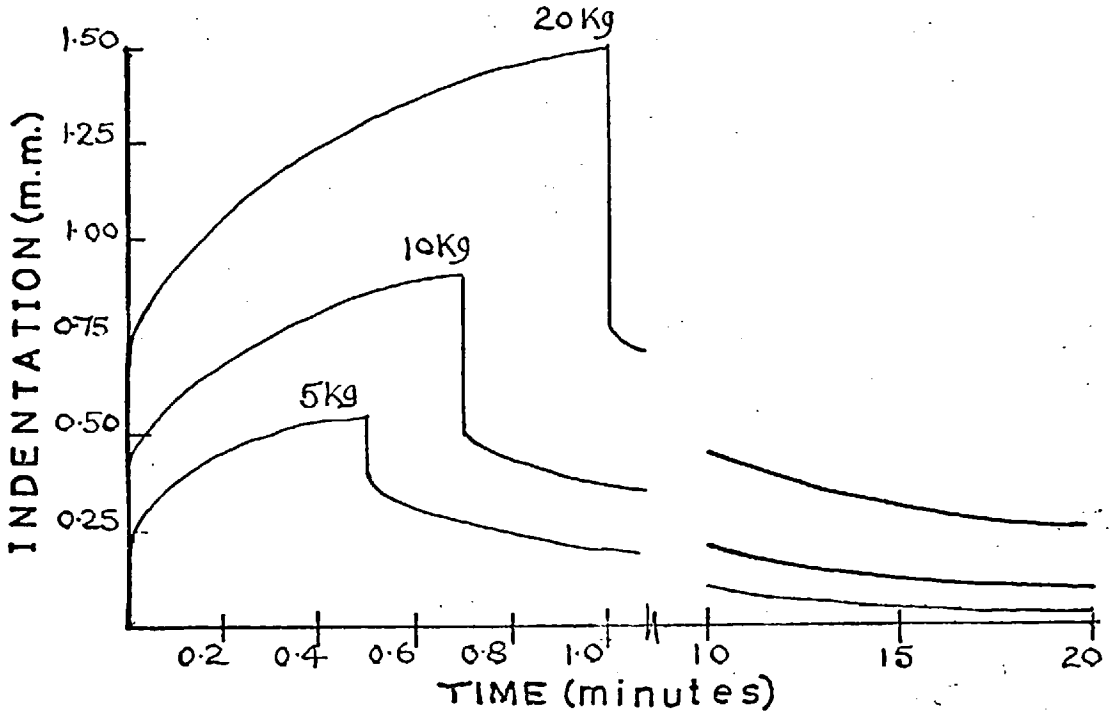


FIG. 4b. INDENTATION-TIME CURVES OBTAINED BY GÖCKE

nected the edges of the joint surface by the shortest route, and with the least curvature. In this view he was not supported by Pauwels (1959) who paid particular attention to the collagen orientation at or near to the articular surface. This he demonstrated using the technique developed by Hültkrantz (1896) from Langer. The surface of the tissue was pricked with a blunt awl and the fissures so produced were joined to form a cleavage line pattern over the joint surface. Zarek and Edwards (1963) however, plotted the principal stress trajectories for a simplified cartilage system, and concluded that their results neither confirmed nor denied the trajectorial collagen orientation in the deeper zones, but they did agree that the surface collagen was probably orientated in the direction of the principal tensile stresses. They also pointed out that experiments had shown that this orientation is settled during embryonic growth, Little et al. (1958) and is therefore probably hereditary.

Influenced by the work of Benninghoff, Bär (1926) and Göcke (1927) both performed indentation tests on articular cartilage, using modified forms of Schäde's elastometer. Typical indentation-time curves produced by these two workers are shown in Figs. 4a and 4b (facing p. 7). Although the stepwise progression of the curve representing the results of Bär, indicates that his apparatus produced considerable friction, nevertheless there is marked similarity between the shapes of these graphs. The results of all subsequent workers, and those of the present investigation, have confirmed the general shape of the load phase of the curves shown in Fig. 4, but as previously stated, it was not realised until relatively recently, by Elmore et al. (1963) that incomplete recovery after removal of the load is an experimental artefact. Therefore the results of more recent work differ in this respect from the results of Bär and Göcke.

The conclusions of Bär were: firstly that the cartilage of joint sockets is softer than that of articular heads. This statement is subject to the same criticism as those of Fürbringer and Lubosch; secondly, areas of cartilage exposed to large mechanical strains are softer than other areas. This must be taken as a very generalised conclusion, since the stress and strain distributions on any one complete joint surface are highly complex, and variable with motion. Furthermore, inasmuch as the load distribution on the femoral head is known at all, the results of the present investigation do not confirm the conclusion of Bär.

The conclusions of Göcke were: firstly that the persistent deformation of cartilage after removal of a load, increases as the applied load is increased. Secondly the elasticity of cartilage, determined by the criterion of Schäde, decreases with age. The work of Sokoloff (1966), and the results of the present investigation using a different criterion do not confirm this conclusion.

In summarising the literature reviewed so far, the following criticisms may be made:

1. All experiments on cartilage were performed in air, with consequent drying of the tissue.
2. Considerable dry friction existed in most of the testing apparatus employed.
3. Experimental controls were lacking, such as: a reproducible method of applying load, temperature, the effect of sub-chondral bone deformation, cartilage thickness measurement and so on.
4. Experiments, in general, were confined to limited areas of one joint surface, and they were not supported by histology or chemical analysis.

Thus, although the work of Benninghoff added a great deal to our understanding of the structure of cartilage, and to a certain extent, of the relationship of structure to the loads which cartilage experiences, the early mechanical tests left a need for much more comprehensive surveys and refined techniques.

(5) More recent investigations

One of the most important contributions, in addition to that of Benninghoff, was made by Hirsch (1944).

It was Hirsch who first paid considerable attention to, and consequently refined, the experimental technique employed in indentation tests of cartilage. In addition, he took the very necessary step of attempting to relate his indentation measurements, to the appearance of histological sections of the tested areas. This was an important development in an attempt to determine the influence of a structural component of cartilage, upon its elasticity. In particular Hirsch attempted to assess the role of chondroitin sulphate, in the resistance of cartilage to a compression load.

Initially Hirsch produced indentation-time curves, under static loads, which confirmed in essence, the previous results of Göcke. However, a persistent deformation after load removal was still a feature of his results, and he showed that the magnitude of this persistent deformation increased, both as the load, and as the duration of the load increased.

The indentations recorded for degenerate areas of cartilage were shown to be considerably greater than those of visually normal cartilage. Histological studies, and chemical analysis, revealed that the chondroitin sulphate content of these degenerate areas was considerably lower than that of visually normal cartilage. But Hirsch also observed that, a considerable

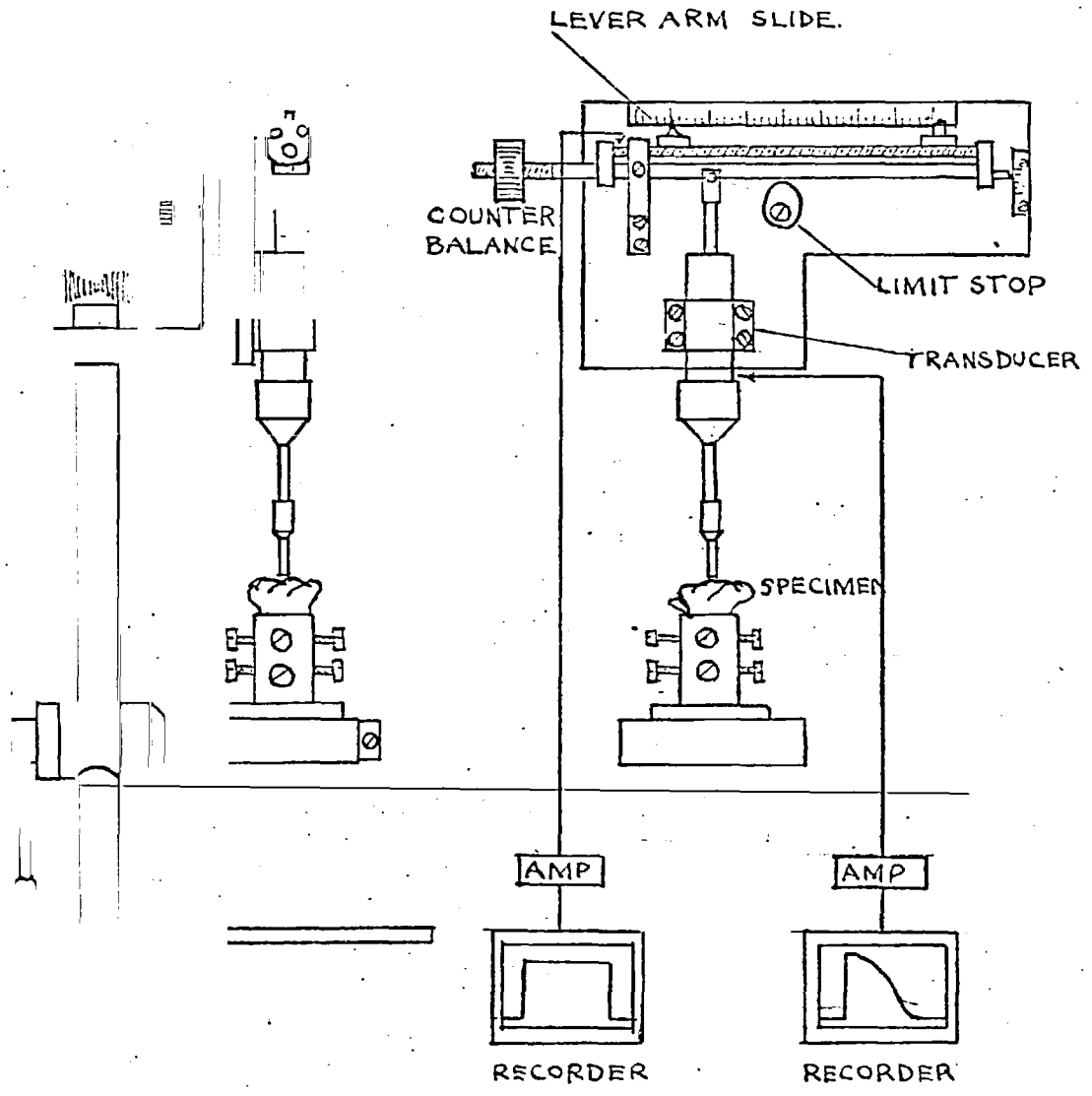


FIG. 5. INDENTATION APPARATUS USED BY ELMORE ET AL. (1963).

reduction in chondroitin sulphate content occurred in some areas, which were otherwise visibly normal. This observation is confirmed, in a slightly modified form, by the present investigation, and it will be discussed in more detail later.

The main criticism of the work performed by Hirsch is, that all his experiments were performed in air. This is a non-physiological environment for cartilage, in which considerable water loss occurs. In addition his work was limited to patellar cartilage, and the comparative studies were essentially between visually normal, and degenerate cartilage. In other words no pattern of stiffness of visibly normal cartilage was demonstrated. It should be realised therefore that there are limitations to the general applicability of the conclusions reached by Hirsch.

The first of three more recent, and highly significant, studies on the mechanical properties of articular cartilage, is that of Elmore et al.(1963). Elmore was puzzled by the incomplete recovery of cartilage recorded by all previous workers, and by Benninghoff's hypothesis that the elastic property of cartilage is due largely to expression and resorption of interstitial water. If the hypothesis were true, it was argued, it would be expected that, resorption of water would eventually restore cartilage to its original thickness. Using the apparatus shown diagrammatically in Fig. 5 (facing p.10), Elmore successfully demonstrated that the incomplete recovery of cartilage, was due to the fact that all the earlier experiments had been performed in air. Hence,water was expressed under load, but some was lost as a result of evaporation and movement away from the loaded area, with the result that resorption was limited,and therefore recovery was incomplete.

Indentation tests performed under balanced, isotonic, Hank's saline solution demonstrated that the deformation almost invariably recovered com-

pletely after removal of the applied load.

Linn and Sokoloff (1965) showed that the shape of curves representing the rate of expression and resorption of water from, and by cartilage, closely resembled the shape of indentation-time curves, but with one important difference. The flow of water from cartilage proceeded continuously with time, from the moment of load application, whereas some deformation invariably occurred 'instantaneously' upon application of the load, and thereafter proceeded continuously. This difference in behaviour was partially explained by the fact that the apparatus for measuring water flow, restricted it on three, of four boundaries, which was not the case in the indentation tests. But Linn and Sokoloff stated that this was only a partial explanation, and they suggested that cartilage possesses an immediate, or springlike deformation, which is independent of water expression.

Finally, Sokoloff (1966), using the same apparatus as that shown in Fig. 5, demonstrated that there was no significant correlation between the indentation of patellar cartilage, and the age of the specimen. In addition he confirmed an earlier result, that large local variations of indentation existed on the patella. However, Sokoloff was unable to show that these variations formed a systematic pattern over the joint surface, nor could he correlate them with the thickness of the tested areas. Typical indentation-time curves recorded by Sokoloff, revealed that after one hour from load application, the indentation had effectively reached an equilibrium value, the creep part of deformation at this time accounted for approximately 68% of the total deformation. Recovery was complete within a similar time period after removal of the load.

Histological sections confirmed that there was a marked reduction

of the chondroitin sulphate content in areas displaying degenerative changes. The indentation of these areas, was also considerably larger than that of visually normal cartilage.

The work of Sokoloff (1966) was the first to take into account such factors as: temperature, pH, and tonicity of the immersion medium, thickness of the indented area and the effect of the method of storage. An attempt was also made to eliminate friction from the testing apparatus, but Sokoloff stated that this was not completely successful.

Despite the obviously refined nature of these experiments, two important experimental points were overlooked.

Firstly, the limit stop in Fig. 5 (facing p. 10), released the beam plus the load in a time of between two to three seconds, and yet the indentations taken to be representative of the response of each area of cartilage to an applied load, were 'instantaneous'[1] indentations. From this, it is not clear how an 'instantaneous' indentation was measured. Neither is it clear how reproducibly the load was applied. Secondly, it was reported that autolytic degradation[2] was frequently observed at the termination of an experiment. But the same authors had shown previously that artificially produced autolytic degradation, caused a marked increase in indentation. Hence there is the possibility that the magnitudesof some recorded indentations were increased by autolytic softening of the tissue.

In addition to the two criticisms described above, the work of Sokoloff is subject to the same limitations as that of Hirsch, namely, that apart from some tests on costal and animal cartilage, most of the results relate to the

---

1. It was shown, in the present investigation, by the use of an oscilloscope, that a suddenly applied load produces a deformation within a few hundredths of a second. In this sense cartilage exhibits an 'instantaneous' deformation.

2. A term used to describe the process of cellular death in vitro. The cell walls rupture releasing enzymes which digest the matrix of cartilage. This action is faster at higher temperatures.

patella; and secondly, indentation and histological studies of different areas were compared mainly between visibly normal and degenerate areas of cartilage. Thus a pattern of changes was not established for visibly normal cartilage.

(6) Objects of the present investigation

A survey of the literature in Para. 5 of this Chapter has illustrated some of the limitations of previous investigations, both with regard to experimental controls, and to the scope of the work. The present investigation sought to rectify these limitations, and in particular:

1. To construct an apparatus, which would enable indentation surveys to be performed over the entire cartilage surface of human femoral heads. Coulomb, or dry friction was to be eliminated as much as possible, and a continuous graphical record of indentation versus time was to be provided.
2. To confirm the main results of previous investigations, and in addition to plot the variation of indentation of visually normal cartilage, on as many femoral heads as possible.
3. To express the shape of an indentation-time curve mathematically.
4. To establish a method for calculating a creep modulus for visibly normal cartilage, which takes into account the finite and variable thickness of the tissue. Finally, to draw layered maps, and histograms of the variation in creep modulus, for all the femoral heads tested.

Having performed these mechanical tests, the results were to be compared with the following variables;

A. The age of each specimen.

B. The chemical components of articular cartilage, for example chon-

droitin sulphate, keratan sulphate, total mucopolysaccharide content and collagen content, measured for the indented areas.

C. Physicochemical measurements of the permeability to water, of the indented areas.

D. Histological studies.

E. The severity of degenerative changes observed in localised, untested areas of cartilage on the femoral heads.

CHAPTER 2

MATERIALS AND EQUIPMENT

(1) Materials

It has been stated in Para. 6, Chapter 1 (p. 13) that the human femoral head was the joint surface which constituted the subject of this investigation. There are four main reasons why this joint was selected:

Firstly, because it is the commonest site of osteoarthrosis; secondly, because no report could be found in the literature, of a systematic study of the mechanical properties of the cartilage of the hip; thirdly, the classification according to Byers refers only to the hip joint and, finally, a normal femoral head is almost spherical in shape, Bullough et al. (1968), therefore it is relatively easy to perform an indentation survey over the entire joint surface.

A total of sixty-two femoral heads were studied in the course of the present investigation. All the femoral heads, except two, were obtained from routine post-mortem examinations. The other two were amputation specimens, obtained and first tested within fifteen minutes after amputation. Of the total number of femoral heads studied, sixteen were subjected to detailed indentation surveys, over the entire cartilage surface. During these surveys five hundred and ten areas of cartilage were indented.

The time lapse between death and post-mortem examination was usually less than twenty-four hours, but in a few cases it was forty-eight hours. During this period the cadavers were stored at $3^{o}C$. All the specimens, except those obtained as a result of amputations, were stored for periods up to a maximum of fourteen days, at $-20^{o}C$ between post-mortem and testing.

Specimens of which the cause of death could have affected the cartilage or sub-chondral bone were rejected.

The details of each specimen are given in Table 1, of Appendix 1, (p. 127), and an analysis of those specimens contributing to each separate section of the total investigation is given in Table 2, of Appendix 1, (p. 129).

(2) Evaluation and classification of each femoral head

Before testing, each femoral head was examined radiographically for any sub-chondral bone changes likely to influence the indentation measurements. Only those heads displaying normal or almost normal X-rays were tested.

Each femoral head was then classified according to the severity of degeneration observed in localised areas of the cartilage surface, after Byers[1] see Figs. 1a to 1e (facing p. 3). By so grading each femoral head, it was intended to attempt to correlate any observed variation of the stiffness pattern for visibly normal cartilage, with the grade number of a head, which represents the severity of localised degeneration.

(3) Apparatus and instrumentation

A testing apparatus was designed and manufactured by the author, in which a femoral head could be rotated about two mutually perpendicular axes, both of which passed through its central point. It was therefore, as previously stated, relatively easy to position a femoral head so that indentation tests normal to the articular surface could be performed over the entire joint surface.

(3.1) Rigidity of the testing apparatus

In order that deformations of the apparatus and equipment other than the specimens are minimised, it is usual to manufacture a materials testing machine which is considerably stiffer than the materials it is likely to test. It was considered that the method of supporting a specimen,

1. Personal communication.

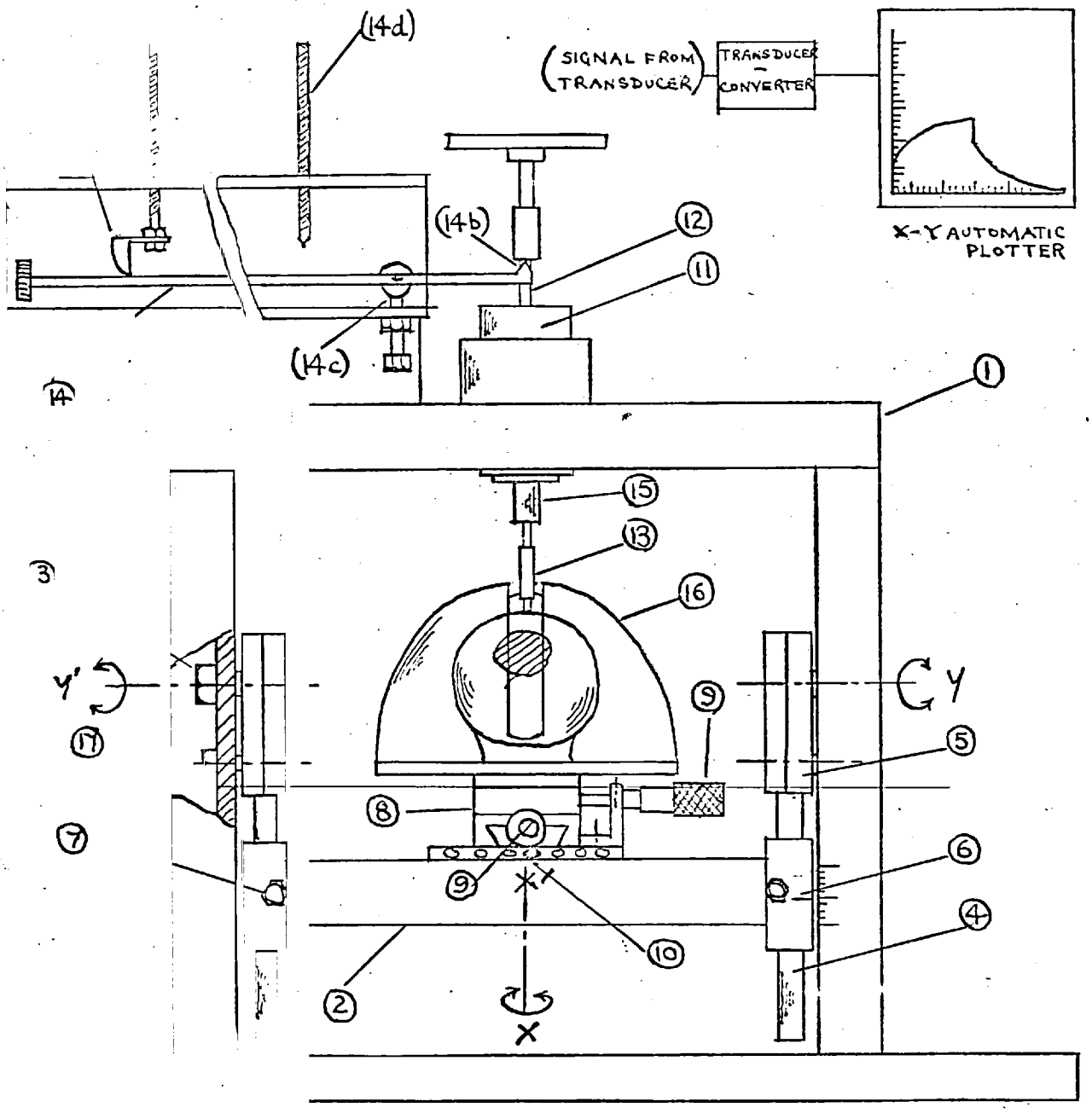


FIG 6. DIAGRAM OF INDENTING APPARATUS.

KEY.

1. FRAME
2. SWINGING PLATFORM
3. TRUNNIONS. ×2.
4. SIDE RODS ×4.
5. UPPER BLOCKS ×2.
6. LOWER " "
7. PINCH BOLTS ×2
8. DOVE-TAILED SLIDES ×2.
9. MICROMETER SCREWS ×2.
10. GRUB SCREW TO LOCK ROTATING BASE (FIXTURE NOT SHOWN).
11. BARREL OF INDENTER GUIDE.
12. PLUNGER " "
13. PERSPEX INDENTER FOOT.
14. SUPPORTING BEAM.
14a. RELEASE CAM.
14b. KNIFE EDGES.
14c. ROLLING PIVOT.
15. DISPLACEMENT TRANSDUCER
16. PERSPEX DOME.
17. LOCKING BOLTS.

used by previous workers, in particular see Fig. 5 (facing p.10), was not sufficiently rigid for the purposes of the present investigation, neither did it allow a particular position to be recorded and reproduced.

Fig. 6 (facing p.17), and working drawings in the folder at the end of this thesis, both show the apparatus which was used. It consisted of a rectangular frame (part 1), fabricated from 3 in. by 1 in. by 0.25 in. aluminium alloy, channel section. A swinging platform (part 2) was supported from two coaxial plain bearings (trunnions) (parts 3), by means of four silver steel rods (parts 4) 0.5 in. in diameter, set at their upper and lower ends in two aluminium alloy blocks (parts 5 and 6 respectively). The upper blocks acted as support points for the two trunnions, whilst the lower blocks were secured to the swinging platform. By unclamping the pinch bolts (parts 7) these two lower blocks, and therefore the platform, could be moved along the four rods and their position recorded by means of a scale. The platform was manufactured from the same channel section as the frame, and the whole apparatus was a comparatively rigid structure. Aluminium alloy was used because it is easy to machine and because it is relatively free sliding on silver steel.

(3.2) Rotational and translational motion

Each femoral head was secured initially on to a circular stainless steel[1] baseplate (described later), which in turn was secured on to a pair of dovetailed slides, manufactured as a complete unit (part 8). These slides were operated by two micrometer screws (parts 9), and they in turn were mounted on a small circular base, which could be rotated through 360° in the horizontal plane, about an axis perpendicular to, and passing through the mid-point of the swinging platform (axis xx'). Sixteen equi-spaced

1. Stainless steel was used to avoid contaminating the saline solution used as an immersion medium.

holes were drilled and tapped around the outer edge of the circular base, and by means of a 4B.A. grub screw (part 10) this base could be locked to the platform in any one of sixteen positions.

Each femoral head could also be rotated about the horizontal axis of the trunnions (axis yy'). Two bolts (parts 17) which passed through the frame, could be screwed into any one of eleven tapped holes on the outer surfaces of each of the upper blocks. Thus the entire swinging platform assembly could be locked in any one of eleven positions, through a total angular movement in the vertical plane of 180°.

Translational motion was achieved in two ways: firstly, by unclamping the two lower blocks (parts 6) and sliding the whole assembly along the side rods as described previously in Para. 3.1 of this Chapter; and secondly by means of the two dovetailed slides. These slides moved in two perpendicular directions and in two planes parallel to the swinging platform. As previously stated, they were operated by two micrometer screws with a total travel of 0.5 inch each. The principal use of the slides was as a fine adjustment, when positioning the indenter normal to the articular surface.

Thus each femoral head was provided with two rotational, and three translational degrees of freedom. It was possible by using the controls, to secure each head in a maximum number of eighty-one positions under the indenter, each of which could be recorded and reproduced when necessary.

(3.3) The indenter

The testing apparatus used by all previous workers had almost invariably presented friction problems either in the indenter guide , or in the apparatus for applying the load. This is a serious problem when attempting to measure indentations of 0.5mm or less, accurately.

The main source of friction in the apparatus seems to have been in the pivots or bearings of the beams, which all the previous workers used to apply load to the specimens. Unfortunately the bearings used were of the type which produce varying amounts of dry, or coulomb friction. This type of friction caused the apparatus to stick at certain points, see Fig. 4a (facing p. 7).

When designing the apparatus used in the present investigation it was decided to eliminate dry friction as much as possible. To this end a finely lapped barrel[1] and plunger, with a length to diameter ratio of approximately seven to one (parts 11 and 12 respectively), lubricated with a fine grade oil, acted as the guide and only bearing surface of the indenter spindle. The plunger was coaxial with, and formed an integral part of this spindle. At its lower end it was drilled and tapped (6B.A.) to accommodate the core piece of a displacement transducer (see Para. 3.5 of this Chapter), which in turn was screwed into a 'Perspex'[3] end piece[2] (part 13). The top end of the plunger was drilled and tapped (4B.A.) to take a small load platform, upon which weights could be placed.

It was an important feature of the indenter guide, that providing a constant supply of oil was maintained the frictional drag was essentially viscous in kind. This was due to the maintenance of a thin film of oil between the sliding surfaces. Because the magnitude of this type of friction depends upon the sliding velocity, which was low in the present case, so the frictional drag was small. It was however essentially constant, and usually no sticking occurred.

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1. The author wishes to thank C.A.V. Ltd., of Acton, who kindly supplied the necessary barrel and plunger.

2. This end piece will usually be termed the indenter.

3. Perspex or Plexiglass.

(3.3.1) Euler buckling load of the indenter

The physical configuration of the indenter spindle was that of a thin strut, loaded at both ends. This raised the problem that it might buckle under the loads used in the present experiments. The following calculation was performed, to evaluate the minimum load which would cause buckling.

The lowest Euler critical load P_{crit} for buckling of a strut of length L, subjected to an axial end load P is given by:-

$$P_{crit} = \pi^2 EI/L^2 \qquad (1)$$

where in the present investigation,

$$I = \frac{\pi}{256 \times 64} \qquad \left[\text{from } I = \frac{\pi d^4}{64} \; ; \; d = 0.125 \text{ in}\right]$$

L = 2 in; (the length of the unsupported indenter spindle)

$E = 16 \times 10^6$ lbf/in^2; (the material at the thinnest section was Brass).

$$P_{crit} = \frac{\pi^2 \times 16 \times 10^6 \times \pi}{4 \times 256 \times 64} = 75.5 \text{ lbf.} \qquad (2)$$

It is immediately obvious from equation (2) above, that the loads of 2 lbf, and 5 lbf, used in the investigation were well below the Euler critical buckling load.

(3.4) Method of load application

In order that the loading conditions of each test should approximate as closely as possible to physiological conditions, and at the same time be reproducible, it was necessary to consider how the load should be applied.

The results of Contini et al. (1964), and Rydell (1964), and Swanson (personal communication) concerning human gait analysis showed that in normal walking the load is effectively applied suddenly, and remains for a period between 0.5 sec. and 1 sec. depending on the speed of each step. It will be

stated later with reasons, that indentations were compared between different areas of cartilage, at a time of two seconds after load application, therefore the method whereby load was applied to the cartilage surface, had to fulfil the following conditions:

1. The load had to be applied suddenly,
2. It had to be applied in a reproducible manner, and,
3. The release mechanism had to be such, that it did not interfere with the indentation-time curve of cartilage at two seconds after load application.

The apparatus designed to meet the above conditions, consisted essentially of a light aluminium alloy beam 0.25 in. sq. in cross section (part 14), with a cut-away cam release device (part 14a).

The essential features of this beam were: firstly two knife edges (parts 14b which located on the under surface of the load platform; secondly a rolling pivot (part 14c), the point of action of which could be adjusted vertically; and lastly the cut-away cam (part 14a) which could be rotated to release the load suddenly and reproducibly. Thus when a load was placed on the platform, the reaction was taken by the three point bending configuration of the beam. The end of the indenter was adjusted to be just above the cartilage surface, by moving the rolling pivot vertically, and finally the load and beam were released by rotating the cam.

(3.5) Displacement transducer and instrumentation

Vertical movement of the indenter and therefore indentation of the cartilage, was measured by means of a displacement transducer (part 15), a Schaevitz L.V.D.T. model 100 MS-LT (L.V.D.T. represents Linear Variable Dis-

placement Transducer), with a total range of $\pm$ 0.100 in., mounted underneath the indenter guide. The core piece of this transducer, which formed part of the spindle of the indenter, moved vertically as the cartilage deformed. By so doing, a voltage output was generated from the transducer which was a measure of the magnitude of the indentation.

The main features of this type of transducer are its linear voltage output with displacement, and the absence of friction.

The signal from the transducer was fed into an amplifier/demodulator (S.E. 449/1), housed in an S.E. 905, two channel oscillator unit. The output was used to drive the Y axis of an automatic X-Y/T recorder (Bryans model 20180).

By means of the apparatus described above, the indentation of the cartilage normal to the articular surface, was amplified and plotted continuously on the X-Y recorder as a function of time, with the X axis acting as a time base.

(3.6) Immersion bath and temperature control

As Elmore et al. (1963) pointed out, it is necessary when performing indentation tests, to immerse the specimen in an isotonic buffered saline solution of physiological composition. The saline solution is necessary to prevent the cartilage drying, and it should ideally be isotonic to avoid swelling or shrinkage which would alter the mechanical response, (Sokoloff, 1963).

A hemispherical 'Perspex' dome (part 16) was made to fit over the femoral head, and this was located on and sealed by a rubber '0' ring housed around the edge of the circular baseplate. A slot 0.5 cm. width was cut in the dome, from slightly beyond the zenith, to a height of approximately 0.75 in. above the base.

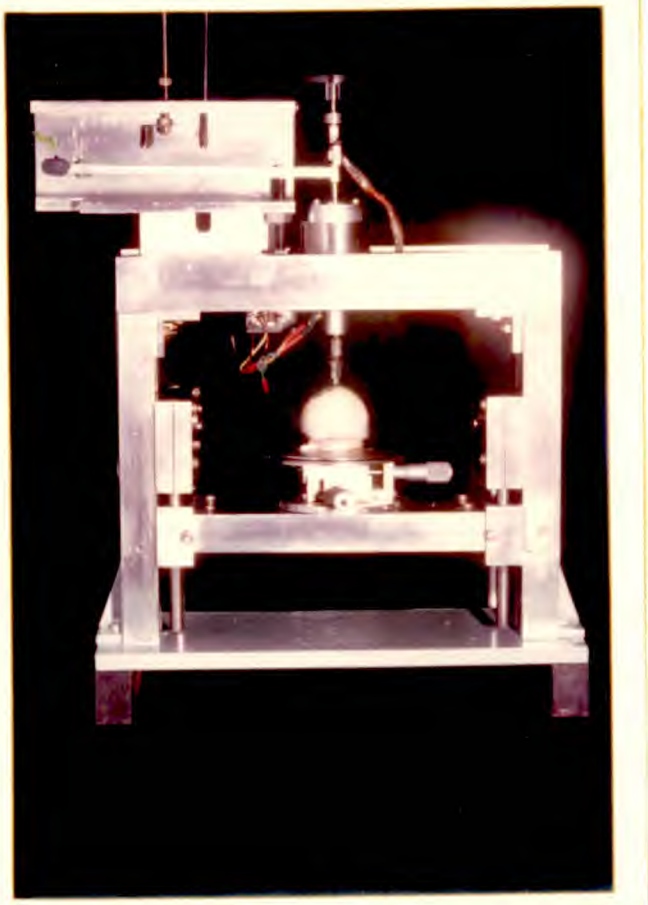


Fig. 7. Indenting Apparatus



Fig. 8. Complete Apparatus with Instrumentation

The dome acted as a container for buffered Ringer's[1] saline solution which was added in sufficient quantity to cover the entire femoral head. The dome was rotated so that the slot allowed the indenter free access to the cartilage surface, and the remaining area of the slot was then sealed with adhesive tape.

The Ringer's solution was heated to a temperature of 37°C, by means of a small heating coil (Electrothermal 'Thermocord') housed on the inner surface of the base of the 'Perspex' dome. The temperature was measured by an iron-constantan thermocouple[2] and controlled by an indicator/controller (Honeywell Servotronik model 02-665/10843) with an indication sensitivity better than 2%F.S.D. and a control sensitivity better than 0.25% F.S.D. Photographs of the indenting apparatus and the complete apparatus with instrumentation are shown in Figs. 7 and 8 respectively (facing p. 23).

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1. For a specification of this solution see Para. 6 , Chapter 4 (pp. 42 to 45.

2. The tip was covered by epoxy-resin.

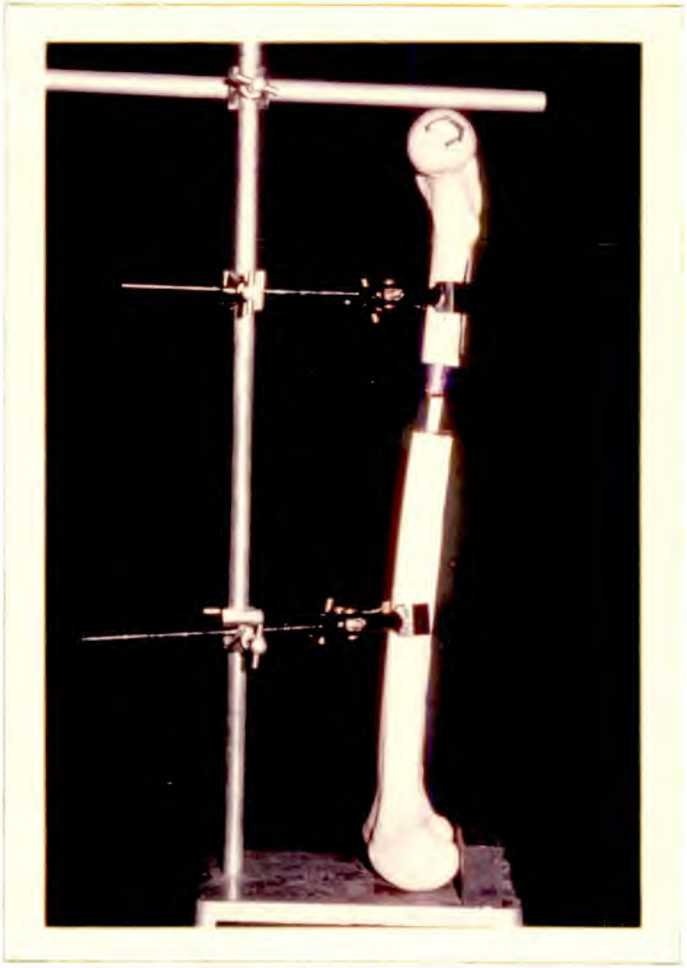


Fig. 9. 'Compound' Femur - Medial View

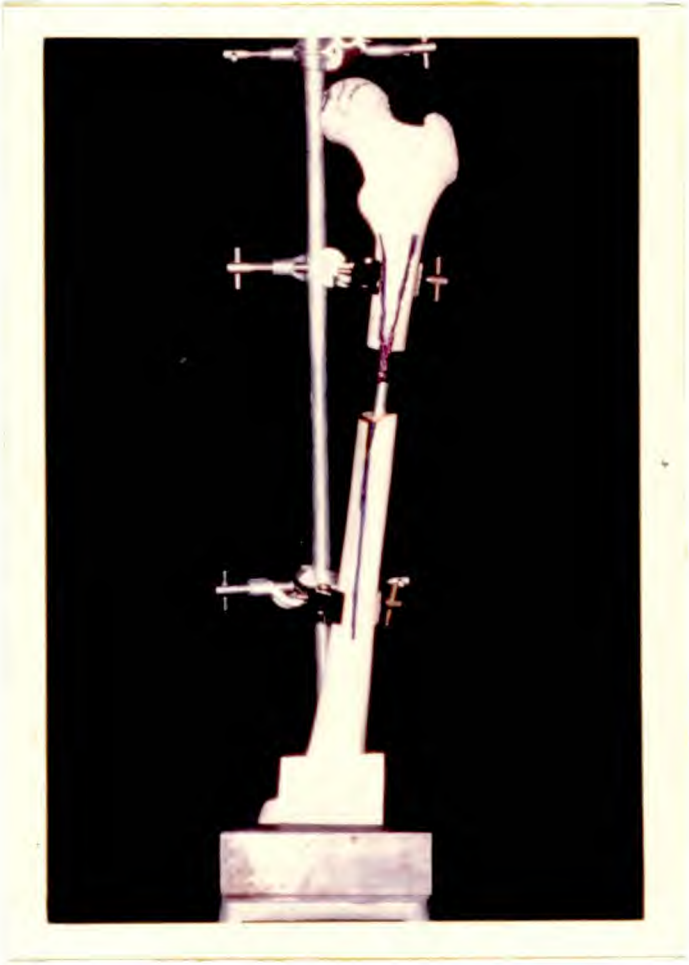


Fig. 10. 'Compound' Femur - Posterior View

CHAPTER 3

EXPERIMENTAL METHODS

(1) Anatomical positioning of a femoral head before testing

During this investigation, femoral heads were indented over the entire cartilage surface. In order that valid comparisons of the results could be made between corresponding areas on different femoral heads, it was necessary to develop a method whereby these corresponding areas of cartilage could be defined. The most obvious way to do this was firstly by setting each femoral head in the anatomically correct position, then marking the posterior-anterior and medial-lateral directions. If these were marked on a part of the femoral neck which remained with the head after sectioning the femoral neck, then a frame of reference would be established to which all indented areas could be referred.

The method of setting a femoral head in its anatomical position involved the use of a 'compound'[1] femur. Figs. 9 and 10 (facing p.24) show a compound right femur viewed from the medial and posterior aspects respectively. The distal two thirds of a macerated femur were clamped in a retort stand, in such a way that the fronts of both femoral condyles rested against a block, which was set at right angles to the edge of the base of the stand. Both condyles were in contact with the base, and the linea aspera (the blue line) therefore pointed in the posterior direction. An aluminium spigot was pushed into the inter-medullary canal at the proximal end of the lower part of the femur, and firmly fixed with 'Araldite'[3] adhesive. Approximately three inches of this spigot were allowed to protrude. The

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1. Unfortunately, it was not possible to obtain complete femurs at post-mortem.

2. Unless otherwise stated all femoral heads were taken from right hip joints.

3. Epoxy-resin.

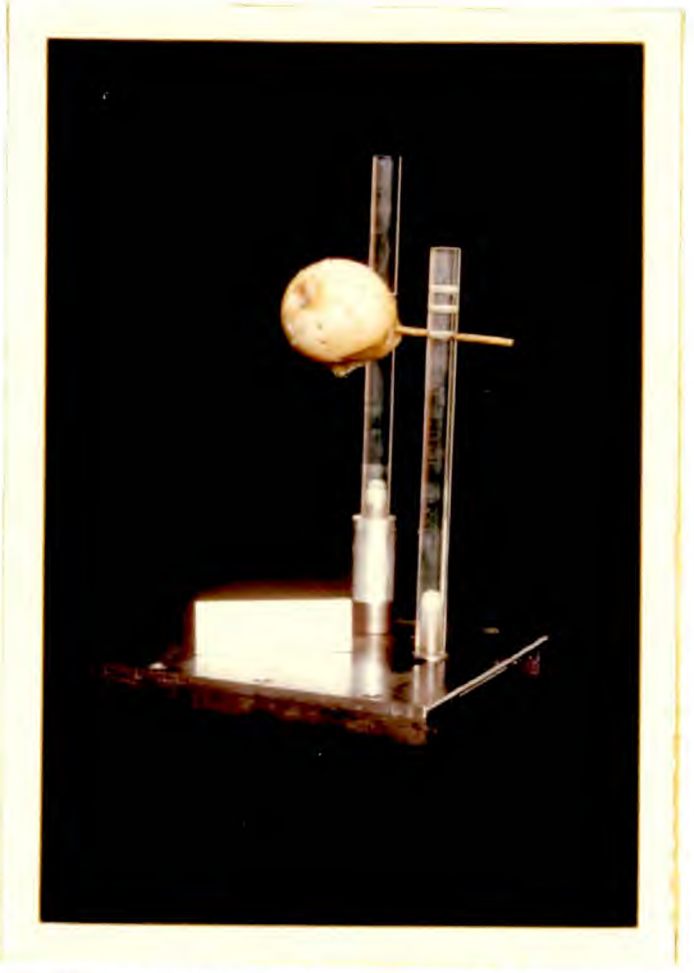


Fig. 11. Femoral Head Mounted on The Jig in The Anatomical Position



Fig. 12. Baseplate Showing Bone Screws and Rubber 'O' Ring at Outer Edge

proximal third of a femur as obtained from post-mortem examination was then located over the spigot so that both the sectioned ends were parallel, and the appearance was that of a complete femur. The linear aspera of the two sections were then aligned, and the compound femur was rotated about the condyles, until the zenith of the femoral head was above the points of contact of the condyles with the base. This is commonly accepted as the anatomical position of an extended hip, with neutral abduction, adduction and rotation. The whole assembly was then clamped rigidly in this position. With the sepcimen held in this position, two holes 0.125 in. in diameter were drilled in the neck of the femur, just medial to the intended line of section of the femoral neck. One hole was drilled from the posterior direction, whilst the other was drilled from the lateral direction. The two holes were drilled in approximately the same plane in two directions at right angles. When the femoral neck was sectioned perpendicularly to the long axis at approximately mid-section, the holes remained with the femoral head, thereby giving the necessary frame of reference.

In addition a small jig was made, which consisted of two vertical 'Perspex' rods, through which two pieces of wooden dowelling 0.125 in. in diameter were passed horizontally. These dowels were then inserted into the holes in the femoral head which was therefore supported, independently of the femur, in the anatomical position as defined above, see Fig. 11 (facing p.25). In this position the femoral head could also be photographed and examined radiographically.

(2) Mounting a femoral head in the apparatus

Having drilled the two holes described in Para. 1 of this Chapter, and sectioned the neck of the femur, approximately in the middle, the femoral

head was then ready to be mounted in the testing apparatus.

Firstly the cut face of the femoral neck was placed on a stainless steel baseplate, and positioned over three holes, the centres of which formed an equilateral triangle. A drill, 0.125 in. in diameter was then introduced to make three guide holes up into the cancellous bone of the femoral head. Finally three countersunk bone screws were used to secure the femoral head firmly to the baseplate, see Fig. 12 (facing p. 25). The rubber 'O' ring which acted as a seal for the 'Perspex' dome, was also located around the periphery of the baseplate. This assembly was then screwed down on to the dove-tailed slides, by four countersunk screws which passed through the baseplate and located in tapped holes on the top surface of the slides. The rigidity of this method of fixation is discussed in Para. 2 of Chapter 4, (pp. 33 to 34).

Having firmly secured the femoral head in the apparatus, the next step was to position its central point on the axis of the trunnion bearings (see Fig. 6). This was performed by sliding the swinging platform along the side rods, and securing it in the required position by means of the two pinch bolts. The accuracy of this position was checked by supporting the indenter just above the cartilage surface, and rotating the platform through 180° in the vertical plane, when the radial gap between the indenter and the articular surface remained sensibly constant, this was considered to be the correct position.

Before the procedures, about to be described in Paras. 2.1 and 2.2 of this Chapter were performed, each femoral head was immersed in Ringer's solution at 37°C for approximately one hour.[1] The reasons for this were:

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1. Except for the amputation specimens which were tested within approximately fifteen minutes of amputation.

firstly to prevent the cartilage drying, see Elmore et al. (1963); and secondly to allow the cartilage to reach equilibrium with its immersion medium before testing.

(2.1) Positioning the indenter normal to the articular surface

It is a necessary condition of any indentation test, that the indenter should be positioned normal to the surface of the material. If this is not so, then according to whether a plane-ended or hemispherically-ended[1] indenter is used either the edge will indent the material first, or the indenter will slip.

The method used to position an indenter normal to the articular surface in this investigation, may be described as an optical method. The plane-ended indenter was initially lowered gently on to the spherical articular surface of the femoral head. When contact was first made between the surface and the end of the indenter, a dark spot could be seen to appear on the latter. The femoral head was then moved, by means of the slides, so that the dark spot was positioned centrally with respect to the circular end of the indenter. In this position the latter was normal to the articular surface. The accuracy of this method was checked by mounting a femoral head on a base, the angle of which to the horizontal, could be varied by small increments. It was found that the optical method described above, clearly showed deviations of two to three degrees from the normal. When the hemispherically-ended indenter was to be used, it was inserted after normal alignment had been accomplished with the plane-ended indenter. In addition

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1. The detailed indentation surveys were divided into two series: the first, employed a cylindrical plane-ended indenter; and the second series, involved the use of a cylindrical hemispherically-ended indenter. Both were 0.125 in. in diameter. These are discussed in more detail, later in the text.

the hemispherically-ended indenter was always pressed gently on to the articular surface before testing, to ensure that no slipping occurred.

(2.2) Recording the no-load position of the articular surface

All indentations were measured from the articular surface and therefore it was necessary to record the position of the latter accurately, under zero load.

The beam used for supporting the load prior to its release, see Part 14 of Fig. 6 (facing p.17) was equipped with a small counterweight at its lateral end. The purpose of this weight was to counterbalance the indenter assembly, so that with no load on the platform and the beam in position, the moment of the indenter about the moveable pivot, was just larger than the moment of the counterweight. Thus the indenter moved slowly down, and if allowed to contact the articular surface, it applied a tare load of approximately two grammes to it. Indeed the tare weight was just sufficient to maintain contact between the end of the indenter and the articular surface.

When the indenter had been positioned normal to the cartilage surface, the hemispherical 'Perspex' dome was placed over the femoral head, and Ringers saline solution was added so that the entire head was immersed. The indenter under the tare weight described above, was then lowered so that it contacted the articular surface.

By moving the outer casing of the indenter guide whilst the indenter itself remained stationary, the core piece of the displacement transducer was positioned centrally so that the voltage output was zero, as indicated by the dial on the oscillator. The X-Y recorder pen was then moved along the Y axis (using the shift control), and a mark was made at a convenient position on the graph paper. The end of the indenter was then raised clear of the articular surface by means of a threaded rod (part 14dof Fig. 6), which

pressed on and lowered the outer section of the supporting beam. After a time lapse of ten minutes the indenter was again lowered on to the cartilage surface, and any change in the position of the latter, was compensated for by moving the recording pen back to its original base-line position. This procedure was repeated until three consecutive readings revealed no change in the position of the pen, and hence no movement of the articular surface.

Having recorded the no-load position of the articular surface the load was placed on the load platform and the reaction taken via the beam, to the cut-away release cam. Load release, by the method described in Para. 3.4 of Chapter 2, (p. 20), was then performed.

(3) Indenter and load selection

Two indenters were used in the detailed indentation surveys. Both were circular in cross section and 0.125 in. in diameter. Both were manufactured from 'Perspex' to avoid contaminating the Ringer's solution. One was made with a plane end, whereas the other was hemispherically-ended (a detailed comparison of the results obtained with both indenters is given in Para. 5 of Chapter 6 (p. 62).

The load to be used with each indenter was selected, so that the average compressive stress applied to the articular surface was approximately equal to the maximum compressive stress experienced in vivo. According to Greenwald (personal communication) this stress is likely to be as high as 400 lbf/in^2 (28.2 Kg/cm^2). With reference to this value, a load of 5 lbf (2.28 Kg) was used with the plane-ended indenter, yielding an average stress of 410 lbf/in^2 (28.9 Kg/cm^2); and a load of 2 lbf (0.92 Kg) was used with the hemispherically-ended indenter. The stress for this shape of indenter



Fig. 13. Femoral Head from which Indented Areas of Cartilage have been Extracted Using the Trapanning Tool

obviously varies with the area of contact, but the maximum experimental value was approximately 400 lbf/in^2.

(4) Extraction of the tested areas of cartilage

To measure the deformation of the sub-chondral bone, and the thickness of each indented area of cartilage, it was necessary to remove these areas from the bone. To this end, a small trepanning tool was made, 0.125 in. in diameter with a sharpened cutting edge, 0.007 in. thick, which could replace the solid ended indenter. Each area of cartilage which had been indented, was then re-positioned under this tool, which was pushed through the cartilage until it contacted the sub-chondral bone; it was then withdrawn. Finally the small cylinders of cartilage were prised off the underlying bone, leaving in almost all cases, a very well defined fracture surface at the bone to cartilage interface. Fig. 13, (facing p. 30), shows a femoral head, from which some areas of cartilage have been removed by the procedure described above.

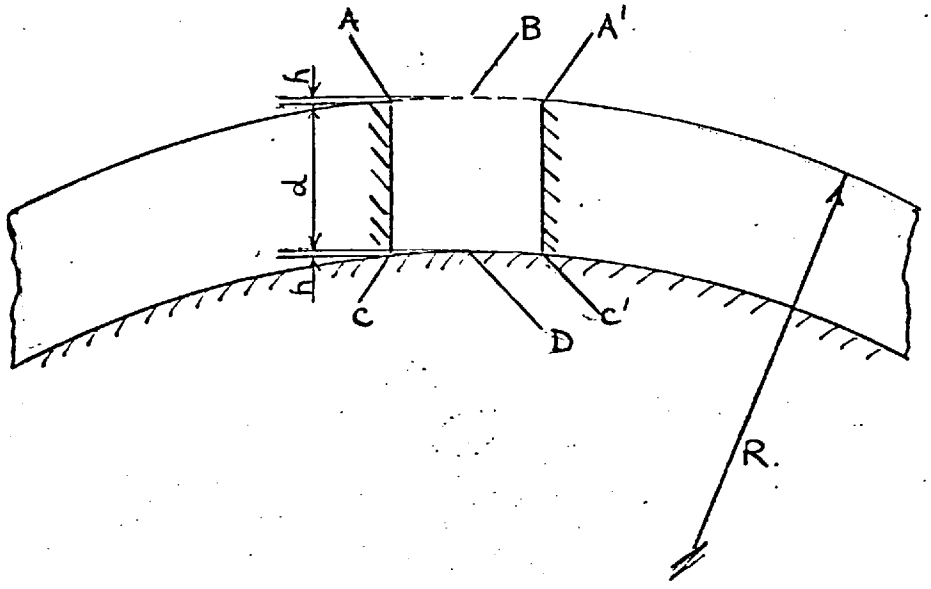


FIG. 14. DIAGRAM OF A "CORED" AREA OF ARTICULAR CARTILAGE (AA'C'C).

CHAPTER 4

EXPERIMENTAL CONTROLS AND ASSOCIATED METHODS

Introduction

In this Chapter seven control experiments will be reported. These were performed in order to ensure reliability and reproducibility of the results obtained in the detailed indentation surveys.

(1) Thickness measurement of tested areas of cartilage

It will be shown later in Para. 5 of Chapter 5 (p.53), that the indentation of cartilage on the femoral head is thickness dependent. This is to be expected, because below a certain thickness the indentation of a relatively soft material such as rubber, is reduced by the restricting effect of the lower boundary.

Before this effect could be accounted for in the results of the indentation surveys, it was necessary to measure the thickness of each indented area of cartilage.

Every tested area of cartilage was removed from the sub-chondral bone by the method described in Para. 4 of Chapter 3, and then two methods were used to measure the thickness:

Method 1 Micrometer screw gauge method

Each small piece of cartilage was placed between the anvils of a micrometer screw gauge, of which the ratchet slipped at an axial load of approximately 25 gm.

With reference to Fig. 14 (facing p.31) the micrometer gauge theoretically measured between point B and the line CC'. It therefore overestimated the thickness predicted by geometry (distance BD) by 'h'.

When the radius of the area of cartilage r =1/16 in. and the radius of an average femoral head R = 1 in. then using the intersecting chords pro-

perty of a circle:

$$r^2 = h\,(2R - h) \tag{3}$$

when $h \ll 2R$ equation (3) may be written in the form

$$r^2 \approx 2Rh \tag{4}$$

from which,

$$h \approx \frac{r^2}{2R} \tag{5}$$

Using the data given above in equation (5) gives h = 0.002 in.

Thus the micrometer screw gauge method of measuring the thickness of cored areas of cartilage, theoretically overestimated the value predicted by geometry, by approximately 0.002 in.

Method 2 Vernier depth gauge method

The second method of measuring the thickness of cartilage, involved the use of a Vernier depth gauge. The central guide of the depth gauge was inserted into the hole left by the cored area of cartilage, and the slide was moved down until it rested across points A and A', see Fig. 14 (facing p. 31). The depth of the hole and therefore theoretically the thickness of the area of cartilage, was read from the Vernier scale. The gauge was also rotated in the hole and measurements were taken at different orientations to ensure that there was no significant variation of the reading.

It can be seen from Fig. 14, that the Vernier gauge measured the distance between the line through points A and A' and point D, it therefore theoretically underestimated the thickness predicted by geometry by h, which has been shown to be approximately equal to 0.002 in.

Method 1 overestimated the thickness by 0.002 in., and Method 2 underestimated the thickness by 0.002 in. Theoretically, since the difference between the two measurements should have been 0.004 in., at most

	CONTROL EXPERIMENT	SPECIMEN	APPLIED LOAD (lbf)
1.	Bone deformation	-	2 and 5
2.	Storage at -20^{o} and prolonged periods at $37^{o}C$	1 38	5 2
3.	Storage for 3 weeks at $-20^{o}C$	63 and 64(RANDL)	2
4.	Repeatability of the apparatus	13,14,15 and 17 20 to 23,25,38,58	5 2
5.	Variation of indentation with temperature	12,17 and 19 58	5 2
6.	Variation of indentation with composition and pH. of Ringer's solution	55,61 and 62	2
7.	Variation of indentation under Synovial fluid and Ringer's solution	Knee, 36,55,58	2

Table 3a: The loads of 2lbf and 5lbf used in the indentation surveys were also used in the control experiments.

this represents an error of approximately 10% in the areas of minimum thickness. But in the majority of areas the error is approximately 5% or less. In practice however, the measurements of thickness by the two methods described rarely differed by more than 0.002 in. This is probably explained by the fact that the diagram shown in Fig. 14 shows an ideal situation in which it is assumed that the cored piece of cartilage retains the shape of the hole, whence it was removed. There is the observation of Abrahams and Duggan (1964) however, that devoid of the constraint of the sub-chondral bone, cartilage uncurls from its normal configuration. It may well be therefore, that the curved surfaces at the top and bottom of an indented area of cartilage become flatter, when it is removed from the bone. The micrometer screw gauge method would then tend to measure the true thickness of the piece of cartilage, whereas the depth gauge would still underestimate the thickness by 'h'. The overall effect however, would be to reduce the difference between the two readings by a factor of 'h' which has been shown to be equal to 0.002 in. This agrees with the experimental observation. The errors quoted previously are therefore halved to a maximum value of approximately 5%, and a mean value of approximately 2.5%.

(2) Deformation of the bone and apparatus.

Loads used in the following control experiments are given in Table 3a opposite.

It was explained in Para. 3.1 of Chapter 2 (p.16), that particular attention was paid at the design stage, to the rigidity of the testing apparatus. However, the deformation produced in the bone and testing apparatus by a load applied to the cartilage surface, had to be determined and subtracted from the total deformation which included that of the cartilage.

Two series of tests were performed on ten femoral heads, using loads of 2 lbf (0.92 Kg) and 5 lbf (2.28 Kg). In each case the plane-ended

indenter was used to apply the load directly to the sub-chondral bone, in areas from which the cartilage had been removed. The hemispherically ended indenter was not used, because it would have produced larger contact stresses than those experienced by the bone with the cartilage in situ. The results of both series of tests are shown in detail in Table 3 , see Appendix 1 (p.130).

Table 4 below, shows only the important overall results, extracted from Table 3.

	Load	
	2lbf(0.92Kg)	5lbf(2.28Kg)
Mean bone deformation (cm)	0.0029	0.0059
Minimum bone deformation (cm)	0.0020	0.0043
Maximum bone deformation (cm)	0.0038	0.0076

Table 4 Condensed results of bone deformation tests, from Table 3. Using a cylindrical plane-ended indenter, 0.125 in. in diameter.

The mean deformations of 0.0029 cm. and 0.0059 cm. recorded for loads of 2 lbf and 5 lbf respectively, were subsequently subtracted from the corresponding indentations recorded in the indentation surveys.

The error caused by using the mean bone deformation values instead of either the maximum or minimum values may be expressed in terms of the lowest mean deformation recorded in both series of indentation surveys. It can be shown on this basis, that the maximum error is approximately $\pm$5%. In most cases the error will be less than this value, since the latter refers to the lowest mean deformation, that is the value which would yield the largest error.

(3) The effect of storage at -20°C and prolonged periods at 37°C

When cartilage is removed from the body, autolytic degradation proceeds as a function of both time and temperature. Sokoloff (1966) in fact observed that autolysis had occurred at the termination of some of his experiments. Such degradation may well influence the mechanical properties of cartilage, because one of the effects is the leaching out of the mucopolysaccharide moiety of the matrix. Autolysis can be prevented chemically by storing a tissue in Formalin, but this also affects the mechanical properties of cartilage, Stücke (1950), Curtis (1963), Elden (1964) and Viidik and Lewin (1965).

It is common practice to store biological materials and in particular cartilage, at a very low temperature. This retards the rate at which autolysis occurs to such an extent, that the tissue may be kept for several months without any significant effects of autolysis being observed.

In the present investigation specimens were stored at -20°C until testing, when they were surrounded by Ringers solution at 37°C for periods of up to 19 hours. It was therefore necessary to demonstrate, that autolytic degradation did not significantly influence the results during this procedure.

Two femoral heads were obtained immediately post-operatively, from hind-quarter amputation specimens. Three areas of cartilage on each femoral head were tested repeatedly and cyclically, the first test being performed within approximately fifteen minutes of amputation. The tests were continued until the cartilage showed signs of systematic and progressive softening, which was indicative of autolytic degradation, (Sokoloff, 1966). The results of these repeated tests are shown in Tables 6 and 7 , see Appendix 1 (p.131-2)together with the time for which each femoral head was surrounded

by Ringer's solution at 37°C, and the total time for which the head was stored at -20°C during the test procedure. The results are summarised below.

(3.1) Summarised results and conclusions

(1) Amputation specimen 1 (Table 6)

A total of 20 repeated indentation tests were performed on three areas of cartilage.

Significant increases in the indentations of all the areas were recorded at the sixth repeat, and confirmed as having increased further at the seventh repeat.

By the time that the sixth repeat revealed softening of the cartilage, the femoral head had been immersed in Ringer's solution at 37°C, for a total of 25 hours. In addition, the head had been stored at -20°C for approximately 95 hours between the testing periods.

(2) Amputation specimen 2 (Table 7)

A total of thirty repeated indentations were performed on three areas of cartilage.

With the exception of area number 3 (see Table 7), no systematic increase in the indentation occurred, even though the femoral head had been immersed in Ringer's solution at 37°C for a total of approximately 24 hours.

Closer inspection of the results relating to area 3 revealed that the sixth repeat deformation was approximately 14% larger than the previous deformations, but thereafter no further systematic increase occurred. The reason for the sudden increase in indentation, was eventually traced to incorrect re-positioning under the indenter, which was perpetuated for all the subsequent tests. In view of this error, the results of the final five indentations of this area are not included, even though they show no

further significant increase.

The total time for which the femoral head was stored at -20°C between tests was 50 hours.

The results summarised above show, that no significant softening of cartilage occurred on either of the two femoral heads, within the time period of fifteen minutes to 24hr. or more, for which the heads had been immersed in Ringer's solution at 37°C. In addition, the results indicate that a femoral head may be stored for fifty hours or more at -20°C between tests, without any change in the compression properties of the articular cartilage.

Table 5 (below) show the time spent at 37°C and -20°C by each of the femoral heads involved in the detailed indentation surveys. It can be seen from Table 5, that no femoral head was kept at 37°C for any longer than 20 hours, which is within the safe period defined above. In addition, no head was kept at -20°C between tests for more than a total of fifty hours, which again is within the safe period shown by Tables 6 and 7.

Spec. Number	2	3	4	5	6	7	8	9	10	11	20	21	22	34	35	57
Time at 37°C (hr)	18	13	14	12	16	16	17	19	18	15	20	11	15	14	12	18
Time at -20°C (hr)	31	17	34	19	18	34	50	49	33	34	50	31	16	37	36	34

Table 5 Total times spent by each femoral head, during indentation surveys at 37°C in Ringers solution and -20°C in storage.

It was concluded from the results shown above, that no significant progressive softening of cartilage due to autolytic degradation, had occurred during the indentation surveys. However, a further check was performed by repeating indentations on at least two areas of cartilage on each femoral

head during the indentation surveys. The results of these repeated indentations are shown in Table 9, see Appendix 1 (p.133-4). From column E of this table, it is apparent that no systematic, progressive softening of the tested areas of cartilage occurred, which confirmed the conclusion realised previously.

(3.2) Long term storage at -20°C

It can be seen from Table 1, see Appendix 1 (p.127-8), that the femoral heads tested in the indentation surveys, were stored at -20°C for periods of up to two weeks, between postmortem examination and testing. It was necessary to show that this procedure did not alter the mechanical properties of the cartilage.

Three areas of cartilage on each of three femoral heads were indented twice using the hemispherically-ended indenter and a load of 2 lbf: the first indentation test was performed within forty eight hours of post-mortem. The femoral heads were then stored at -20°C for three weeks. After this period the indentations were repeated.

In order to be consistent with the results of the main indentation surveys, indentations recorded at two seconds after application of the load were extracted from the indentation-time curves and compared. These values for all of the nine tested areas are set out in Table 8, over.

It can be seen from Table 8, that all the repeated indentations were within ±5% of the initial values. The difference was within the limit of repeatability for the apparatus, to be established in Para. 5 of this Chapter, (p. 41).

It was concluded from these results, that femoral heads may be stored for at least three weeks at -20°C, without any significant change in the indentation response of the cartilage. The periods for which the femoral

heads were stored at -20°C in the indentation surveys have been therefore shown to be acceptable.

Table 8 Results of long term storage at -20°C

Specimen number	Position	Indentation (cm)		Difference as a percentage of the initial
		Initial	3 weeks after P.M.	
63.	1	0.0356	0.0368	+3.37
	2	0.0373	0.0381	+2.16
	3	0.0422	0.0402	-4.75
64. Right	1	0.0310	0.0310	0.00
	2	0.0294	0.0297	+1.02
	3	0.0340	0.0330	+2.94
64. Left	1	0.0254	0.0264	+3.94
	2	0.0264	0.0264	0.00
	3	0.0323	0.0330	+2.79

(4) Repeatability of the apparatus

During the course of an indentation survey, each femoral head was necessarily moved many times. This fact raised the question of how accurately an indentation-time curve could be repeated for each area of cartilage, after the femoral head had been moved, and then re-positioned under the indenter.

To determine the repeatability of the apparatus, two groups of tests were performed: in the first group a total of nineteen areas of cartilage were repeated once, and seven areas were repeated twice or more;

in the second group repeated indentations were performed on the two femoral heads obtained from amputated hips, in the course of assessing the effect of autolytic degradation. In addition two areas on each femoral head were tested at least twice (during the indentation survey).

The results of the first group of repeatability tests are shown in Table 9 Appendix 1 (p.133-4)and those results relating to the second group of tests are shown in Table 10, see Appendix 1 (p.135). All the indentations shown are those recorded at two seconds after load application.

(4.1) Summarised results

(1) Group 1 tests (Table 9):

The principal results of the first group of tests have been extracted from Table 9 and they are shown below in Table 11.

Total number of repeated indentations	25
Number of repeats differing by $\pm$5%, or less from the original deformation	20
Number of repeats differing from the original deformation by less than $\pm$10%	25

Table 11 Principal results of repeatability tests, for group 1, extracted from Table 9

The above data shows that approximately 80% of the repeated indentations differed by $\pm$5% or less from the original value. All the repeated indentations are within $\pm$10% of the original values.

(2) Group 2 tests (Table10)

The principal results have again been extracted, in this case from Table 10, and they are shown in Table 12, see over.

Total number of repeated indentations	88
Number of repeats differing from the original deformations by $\pm$5%, or less	78
Number of repeats differing from the original deformation by less than $\pm$10%	86

Table 12 Principal results from repeated indentations of group 2 tests, extracted from Table 10

The above results show, that approximately 89% of the repeated indentations differed by $\pm$5% or less from the original values. Approximately 98% of the repeated indentations were within $\pm$10% of the original values. Only two repeats differed by more than $\pm$10% from the original values. After closer inspection of the results relating to these two areas, it was concluded that an error had occurred in re-positioning them under the indenter.

(4.2) Overall conclusion

Consideration of the results of both groups of tests together, shows that a total number of 113 areas of cartilage were subjected to repeated indentation tests; of these, 98 or approximately 87%, yielded repeat indentations differing from the original values by $\pm$5% or less. All but two of the total number of repeats, were within $\pm$10% of the original deformation.

On the basis of these results, the repeatability of an indentation recorded at two seconds after load application, was taken to be $\pm$5% of the original value.

(5) Temperature variation of the Ringer's solution

It was stated by Elmore et al. (1963), that a variation in the temperature of the immersion solution from 13°C to 50°C, produced a neg-

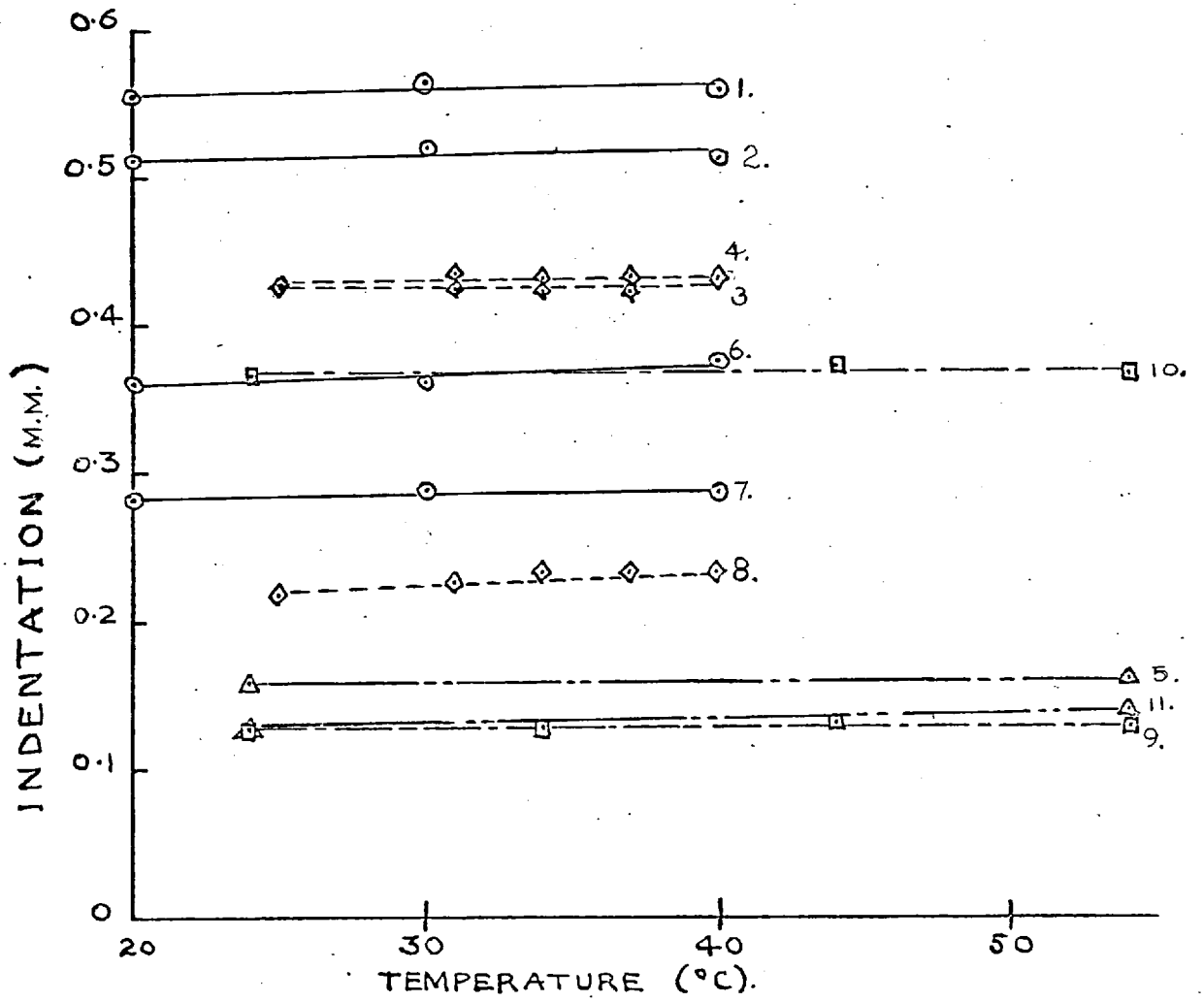


FIG. 15. VARIATION OF INDENTATION WITH TEMPERATURE.

SYMBOL	SPECIMEN Nº.	GRAPH NUMBER. ORDER OF DECREASING IND'TN.
⊙	58	1, 2, 6, 7.
⊡	19	10, 9.
△	17	5, 11.
◇	12	4, 3, 8.

ligible effect on the indentation of cartilage. It was decided to attempt to confirm this statement experimentally, for cartilage on the femoral head.

Eleven areas of cartilage on four femoral heads, were subjected to repeated indentation tests, whilst the temperature of the surrounding Ringers solution was increased in discrete steps, from between 20°C to 25°C up to 40°C or 50°C. Indentation-time curves were plotted for each area at each temperature, and values of the indentation at two seconds after load application were extracted. Graphs of these indentations against temperature were plotted, and these are reproduced in Fig. 15, (facing p. 42). Table 13 see Appendix 1 (p.136) contains the numerical data from which Fig. 15 was drawn.

(5.1) Summarised results of temperature variation tests

Inspection of the graphs shown in Fig. 15 reveals, that the variation of indentation with increasing temperature was extremely small. There was however a slight tendency for the indentation to increase with increasing temperature.

Table 14 (see over) shows the average, and maximum variation of indentation per degree Centigrade, and the difference between the indentation at the maximum and minimum temperatures, as a percentage of the latter.

The results shown in Table 14 confirm the conclusion stated above, that the variation of indentation with temperature is small. The results also indicate that the variation of indentation which could have occurred during the indentation surveys due to temperature variation of the Ringers solution, was negligible, since the temperature was controlled to within $\pm 1^{\circ}C$ at most.

(6) The effect of variation of the composition and pH value of Ringer's solution

During the indentation surveys it was decided that in a few cases

e Number . Fig. 15)	A Temperature Range ^{o}C	B Average variation of indentation with temperature (cm/^{o}C) $\times 10^{-5}$	C Maximum variation of indentation with temperature (cm/^{o}C) $\times 10^{-5}$	D Difference between indentations at maximum and minimum temperatures, as a percentage of the latter
1	20 to 40	2.0	10.0	+0.72
2	"	0.5	8.0	0.20
6	"	7.5	7.5	0.42
7	"	2.5	7.0	1.80
10	24 to 54	1.7	1.7	1.60
5	"	4.3	4.3	1.40
9	"	0.67	3.5	3.20
11	"	1.7	5	9.40
4	25 to 40	5.3	5.3	1.90
3	"	7.3	7.3	2.60
8	"	10.7	17.5	7.30

Table 14 The effect of temperature variation on the indentation of cartilage (results extracted from Table 13 see Appendix 1 (p. 136)).

the possibility could not be ruled out that small amounts of tap water had contaminated the Ringer's solution which was made with distilled water. It was then further decided to perform an investigation in which the Ringer's solution was made up entirely with ordinary tap water and with distilled water. These two solutions would then be analysed for metallic ions and the pH values would be measured. The results of repeated indentation tests would be compared with each of the solutions as the immersion medium.

Twelve areas of cartilage on three femoral heads were indented

repeatedly, whilst immersed in Ringer's solution which was made alternatively with tap water, and with distilled water. The two solutions were analysed (see Table 17 , p. 45), and the pH values were measured before and after use. The order in which the solutions were used was reversed; so that referring to Table 16 (p. 44)Group 1 results relate to the order: Distilled Ringer's - Tap Ringer's - Distilled Ringer's; and Group 2 results relate to the order: Tap Ringer's - Distilled Ringer's - Tap Ringer's. Each area of cartilage was indented three times, and the first and last indentations were performed under identical solutions.

The values of the indentations recorded at two seconds after load application, together with the pH values, are set out in detail in Table 15 see Appendix 1 (p. 137). The principal results are given in Table 16 below.

	Group 1		Group 2	
	Max %	Mean %	Max %	Mean %
First and second solutions - percentage difference in indentations (Numerical value)	4.8	2.6	3.4	2.6
First and final solutions - percentage difference in indentations (Numerical value)	1.3	0.9	3.5	2.1
	pH units			
pH of fresh Ringer's solution - distilled water	6.9		6.9	
pH of used Ringer's solution - distilled water	7.1		6.9	
pH of fresh Ringer's solution - tap water	7.9		7.7	
pH of used Ringer's solution - tap water	7.8		8.1	

Table 16: Principal results of the effect of variation of the composition and pH of Ringer's solution.

Table 17 below shows the composition of both solutions, determined by chemical analysis.

Constituent	Tap water Ringer's	Distilled water Ringer's	Minimum quantity detectable
	MAIN CONSTITUENTS		
Sodium chloride	9.1 gm/litre	9.0 gm/litre	
Potassium chloride	0.26 "	0.053 "	
Calcium chloride	0.053 "	0.030 "	
Sodium bicarbonate	0.08 "	0.025 "	
	TRACE ELEMENTS		
Magnesium	Between 4 and 8 mg/litre	Less than 0.05 mg/litre	
Tin	1 mg/litre	Not detected	
Copper	0.3 mg/litre	Not detected	
Iron	Less than 0.1 mg/litre	Less than 0.1 mg/litre	
Cobalt	Not detected	Not detected	0.5 mg/litre
Chromium			0.25 "
Manganese			0.02 "
Nickel			0.02 "

Table 17 The composition of Ringer's solution made with distilled water and tap water.

(6.1) Discussion

It is possible to draw the following conclusions from the results of this Paragraph:

1. The indentation of articular cartilage is not significantly altered, by a variation of the Ringer's solution within the following limits:

Constituent	Limits of variation in Ringer's solution
Sodium chloride	9.0 to 9.1 gm/litre
Potassium chloride	0.026 to 0.053 gm/litre
Calcium chloride	0.030 to 0.053 gm/litre
Sodium bicarbonate	0.08 to 0.025 mg/litre
Magnesium	(4 to 8) to 0.05 mg/litre
Tin	0 to 1 mg/litre
Copper	0 to 0.3 mg/litre

Table 18 Limits of Variation of main constituents of Ringer's solution

2. The indentation of cartilage is not significantly altered by a variation in pH of the Ringer's solution, of one unit. This confirms the observation of Elmore et al. (1963), that the indentation of cartilage was not significantly altered by a pH change from 3.5 to 8.3 units.

On the basis of these results it is possible to conclude that the amount of contamination of the Ringer's solution by tap water which may have occurred during the indentation surveys, did not significantly alter the indentation of cartilage.

Throughout this investigation the term Ringer's solution may be interpreted as a saline solution having a composition within the limits indicated in Table 18.

(7) Comparison of indentations with synovial fluid and Ringer's solution as the immersion media

The final control experiment was performed to determine whether the indentation of cartilage surrounded by synovial fluid, is different from the indentation under Ringer's solution.

Nine areas of cartilage on four femoral heads were subjected to repeated indentations, initially with synovial fluid surrounding the tested area, and then with Ringer's solution.

In order that the film of synovial fluid covering the articular surface of each femoral head was not disturbed, care was taken not to touch the cartilage between disarticulation and mounting the femoral head in the testing apparatus. Surplus synovial fluid was collected from the acetabulum in a syringe, and this was used to surround the end of the indenter during a test. Ringer's solution was not used with any femoral head until all the selected areas of cartilage had been tested with synovial fluid.

Indentations were compared at two seconds after load application between the two immersion media, and the results are shown in Table 19, see over.

(7.1) Discussion of results

The results of Table 19 show, that the difference between the indentations measured under the two solutions, was within the limit of $\pm$5% of repeatability of the apparatus.

It was concluded that the stiffness of articular cartilage is the same in Ringer's solution, as it is in the synovial fluid.

Indentation (cm)		Difference between (1) and (2) as a percentage of (1)
(1) With Synovial Fluid	(2) With Ringer's Solution	
0.0612	0.0585	-4.3
0.0781	0.0821	+5.0
0.0218	0.0226	+3.7
0.0233	0.0238	+2.2
0.0076	0.0074	-3.3
0.0132	0.0127	-3.8
0.0185	0.0178	-3.8
0.0216	0.0213	-1.5
0.0500	0.0520	+4.0
0.0582	0.0561	-3.5
0.0356	0.0366	+2.8
0.0270	0.0283	+4.8

Table 19 Results of tests performed to determine the difference between indentations performed under synovial fluid and then Ringer's solution.

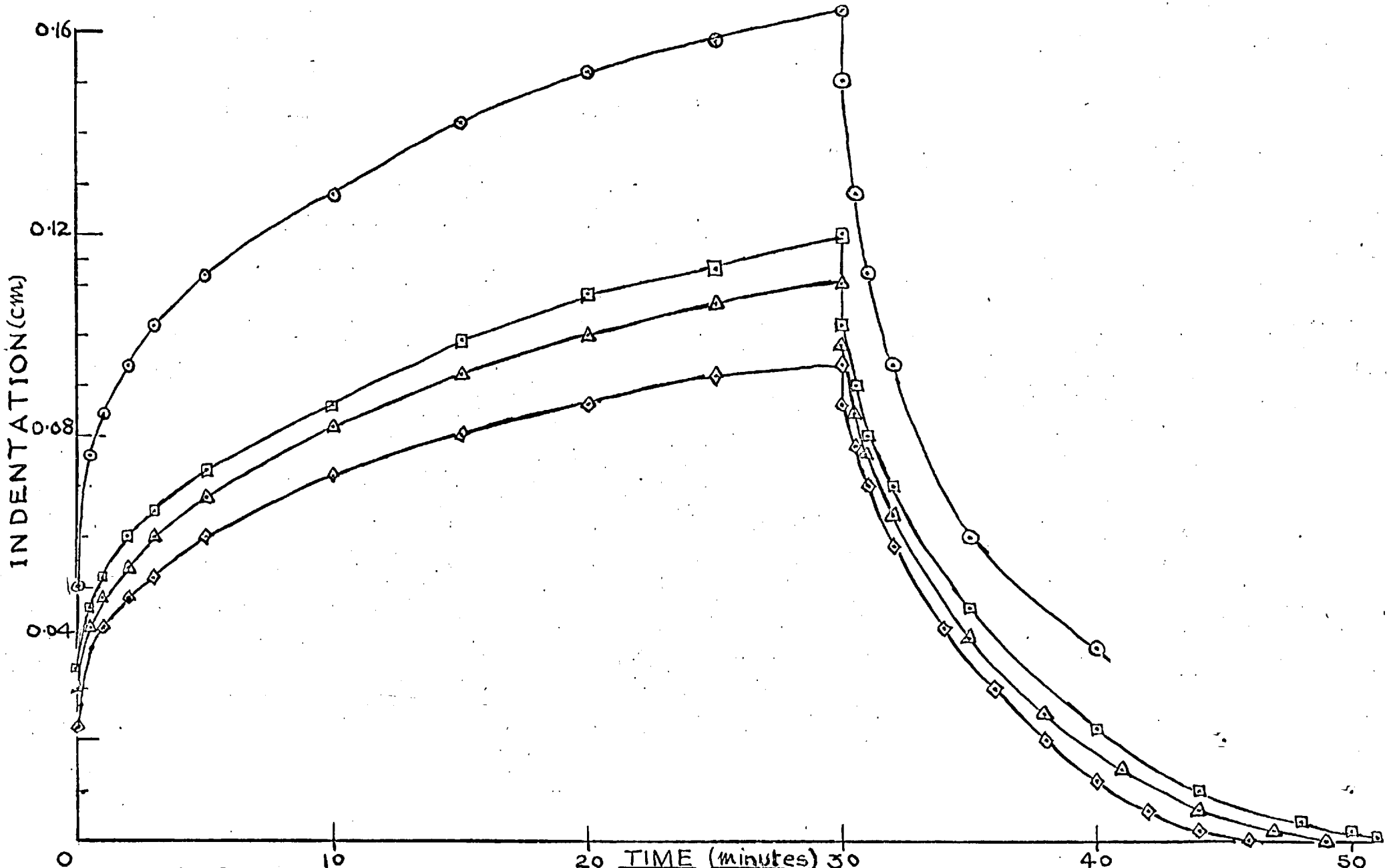


FIG 16. TYPICAL INDENTATION CURVES: [SPEC. Nº 7, PLANE ENDED-INDENTER, LOAD 5 lbf (2·28 Kgf)]

CHAPTER 5

DESCRIPTION OF INDENTATION TESTS AND PRESENTATION OF OBSERVATIONS

(1) The shape of an indentation versus time curve

Before the detailed indentation surveys were performed, the observations of which are given briefly in Para. 4 of this Chapter and discussed together with the results in Chapter 9, the shape of a typical indentation-time curve was considered for three reasons:

Firstly, to attempt to confirm the results of previous workers, or if differences existed then to define and explain such differences;

Secondly, to explain the characteristics of a typical curve in terms of the structure of cartilage and;

Finally, to derive a mathematical expression fitting the experimental points of a representative number of curves, from which to define a time at which indentations could be reliably compared, and to define which parameters of the expression vary with the magnitude of the indentations.

Fig. 16 (facing p. 49) shows the indentation-time curves for four different areas of cartilage. The shape of the curves is typical of all the areas tested in the indentation surveys, irrespective of which indenter was used. There is a marked similarity between the load phase of each curve and those obtained by other investigators (see Chapter 1, and Bibliography). The unload phase however is similar in shape to the results of only Elmore et al. (1963) because all other previous experiments were performed in air (see Chapter 1).

A more detailed description and discussion of the shape of an indentation-time curve is given in Chapter 9, Paras. 1, 1.1 and 1.2 (see pp. 76 to 84).

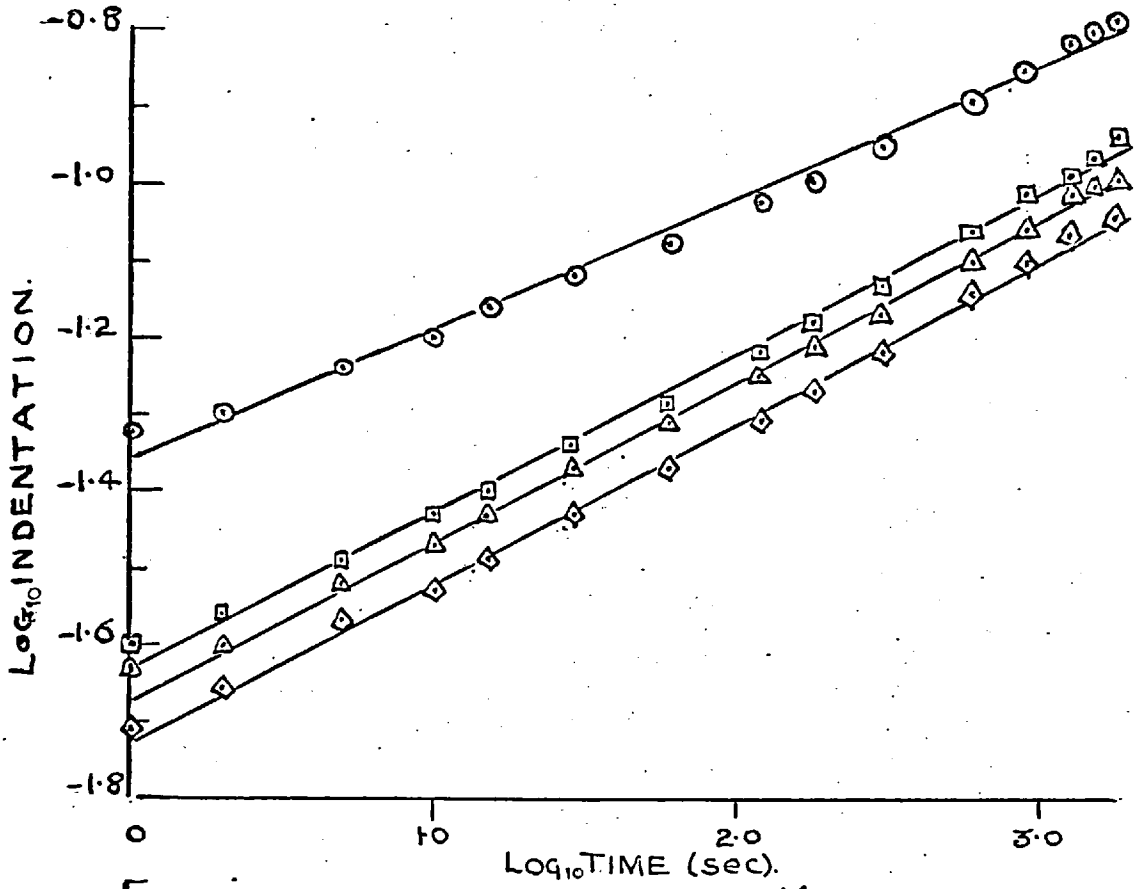


FIG. 17. INDENTATION CURVES OF FIG. 16 PLOTTED LOGARITHMICALLY.

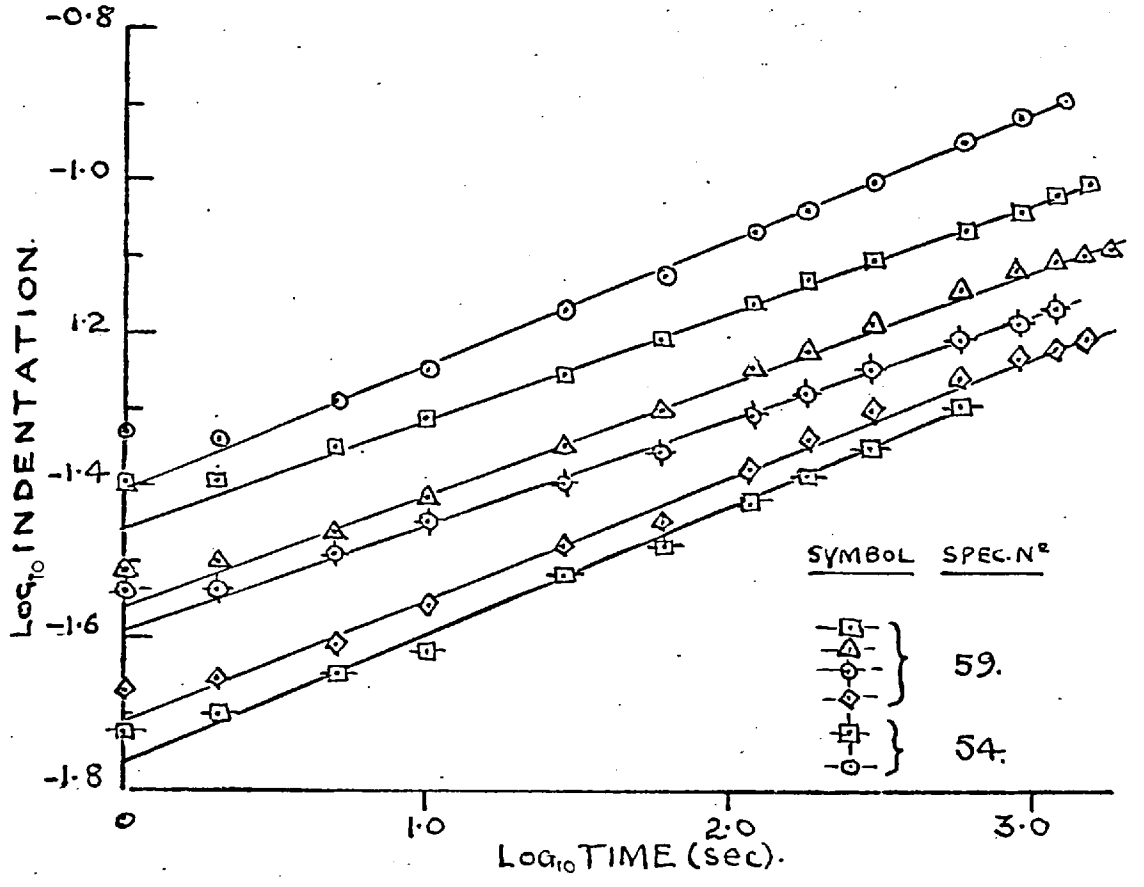


FIG. 18. ADDITIONAL INDENTATION CURVES PLOTTED LOGARITHMICALLY.

(2) Selection of a time at which to compare indentations

The experimental curves of indentation versus time given in Fig. 16 (facing p. 49) show that the indentation of cartilage is time dependent. It was necessary therefore to select a common time at which to compare the indentations of different and corresponding areas of cartilage.

It will be appreciated, that so far in this investigation other variable quantities, such as the applied stress, the immersion medium and the temperature of the latter, were controlled as closely as possible to physiological conditions. In order to be consistent, it was decided to compare all indentations in the detailed surveys at a common time, which was representative of the duration of the load phase in normal walking. Although the hip joint is subjected to many different modes of loading in vivo, it was decided to consider the conditions of walking, since this activity forms a considerable proportion of the complete range of movement of a normal hip joint.

The results of Rydell (1964), Contini et al. (1964) and Swanson (personal communication) all indicate, that the duration of the load phase (heel strike to toe off) of a step taken whilst walking at normal speed, is between 0.5 sec. and 1.0 sec., depending on what constitutes a normal walking speed for different individuals.

The graphs shown in Figs. 17 and 18 (facing p. 50). and discussed in Para. 1.2 of Chapter 9 (p. 80) show that the curves of indentation versus time for cartilage are not fully developed until two seconds after application of the load. In view of this fact it was decided that indentations should be compared at two seconds after load application

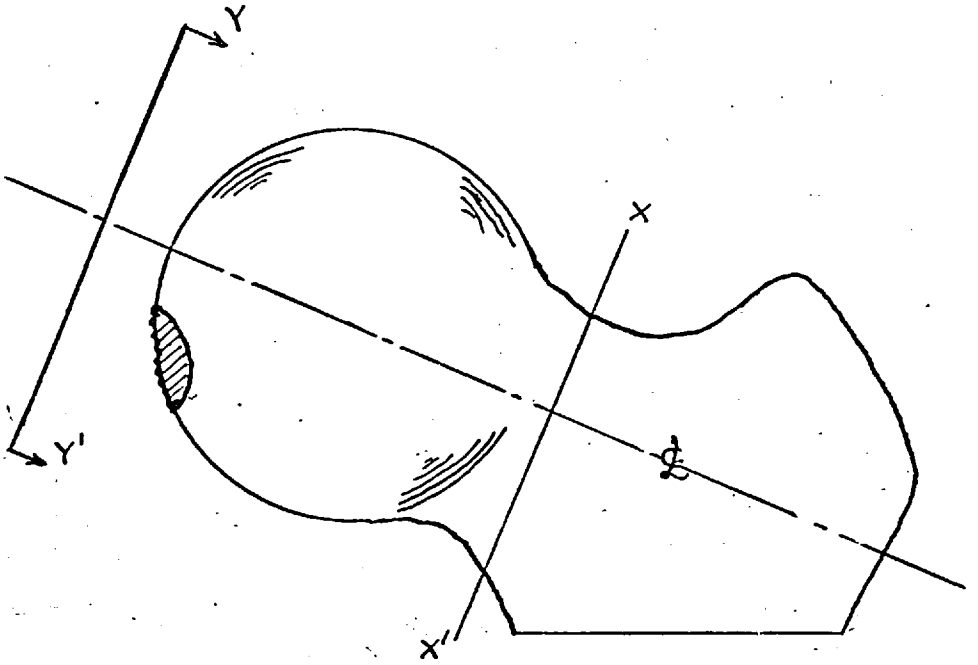
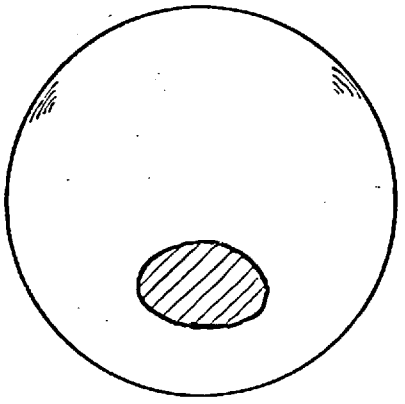
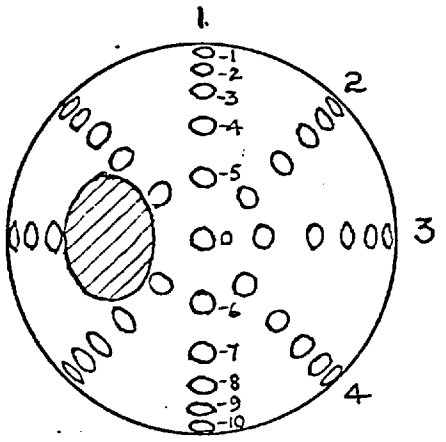


FIG. 20a.



(1). VIEW ON YY'



(2.) PLAN VIEW OF FEMORAL HEAD AS MOUNTED INITIALLY IN THE TESTING APPARATUS (VIEW(1.) ROTATED 90° CLOCKWISE]. SMALL CIRCLES & ELLIPSES REPRESENT INDENTED AREAS.

FIG. 20b. METHOD OF REPRESENTING TESTED AREAS.

this being the time which most nearly satisfies both the physiological and mathematical conditions.

One other reason for selecting the time of two seconds at which to compare indentations, was that removal of the load at this time results in very rapid recovery. In other words the elastic part of the indentation forms the major part of the total indentation, at two seconds after load application. The importance of this fact is described in greater detail in Para. 2 of Chapter 6, (p. 58).

Finally, the shorter the duration of any one indentation test, the larger was the number of areas which could be studied on a femoral head, before autolytic degradation interfered with the results.

(3) Method of displaying the observations

The testing apparatus previously described in Para. 3 of Chapter 2, enabled each femoral head to be indented in a maximum number of eighty-one areas. In practice due to the ever present threat of autolytic degradation, only thirty to forty areas could be studied. These areas were 0.125 in. in diameter (the diameter of each indenter), and they were arranged at equal intervals on four equi-spaced meridians over each femoral head, assuming this joint surface to be nearly spherical, Bullough et al. (1968).

Fig. 20a (facing p. 51) shows diagrammatically the proximal section of a femur, incorporating the femoral head, femoral neck and greater trochanter. View (1) is that seen by looking along the direction YY' from the medial aspect, (from inside the acetabulum). In fact this is the view of each femoral head represented in the layered maps of Figs. 21, 23; 29 and 31 (between pp. 83-4 and 85-6), with small variations, to account for the variation in position of the medial-lateral line in each femoral head.

Fig. 20b (facing p.51) shows the same view of the femoral head from YY', but this time the femoral head is rotated approximately 90° clockwise. This is the position in which each femoral head was initially mounted in the testing apparatus. Each indented area of cartilage is represented by a circle or an ellipse, depending on its distance from the 'pole' point O. Each meridian was numbered from one to four, and the indented areas were numbered from one to ten as shown, except the 'pole' point which was denoted by the symbol 'O'. By this numbering system the position of each indented area was recorded.

Having plotted the indentation-time curve for each area of cartilage, and then extracted the indentations at two seconds after application of the load, it was possible to divide the total range of indentation for all the femoral heads into five smaller ranges see Fig. A (facing p.84). To each range a particular shading was then allocated. The appropriate shading was then allocated to each indented area. Areas of similar shading were then joined, to produce layered maps of indentation over all of the femoral heads. Corresponding areas were defined by referring to the drilled holes described in Para. 1 of Chapter 3, (p. 24), the line between then delineating the medial-lateral direction.

In addition, histograms of the cartilage indentation values were drawn, the vertical axis representing the number of indented areas, and the horizontal axis representing the indentation ranges. The mean indentation for each femoral head was also calculated, and is presented with the corresponding histogram.

(4) Observations

Sixteen femoral heads were studied within the age range of nineteen to eighty two years. A total number of five hundred and ten areas of visibly

normal cartilage were indented in two series of tests. In the first series a plane-ended cylindrical indenter was used, 0.125 in. in diameter, with a load of 5 lbf. In the second series six femoral heads were indented by a hemispherically-ended cylindrical indenter of the same diameter as the plane-ended indenter, with a load of 2 lbf.

Fig. A (facing p. 84) shows the indentation ranges and corresponding shading which were used in the layered maps and histograms of indentation.

Figs. 21 and 22 (between pp. 83-84) show the layered maps and histograms of indentation, which relate to the first series of tests in which the plane-ended indenter was used.

Figs. 23 and 24 (between pp. 83-84) show the similar maps and histograms of indentation relating to the second series of tests, in which the hemispherically-ended indenter was used.

Columns A and B of Tables 20 and 21 (see Appendix 1, pp. 138 - 153) give the recorded thickness and indentation, for all of the five hundred and ten indented areas. It is from these values that the maps and histograms were constructed.

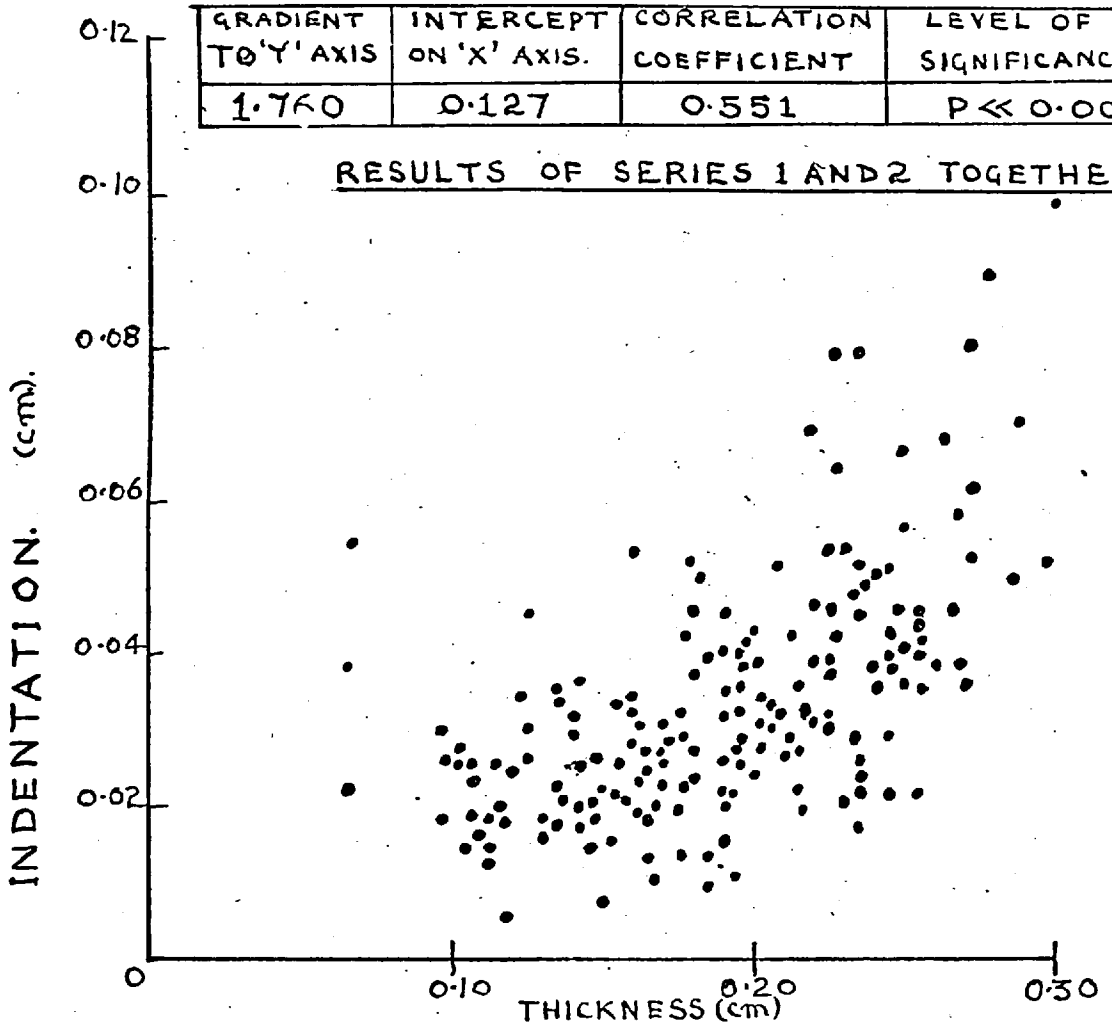
The observations made from Figs. 21 to 24 are explained and discussed in detail in Para. 2 of Chapter 9 (p. 83).

(5) Correlation of indentation with thickness

As Tables 20 and 21 (see Appendix 1, pp. 138 to 153) reveal, the cartilage covering the human femoral head is between approximately one and three millimetres thick. At this order of magnitude of thickness the restrictive effect of the base, which in this case is sub-chondral bone, may be expected to influence the recorded indentation.

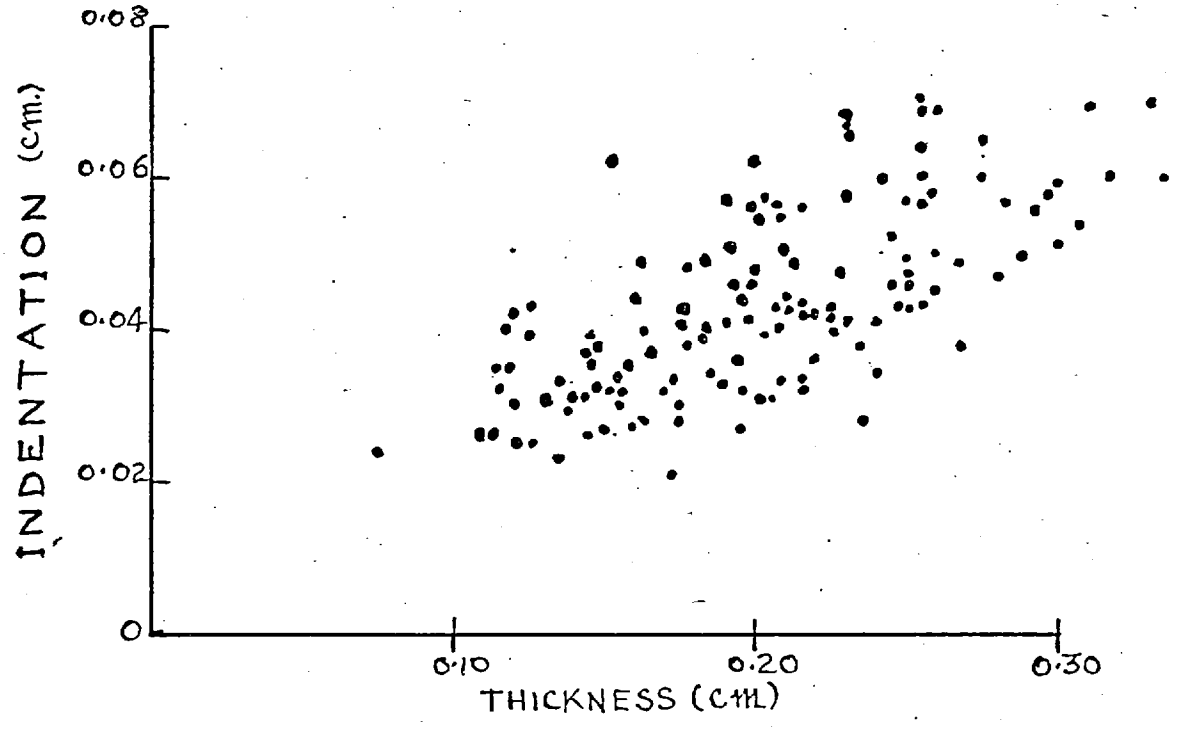
GRADIENT TO 'Y' AXIS	INTERCEPT ON 'X' AXIS.	CORRELATION COEFFICIENT	LEVEL OF SIGNIFICANCE.
1·760	0·127	0·551	$P \ll 0.001$

RESULTS OF SERIES 1 AND 2 TOGETHER.



SERIES 1.

FIG. 25. INDENTATION vs THICKNESS: PLANE ENDED INDENTER LOAD 5lbf (2·28Kg).



SERIES 2.

FIG. 26. INDENTATION vs THICKNESS: HEMISPHERICALLY ENDED INDENTER, LOAD 2lbf (0·91 Kg).

Sokoloff (1966) showed that the 'instantaneous' indentation of rubber specimens under an average indenting stress of 8.41 Kg/cm^2 increased as the thickness increased up to two millimetres. At thicknesses greater than this, the indentation was sensibly constant. Sokoloff chose natural rubber, of which the 'instantaneous' indentation was similar to that of normal articular cartilage, to illustrate how indentation was related to thickness. He concluded that no compensation for thickness was necessary for his results on patellar cartilage because in general cartilage on this joint surface is more than two millimetres thick.

In order to determine the correlation between indentation and thickness for the results of the present indentation surveys, the results were processed in three ways: firstly, graphs of indentation versus thickness were plotted for both of the two series of tests, these are given in Figs. 25 and 26 (facing p.54); secondly a computer program was written (see Appendix 2, p.161), which enabled a straight line to be fitted to the experimental points, according to the method of least squares. This also gave the correlation coefficient 'r' between the two variables. Finally, the best line and correlation coefficient were determined for each femoral head, these results are presented in Table 22, Appendix 1 (p.154). The significance of a particular value of 'r' was expressed as a probability P, (the smaller the value of P, the greater the significance of r), see Davies O.L. (1961) Table E , p.372). [see Bibliography].

Both Figs. 25 and 26, and the overall correlation coefficient of 0.55 show that there was a significant correlation between indentation and thickness of cartilage on the femoral head. It was concluded, that on the basis of this observation, the indentation surveys do not give a completely

accurate picture of the relative stiffness of different areas of cartilage, since no account was taken of the variable thickness of the tissue.

Having reached this conclusion, it was necessary to find a method of calculating the stiffness of cartilage, irrespective of the thickness.

(5.1) Method of compensating for the variable, finite thickness of cartilage

Several methods of compensating for the variable thickness of cartilage were investigated. These will now be discussed to show why only one method was selected.

Initially the possibility of obtaining an indentation versus thickness curve for each indented area of cartilage, was considered. However this was rejected for two main reasons:

1. Firstly, it would have meant that only a few areas of cartilage could have been investigated, because of the limited time for which a femoral head could remain at 37°C, before autolytic degradation occurred.

2. Secondly, in order to obtain curves of indentation versus thickness, it would have been necessary to slice the areas of cartilage parallel to the articular surface. Each layer of cartilage would then have presented a different structural arrangement to the indenter and there are good grounds for supposing, at least for the superficial zone, that these structural differences would probably influence the indentation, irrespective of the thickness of the tested area. It would not have been possible therefore to separate the two effects when presenting the results.

   In addition, it was not possible to plot the indentations recorded for different areas and obtain a single valued function of inden-

tation versus thickness. These graphs have already been presented in Figs. 25 and 26, but because the experimental scatter is quite considerable, and the stiffness probably varies as well, so once again the two effects of stiffness and thickness on the indentation of cartilage could not be separated.

Thus, both of these attempts to compensate the indentation results for the variable thickness of cartilage, were hampered by the difficulties described above.

At this stage a similarity between the behaviour of natural rubber and cartilage was noticed. It was also recalled that Sokoloff (1966) had used natural rubber to show, that at thicknesses greater than two millimetres, the indentation of cartilage is not thickness dependent. Consideration of the published work of Waters (1965)$_{1,2}$ for the indentation of thin sheets of natural rubber revealed, that providing the material under consideration possessed certain characteristics in common with rubber, then the results of his investigations could be applied to such a material, for example cartilage[1], with some justification. The main characteristics which the material must possess are:

1. Elastic reversibility and,
2. Isotropy.

The grounds for assuming that cartilage possesses these characteristics will now be discussed, together with the derivation of a creep modulus to represent the stiffness of cartilage, irrespective of thickness.

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1. This was confirmed by personal communication with Mr. Waters.

CHAPTER 6

CALCULATION OF A CREEP MODULUS FOR CARTILAGE AND PRESENTATION OF RESULTS

(1) General background and assumptions

The theoretical solution to the problem of the pressure distribution between two spherical bodies in contact, was first given by Hertz (1881). The solution has been verified experimentally, for materials which obey Hookes Law[1] within the elastic limit. However, when the solution is applied to the problem of a rigid indenter pressed into a relatively soft elastic medium, the assumption is usually made that the medium is semi-infinite in extent; that is to say the boundaries are stress-free, except that which is in contact with the indenter.

Waters (1965)$_{1,2}$ pointed out, that although analytical and in some cases numerical solutions have been obtained for sheets of finite thickness, for a variety of boundary conditions [Sneddon (1951)], the solutions for cylindrical plane-ended and hemispherically-ended rigid indenters, did not exist in the literature. However, the relationship between indentation hardness of vulcanised rubbers and Young's modulus was shown by Gent (1958) to follow from the work of Hertz, with the following assumptions:

1. That the rubber is elastically reversible.
2. The rubber is isotropic, and
3. The indentation is small enough to assume that the boundaries are stress-free.

Waters initially considered why the behaviour of thin sheets of rubber should be different from that of semi-infinite sheets of the same material. He reached the conclusion theoretically, that departure from the ideal behaviour could be due to either.

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1. Hooke's Law for elastic materials states that stress is proportional to strain, the constant of proportionality being E, the Young's modulus for the material.

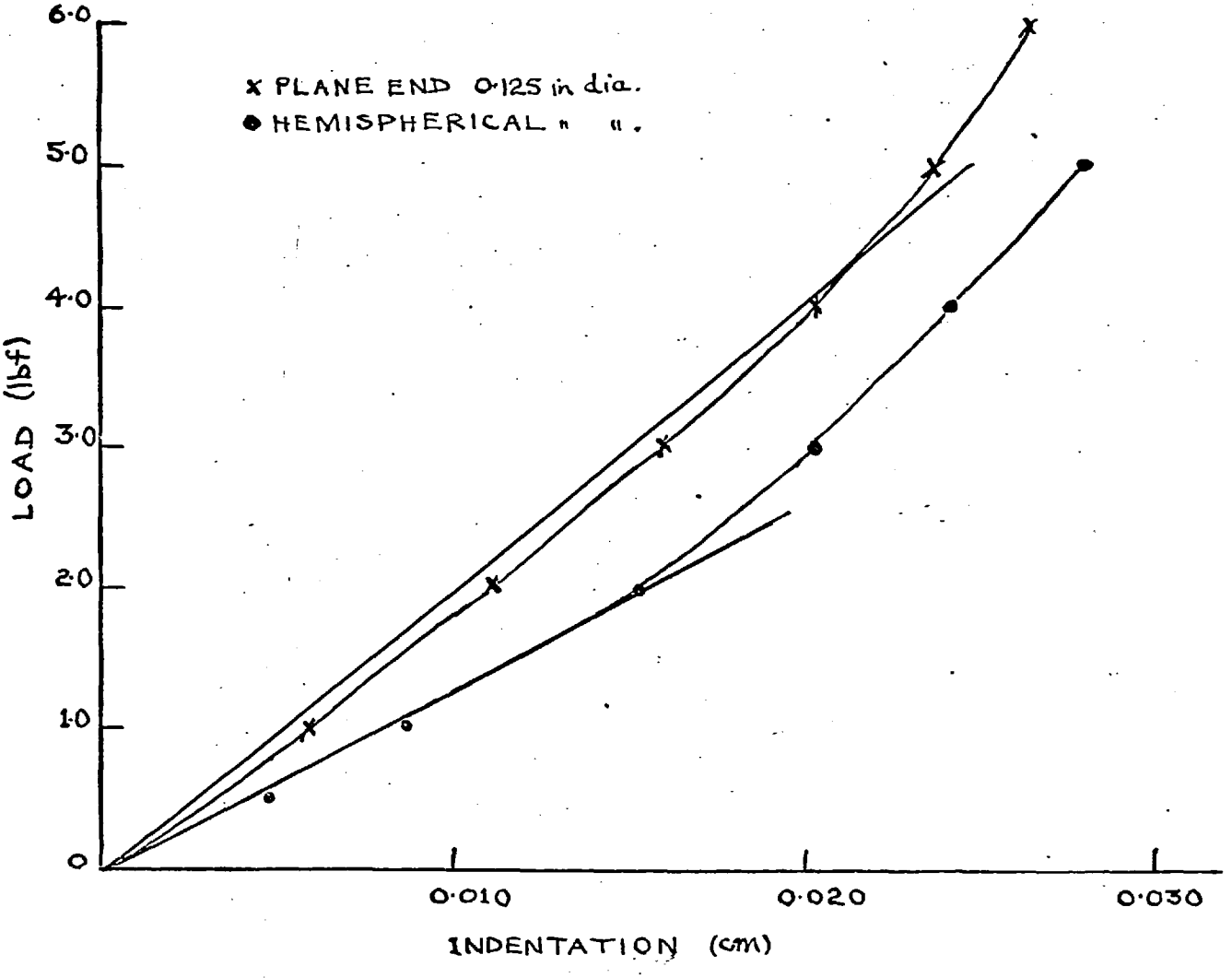


FIG. 26a CURVES OF LOAD versus INDENTATION. FOR BOTH INDENTERS.

1. Deformation of the rubber producing strains outside the elastic limit or,

2. Distortion of the stress pattern due to the finite thickness of the sheet.

Waters (1965)[1,2] concluded that the second of these two factors exerted the predominant influence, and therefore the indentation of a thin sheet of rubber differs from that of a semi-infinite sheet of the same material, mainly because the stress pattern is distorted by the proximity of the lower boundary.

Waters (1965)[1,2] performed two experimental investigations, from which he was able to calculate an elastic modulus for thin sheets of rubber using either a plane-ended or hemispherically-ended indenter. Before the results of these investigations could be applied to indentation measurements on cartilage, it was necessary to consider the validity of the underlying assumptions.

(2) Discussion of the assumptions

Considering the first assumption that the material should be elastically reversible, this is justifiable for cartilage only at short times after load application. Within this region of the indentation-time curve the 'instantaneous' indentation, and recovery if the load is removed, constitute the major part of the total indentation. This is because the amount of water which is expressed is small initially, and increases as a function of time. Fig. 26a (facing p.58). shows graphs of load versus indentation at two seconds for two areas of cartilage using both the plane-ended and hemispherical indenter of 0.125 in. diameter. It will be seen that cartilage displays an essentially non-linear load indentation curve. However, the non-

linearity is most noticeable at the upper end of the load range. The curve relating to the hemispherical indenter is fitted quite closely by a straight line up to a load of 2 lbf; that of the plane-ended indenter is fitted by a straight line again quite closely, up to a load of 5 lbf.

On the basis of these results it was decided that the assumption of elastic reversibility was justified for the two loads given above, for cartilage at two seconds after application of the load. This also constituted the third and final reason why two seconds was the time selected at which to compare indentations.

The second assumption which has to be considered is that of isotropy:

1. in the bulk of cartilage, beneath the surface layer, where it will largely determine whether the stress distributions are geometrically similar for the same ratio of sheet thickness to the radius of the contact area between the indenter and cartilage surface. This is one of the fundamental assumptions made by Waters;
2. in the surface layer, where it will determine whether the projected contact area is circular or approximately elliptical.

   Cartilage consists of collagen fibres and chondrocytes embedded in a gel of mucopolysaccharides and water. In the bulk material, the collagen fibres display an approximately random distribution. This can be seen in electron micrographs of cartilage, Weiss et al. (1968), and Maroudas and Bullough (1968). There is a tendency for the fibres in the deep zone to be orientated perpendicularly to the sub-chondral bone, but this is not a very strong tendency. The gel of mucopolysaccharides, at the molecular level, consists of an essentially random, three-dimensional, network of linear polyanions, Maroudas (1968), and it is commonly accepted that, in healthy cartilage, the mucopolysaccharides are distributed essentially

uniformly throughout the matrix. The chondrocytes occupy an extremely small percentage of the volume of cartilage, between 2 and 5 per cent, Meachim and Collins (1962) and Stockwell (1967), and it is extremely unlikely that they contribute significantly to the mechanical properties.

It appears, therefore, that the major constituents in the bulk of cartilage are distributed approximately randomly and the assumption of isotropy is justifiable. Tension tests performed by the author, parallel to the articular surface at increasing distance below the surface, support the assumption of isotropy in planes parallel to the surface, in the bulk of cartilage. (see pp. 169 to 171 in folder at rear).

The surface layer of cartilage consists of a network of collagen fibres, parallel to the articular surface, with a well-ordered orientation. As section 3 of this thesis (p.97 et seq.) reveals, it is anisotropic, being stiffer in the direction of the fibres than perpendicular to them. The projected area of contact will therefore be approximately elliptical. This has been accepted in the present tests, because it will have little effect on the depth of the indentation.

The effect of varying Poisson's ratio must now be discussed.

Equations (6) and (7) for calculating the Creep Modulus of cartilage are given on pages 60 and 61.

In this investigation indentations were measured at 2 seconds following load application. At this time approximately 90% of the indentation is "instantaneously" recoverable. Constant volume deformation was considered to be an essentially valid assumption, for which the Poisson's ratio $\nu = \frac{1}{2}$. Some tension tests, performed parallel to the articular surface, supported this value in tension. However, since some sponge-like materials possess a value of ν greater than $\frac{1}{2}$, and for many metals $\nu \approx \frac{1}{3}$, calculations were performed to determine the effect, on the Creep Modulus, of ν taking the values of $\frac{1}{3}$ or $\frac{2}{3}$ instead of $\frac{1}{2}$. Table 19a, over, gives the results:

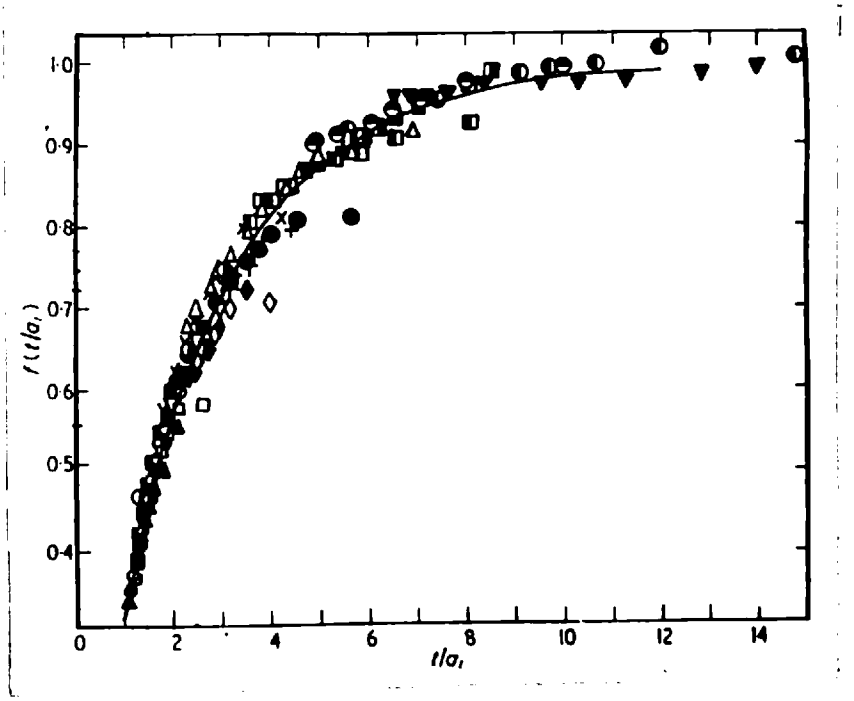


Fig. 4a. Curve of $f(^{t}/a_{t})$ vs $^{t}/a_{t}$ for Thin Sheets of Natural Rubber-Hemispherical Indenters

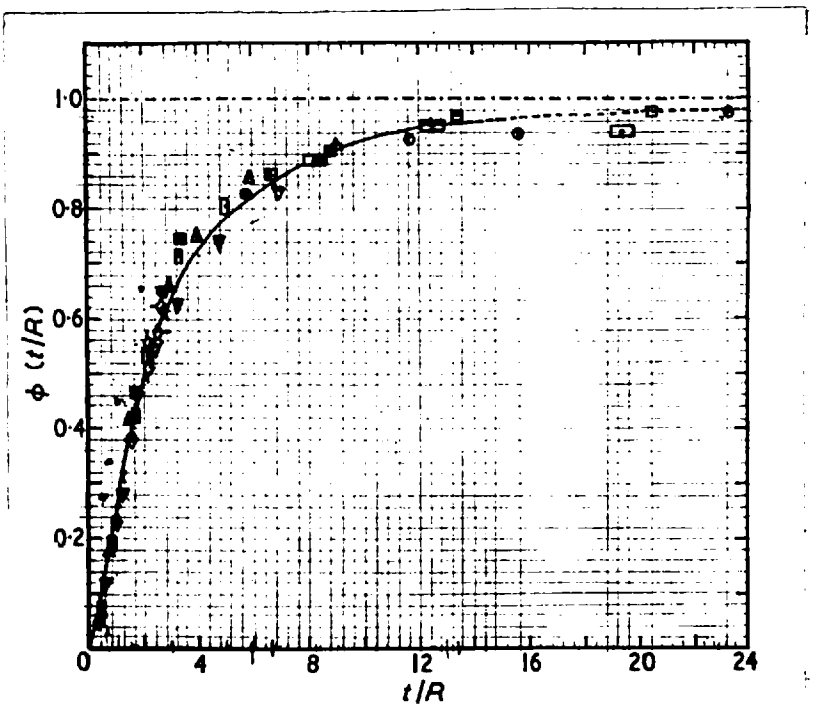


Fig. 4b. Curve of $\varphi(^{t}/R)$ vs $^{t}/R$ for Thin Sheets of Natural Rubber - Plane-Ended Indenters

* Both curves are reproduced from the work of Mr. N.E. Waters (1965) [see Bibliography], by kind permission of the author.

ν	$(K_1)^{\frac{3}{2}}$ from Eq'n (6)	$(1-\nu^2)$ from Eq'n (7)	Percentage difference in 'E'
$\frac{1}{2}$	$\frac{9}{16}$	$\frac{3}{4}$	ORIGINAL
$\frac{1}{3}$	$\frac{2}{3}$	$\frac{8}{9}$	+ 18.5
$\frac{2}{3}$	$\frac{5}{12}$	$\frac{5}{9}$	- 26

Table 19a. The effect on Creep Modulus of Varying Poisson's ratio

If the value of Poisson's ratio wasuniform over an entire femoral head then the maps of Creep Modulus, which are described later, would not be changed, except in so far as the individual values would be changed by a constant factor. Alternatively if Poisson's ratio was $\frac{1}{3}$ or $\frac{2}{3}$ instead of $\frac{1}{2}$ in some areas only, then the pattern of variation of Creep Modulus would be changed in the middle ranges but the main conclusions, which relate to the stiffest and softest regions, would not be altered.

It is possible to conclude therefore that even a large variation in Poisson's ratio would not significantly alter the conclusions of this investigation.

(3) Calculation of a creep modulus for cartilage

For a detailed derivation of the equations from which an elastic modulus for thin rubber sheets was calculated according to Waters (1965)[1,2] the reader is referred to Appendix 3 (pp.162 to 166). At this stage only the final equations are quoted, which were used directly in calculating a creep modulus for cartilage.

1. Hemispherically-ended indenter

$$E = 1.28 \left[\frac{1 - \text{exp.}\ (-0.42\ {}^{t}/_{a_t})}{d_t} \right]^{3/2} \qquad (6)$$

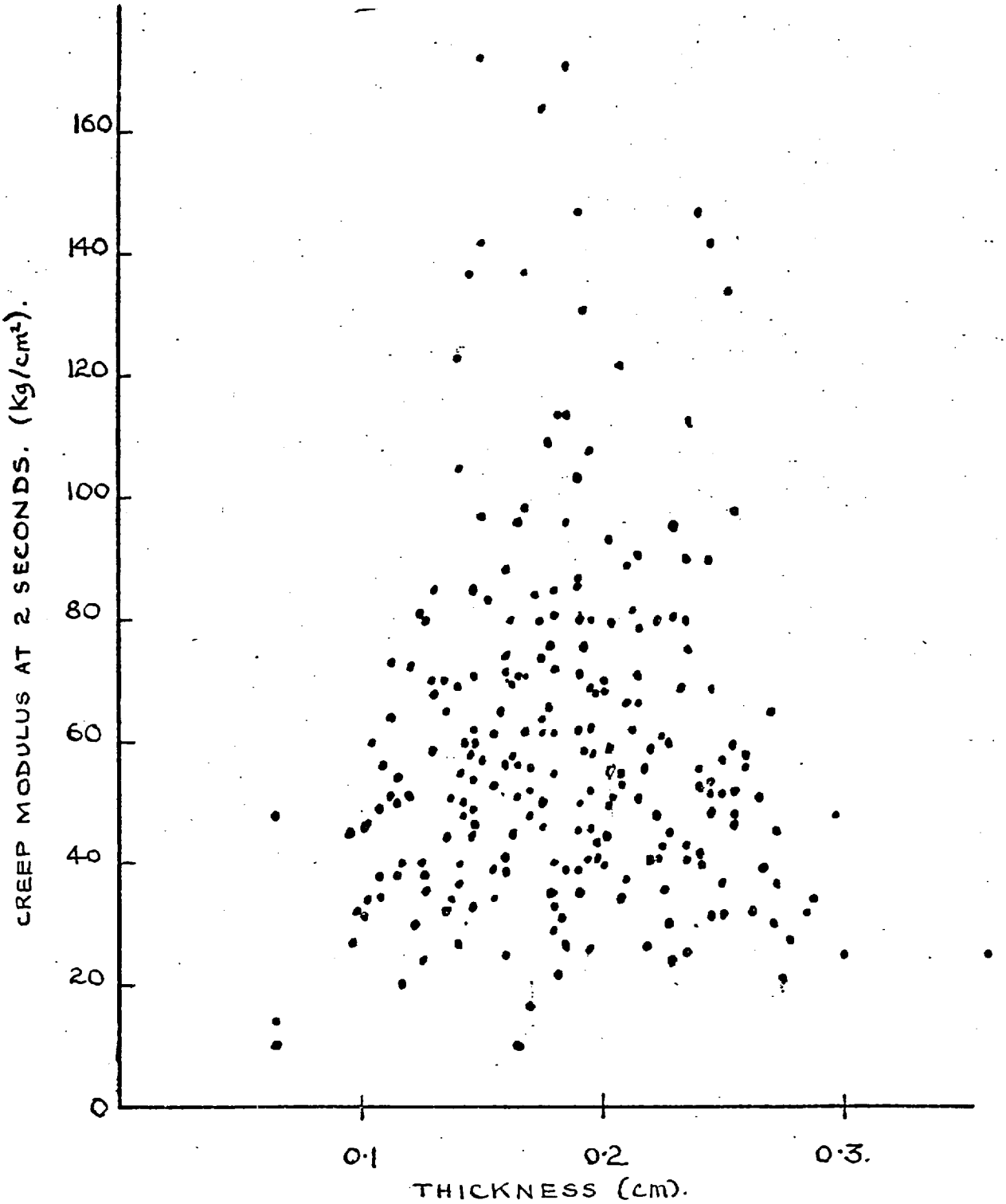
where t = thickness of indented area of cartilage (cm).

a_t = radius of contact area between cartilage and indenter (cm).

d_t = indentation (cm).

E = creep modulus for cartilage.

Fig. 41a (facing p.60) shows the curve obtained by Waters for $f({}^{t}/_{a_t}) = 1 - \exp(-0.42\ {}^{t}/_{a_t})$ versus $({}^{t}/_{a_t})$.



SERIES 1.

FIG. 33. CREEP MODULUS AT 2 SECONDS vs. THICKNESS. PLANE-ENDED INDENTER, LOAD 5lbf (2·28 Kg).

GRADIENT TO 'Y' AXIS	INTERCEPT ON 'X' AXIS.	CORRELATION COEFFICIENT.	LEVEL OF SIGNIFICANCE.
0·000	0·198.	−0·086	$P > 0·1$

RESULTS FOR SERIES 1 AND 2 TOGETHER.

2. Plane-ended indenter

$$E = 5.37 \left(\frac{\phi(\frac{t}{R})}{d_t}\right) \qquad (7)$$

where the symbols denote the same quantities as those used in equation (6) above, and $\phi = \phi(^t/R)$ where R = radius of indenter (cm). The value of this function was obtained using the graph given in Fig. 41b (facing p. 60).

The values of creep modulus at two seconds after load application, which were calculated for all the areas of cartilage tested in the indentation surveys, are tabulated in column C of Tables 20 and 21 of Appendix 1 (pp.138-53). They are also presented as layered maps and histograms see Figs. 29 - 32 (between pp.85 and86) identical in form to those in which the variation of indentation was displayed.

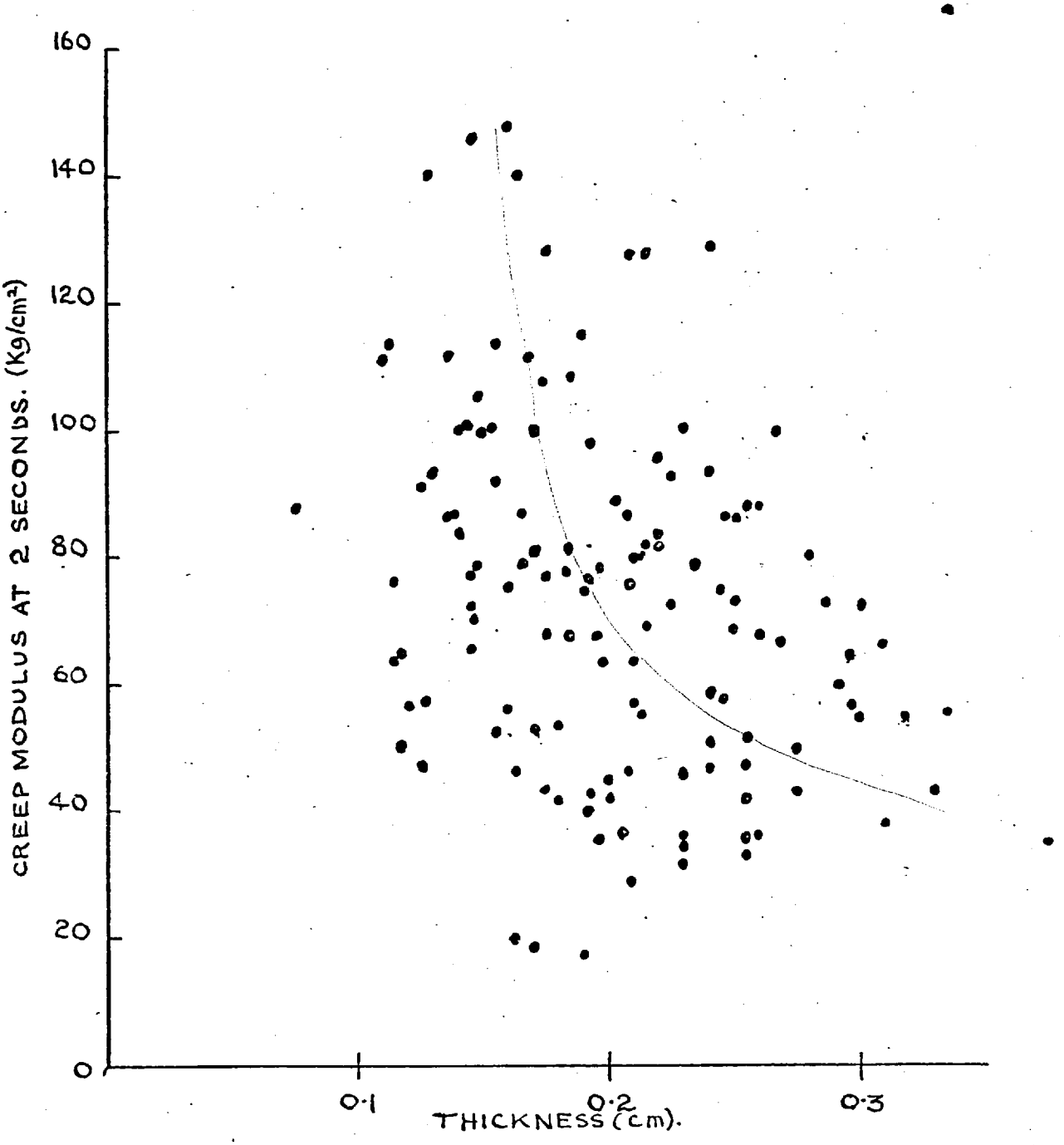
The results given in Para. 5 of this Chapter (p. 62) show, that both indenters gave creep modulus values for the same areas of cartilage, which were in close agreement. In addition, varying the applied load on the hemispherically-ended indenter, did not significantly change the creep modulus value for any one area of cartilage. The results of both series of indentation tests, when expressed in terms of the variation of creep modulus, may therefore be reliably compared.

The results shown in Figs. 29 to 32 are explained and discussed in Chapter 9.

(4) Correlation of creep modulus with thickness

Having defined a creep modulus at two seconds for cartilage, and stated that it is representative of the mechanical stiffness of the tissue irrespective of thickness, it was necessary to show that the creep modulus itself was independent of thickness.

Figs. 33 and 34 (facing pp. 61 and 62 respectively) show the graphs of creep modulus versus thickness, for the areas of cartilage tested in the



SERIES 2

FIG. 34. CREEP MODULUS AT 2 SECONDS VS. THICKNESS: HEMISPHERICALLY-ENDED INDENTER, LOAD 2 lbf (0·91 Kg).

indentation surveys; these graphs may be compared to Figs. 25 and 26 (facing page 54) of indentation versus thickness. It is clear from Figs. 33 and 34 that the correlation between creep modulus and thickness is considerably less significant than that between indentation and thickness. The experimental results were then processed, using the computer program given in Appendix 2 (see p. 161), the resulting fitted best straight lines, and correlation coefficients for each femoral head are tabulated in Table 23 of Appendix 1 (p. 155). It can be seen from Table 23, that the correlation between creep modulus and thickness for each femoral head was not very significant.

(5) Comparison between creep modulus values calculated from the results for both indenters

It has been shown so far in this Chapter, that the creep modulus of different areas of cartilage may be compared within each of the two series of tests. It has not been shown that the creep modulus for corresponding areas of cartilage may be compared between the two series of tests.

In order to do this, thirty areas of cartilage were indented using the hemispherical indenter with a load of 2 lbf (0.92 Kg), and the plane-ended indenter with a load of 5 lbf (2.28 Kg). The order in which the indenters were used was reversed so that it did not influence the results. Creep modulus values were calculated using equations (6) and (7). The results are plotted in Fig. 35 (facing p. 63), and tabulated in line 1 of Table 25 over. The data from which Fig. 35 was plotted are given in Table 24 of Appendix 1 (p. 156).

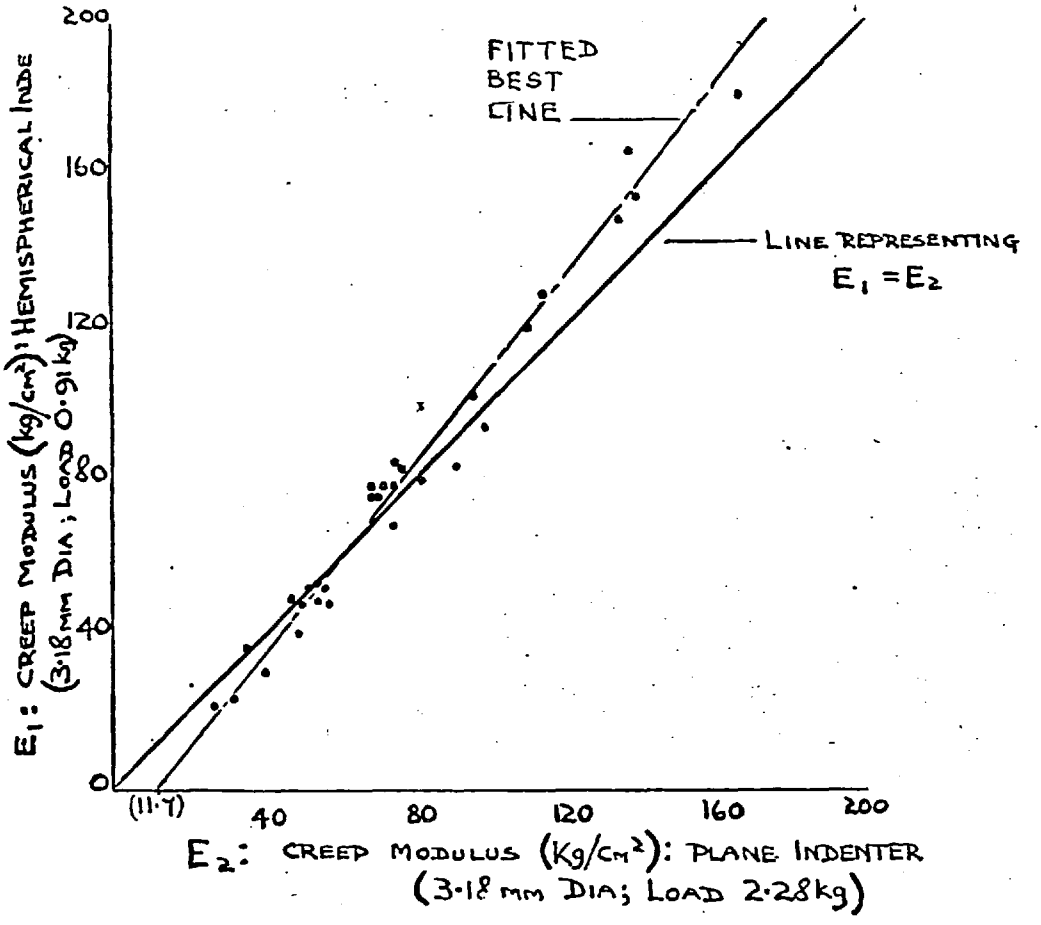


FIG. 35. GRAPH OF CREEP MODULI CALCULATED USING A HEMISPHERICAL INDENTER (LOAD 0·91 kg)(2 lbf) AND A PLANE-ENDED INDENTER (LOAD 2·28 kg)(5 lbf)

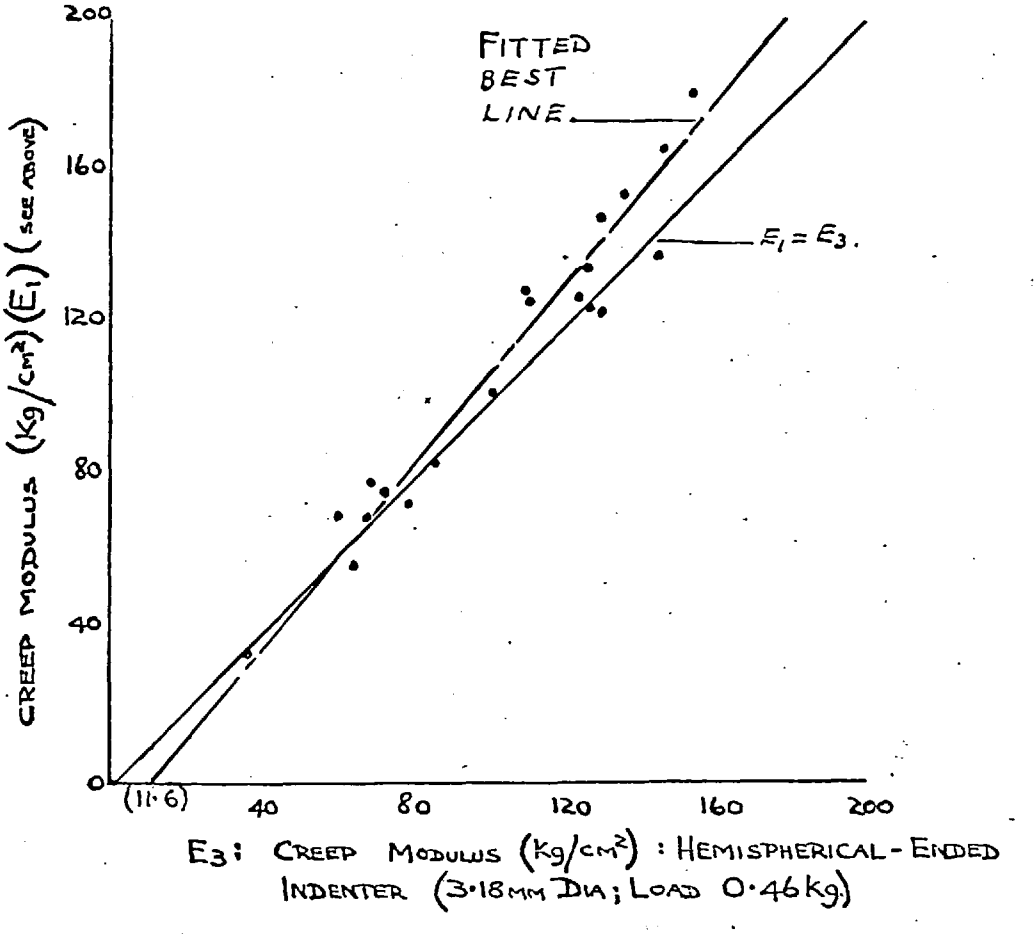


FIG. 36. GRAPH OF CREEP MODULI. 0·91 kg AND 0·46 kg; HEMISPHERICAL INDENTER

	Gradient to 'Y' Axis	Intercept on 'X' Axis	Correlation Coefficient
1.	0.81532	11.71525	0.98841
2.	0.84069	11.62680	0.97149

Table 25 Fitted Lines and Correlation Coefficients relating to Figs. 35 and 36.

Both Fig. 35 and the table above show, that there was close agreement between the two sets of creep modulus values. Below a creep modulus of approximately 100 Kg/cm^2 there is apparently random scatter of the experimental points about the line representing equal values of creep modulus. Above 100 Kg /cm^2 however, modulus values calculated for the hemispherical indenter are consistently higher than those of the plane-ended indenter. However, the difference is rarely more than ten per cent. These results partially justify the assumptions which were made for cartilage in Para. 2 of this Chapter. This was reassuring because the creep modulus values were determined from two independent sets of experimental data, and from equations originally formulated for natural rubber. It has also been shown that the resultsof both series of tests may be compared, when presented in the form of creep modulus values.

As a second check on the validity of a creep modulus for cartilage, the load was changed on the hemispherical indenter from 2 lbf to 1 lbf and it was shown that this did not alter the calculated value of creep modulus.

Twenty areas of cartilage on five femoral heads were indented using the hemispherical indenter, using loads of 2 lbf and 1 lbf (0.92 Kg and 0.46 Kg). Indentations at two seconds were measured, and creep modulus values calculated. These results are shown in Fig. 36 (facing p. 63), and in line 2 of Table 25 (p. 63). Table 26 of Appendix 1 (p. 157) gives the data from which Fig. 36 was plotted. The results, show that close agreement existed between the values of creep modulus calculated for the two loads.

From the results of this paragraph it was concluded, that creep modulus values calculated for a plane-ended indenter of 0.125 in. diameter with a load of 5 lbf (2.28 Kg), may be reliably compared with the values calculated for a hemispherical indenter of the same diameter, under a load of 2 lbf (0.92 Kg). In addition, varying the load on the hemispherical indenter from 2 lbf to 1 lbf does not significantly change the creep modulus, which helps to justify the use of the latter.

SECTION 2

Two investigations were performed, independently of the main indentation surveys, to determine the relationship between the indentation stiffness of cartilage and:

1. The chemical components of the tissue,

2. The permeability to water.

In the first investigation, the creep modulus values of a number of areas of cartilage were determined by the author, according to the method described in Section 1 of this thesis. These indented areas were then chemically analysed by Dr. H. Muir of the Kennedy Institute of Rheumatology. Histological sections were also prepared by Dr. P. Bullough lately of The Nuffield Orthopaedic Hospital, Oxford but now of The Hospital for Special Surgery, New York.

In the second investigation, the permeability of indented areas of cartilage was determined by Mr. J. Read (F.R.C.S.) in conjunction with, and using the technique developed by Dr. A. Maroudas of The Biomechanics Unit, Imperial College.

CHAPTER 7

THE RELATIONSHIP BETWEEN THE CREEP MODULUS (STIFFNESS) AND CHEMICAL COMPOSITION OF CARTILAGE

(1) Introduction

According to the published literature, the results of indentation tests performed on cartilage have been related to the chemical components of the tissue, mainly by histological studies. Indeed, reports of any such correlation are rare, and appear to concentrate on the contribution of chondroitin sulphate to the stiffness of the tissue.

Histological staining techniques are at best only semi-quantitive, which emphasised the need for a more accurate technique for determining the chemical components of cartilage.

A combined investigation was performed with Muir[1], to determine the relationship between the creep modulus of cartilage on human femoral heads and the following chemical components:-

1. Chondroitin sulphate content,
2. Keratan sulphate content,
3. Total mucopolysaccharide content,
4. Collagen content.

The results are presented in graphical form in Figs. 37 to 40 (facing pp. 91 to 93). The best straight line fit to each graph was determined, to gether with the partial correlation coefficients, using the computer program given in Appendix 2 (p. 161).[2] The data from which Figs. 37 to 40 were plotted are presented in Table 27 of Appendix 1.

The results are explained and discussed in detail in Para. 4 of Chapter 9. (pp. 90 to 92).

(2) Materials and Methods

Forty seven areas of cartilage were studied, on eight femoral heads,

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1. See Acknowledgements. 2. See Appendix 3 (pp. 162 - 166) also.

within the age range of 21 to 77 years, for details see Tables 1 and 2 of Appendix 1. All the femoral heads were obtained at routine post-mortem examinations, and stored in the manner indicated in Para. 1 of Chapter 2 (p. 15).

(3) Indentation tests

The areas of cartilage to be indented, on each femoral head, were selected with reference to the results of the main indentation survey, so that the range of creep modulus values would be as large as possible.

The indentation tests were performed using the same apparatus and employing the same techniques as those described in Chapters 2 and 3 (pp. 15 to 30). The hemispherically-ended indenter, 0.125 in. in diameter was used, with loads of 1 lbf and 2 lbf, the selection being arbitrary.

After testing the selected number of areas on each femoral head, the solid-ended indenter was replaced by the trepanning tool of the same diameter, see Para. 4 of Chapter 2 (p. 16), and each area of cartilage was extracted. After the thickness of each area of cartilage had been measured it was passed to Muir for chemical analysis.

The creep modulus of each indented area of cartilage was calculated from the equations given in Chapter 6 (pp. 57 to 64).

(4) Chemical Analysis

The Hexosamine and Uronic Acid contents of each specimen of cartilage were determined by the method described by Maroudas, Muir and Wingham (1969).

The specimens were too small to allow separate hydrolyses to be performed for both hexosamine and hydroxyproline, therefore the hydroxyproline content was determined from aliquots of the hydrolysate used for hexosamine analysis (4N - HCl, 4 hours at 95^{o}). Incomplete hydrolysis of collagen was

accounted for by hydrolysis curves. It was determined from these curves that 79.5% of the hydroxyproline was released under these conditions and therefore a correction factor of 1.26 was applied to account for incomplete hydrolysis.

1. Chondroitin sulphate content

Having found the content of uronic acid in μg/mg dry weight, the next step was to calculate it in μM/mg dry weight, by dividing by the molecular weight of glucoronolactone

$$\frac{\text{uronic acid value}}{\text{M.W. of glucoronalactone}} = \frac{y}{176} \qquad (8)$$

From equation (8) the milli-equivalents of negative groups due to chondroitin sulphate are given by:

$$\frac{y}{176} \times 2$$

Hence, the content of chondroitin sulphate in μg/mg dry weight, is given by

$$\frac{y}{176} \times \text{dissaccharide weight.}$$

which gives $\frac{y}{176} \times 503$ = chondroitin sulphate content in μg/mg dry weight.

2. Keratan sulphate content (see note at end of Chapter)

In a manner similar to the estimation of chondroitin sulphate given above, from the hexosamine content, in μg/mg dry weight, the content in μM per mg dry weight can be calculated using the expression

$$\frac{\text{hexosamine value}}{\text{M.W. of glucosamine hydrochloride}} = \frac{x}{215.5} \qquad (9)$$

From equation (9) above the milli-equivalents of negative groups due to keratan sulphate are given by

$$\frac{x}{215.5} = \frac{y}{176} \times 1 = z$$

and finally

z x 435 = Keratan sulphate content, in μg/mg dry weight.

3. Collagen content

It has been determined, that mammalian collagen contains approximately 14.4% by weight of hydroxyproline, - (Jackson and Cleary 1967). Therefore the following calculation enabled the percentage of collagen to be calculated from a measurement of the hydroxyproline content:

(percentage collagen) = (hydroxyproline content) x 6.94 x 10^{-1}

where hydroxyproline content is measured in micrograms per milligram of the dry weight.

Note

The chondroitin sulphate in the specimens would be estimated by hexuronic acid, and both chondroitin sulphate and keratan sulphate by hexosamine, assuming that any hexosamine due to glycoprotins was negligible. Subtracting hexuronic acid from the values for total hexosamine would thus provide an approximate estimate of keratan sulphate. A value for the total negative charge per unit weight of cartilage contributed by each glycosaminoglycan was derived by making the following approximations; that chondroitin sulphate provides two negative groups, and keratan sulphate one per dissaccharide repeating unit.

Accuracy of the method

The method of Maroudas, Muir and Wingham (1969), which was used in this investigation, for estimating hexosamine and hexuronic acid was a slightly modified form of the automated method developed by Balazs et al. (1965). The results from the automated method of estimating hydroxyproline were compared to those obtained using the methods of Prockop and Udenfriend (1960).

Standard curves were determined during every analysis.

The accuracy of estimating hexosamine was $\pm$ 3%, Balazs et al. (1965_2), and that of hexuronic acid within $\pm$ 5%, Balazs et al. (1965_1). The accuracy of estimating hydroxyproline, by the method of Prockop and Udenfriend (1960), was within $\pm$ 4%. The automated procedure yielded results between 2.7 to 11.2% higher than those of Prockop and Udenfriend, for cartilage of femoral condyles, Muir et al. (1970), with an average difference of 6.34%. These results were confirmed on one femoral head in this investigation. The assumptions made when calculating the quantities of chondroitin sulphate, keratan sulphate and collagen, from measurements of hexuronic acid, total hexosamine and hydroxyproline (see p. 69), introduce unavoidable approximations. The inaccuracy so produced on any one constituent will, however, be approximately constant, in one direction and is unlikely to exceed 5% (Muir, personal communication).

Reproducibility data from duplicate analyses were:

Hexuronic acid $\pm$ 3%

Hexosamine)
Hydroxyproline) $\pm$ 2.2%

CHAPTER 8

THE RELATIONSHIP BETWEEN THE CREEP MODULUS AND PERMEABILITY OF CARTILAGE

(1) Introduction

It has been shown by Maroudas and Muir (1969), that the fixed negative charge density of articular cartilage is proportional to the total mucopolysaccharide content. In addition Maroudas (1968) has also shown, that as the fixed negative charge density increases, so the permeability of cartilage to water decreases.

From the results of these two investigations it appears that the permeability of cartilage decreases as the total mucopolysaccharide content increases.

The results of chemical analysis which was performed on areas of cartilage which had previously been indented have shown, that a significant positive partial correlation exists between the creep modulus and total mucopolysaccharide content of cartilage, see Chapter 7. It should be possible therefore to show, that the creep modulus of cartilage increases as the permeability decreases, and thereby confirm the results of chemical analysis. In addition, if the correlation between creep modulus and permeability should prove to be highly significant then the possibility should exist of using, either a modified indentation test in vivo, or a permeability test on extremely small areas of cartilage, as a measurement of the mucopolysaccharide content in the diagnosis of cartilage degeneration.

(2) Summary and objects of the investigation

A combined investigation was performed with Read (F.R.C.S.), and Maroudas (1968 and 1969), in which selected areas of cartilage on cadaveric human femoral heads, were subjected to indentation tests by the author in the

manner described in Chapter 3. These areas of cartilage were then trepanned from the femoral head together with approximately 0.25 in. of the sub-chondral bone. The thickness of each area of cartilage, and the permeability of slices 200μ thick taken at selected distances below the articular surface, were then measured by Read using the apparatus and techniques designed by Maroudas (1968 and 1969).

There were three purposes in performing this investigation:

1. To determine the relationship between the creep modulus of cartilage on the femoral head, and its permeability to water, and
2. Secondly, to confirm or deny the main result shown by chemical analysis, that the creep modulus depends directly on the total mucopolysaccharide content of cartilage.
3. Finally, if a highly significant correlation should be shown to exist between the creep modulus and permeability of cartilage, then to discuss the possible diagnostic use of either or both measurements.

The results of the investigation are presented in the form of a graph of creep modulus versus permeability coefficient, shown in Fig. 43 (facing p. 96).

The experimental observations from which Fig. 43 was drawn are tabulated in Table 28 of Appendix 1 (p. 160). The best straight line according to the method of least squares analysis was fitted to the experimental points, and the correlation coefficient was calculated. These results are discussed in detail in Para. 7 of Chapter 9 (pp.95 to 96).

(3) Materials and Methods

Thirty two areas of cartilage were studied from nine femoral heads, within the age range 23 to 78 years, see Tables 1 and 2, Appendix 1 (p. 127 to 129).

All the specimens were obtained from routine post-mortem examinations, and stored in the manner indicated in Para. 1 of Chapter 2 (p. 15).

(4) Indentation tests

The first part of the combined investigation consisted of indenting a number of selected areas of cartilage on each femoral head. These areas were selected according to the results of the indentation surveys which are discussed in Chapter 9, so that the range of creep modulus values was theoretically as large as possible.

The indentation tests were performed using the same apparatus, and employing the same techniques as those described in Chapters 2 and 3 (pp. 15 to 30). Both the plane-ended and hemispherically-ended indenters were used, with loads of 5 lbf, and 2 lbf and 1 lbf respectively, to ensure that the results were not influenced by the choice of indenter or load.

The creep modulus of each indented area of cartilage, at two seconds after load application, was calculated according to the procedure and equations described in Chapter 6 (pp. 57 to 64).

(5) Permeability tests

When the selected areas had been indented on any one femoral head, the head was passed on to Read, who initially trepanned each area of cartilage 0.125 in. in diameter, and sufficient surrounding cartilage to grip in the permeability apparatus, together with at least 0.25 in. thickness of the sub-chondral bone. The latter was then gripped in the vice of a sliding microtome, and the cartilage was sliced into layers approximately 200 μ thick parallel to the articular surface. In this way each area of cartilage was prepared for the permeability apparatus. It was inconvenient to test each slice, because in some cases there were ten or twelve such slices to each area of cartilage. Usually, alternate slices were selected for permeability measurements. This procedure was justified by the observation of Maroudas and

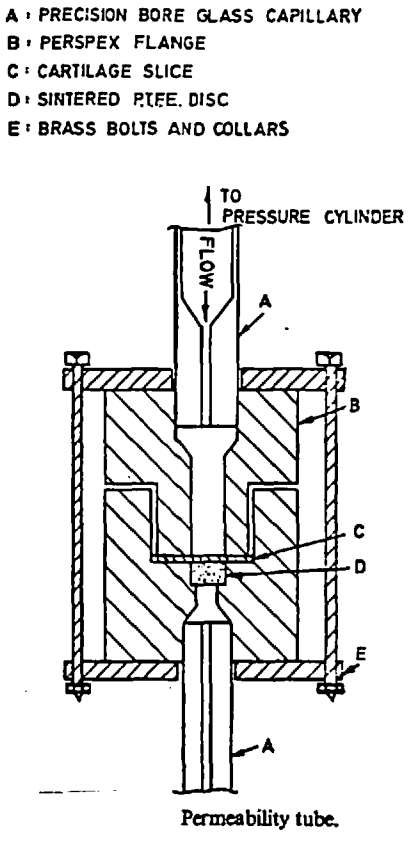


Fig. 44. Permeability Apparatus - from the work of Dr. A. Maroudas (1968), reproduced by permission of the author.

Bullough (1968), that the permeability of cartilage varies continuously and systematically with distance below the articular surface. Each slice of cartilage, which was circular in shape and approximately 0.375 in. in diameter, was then clamped around its periphery, in the apparatus designed by Maroudas for measuring the permeability of cartilage. A diagram of this apparatus is shown in Fig. 44, and for a full description of its use the reader is referred to the work of Maroudas (1968). Briefly, the apparatus functioned in the following manner: firstly the cartilage disc was clamped as explained above, leaving the central area exposed, which corresponded to either the whole or part of the indented region. Clamped in this way, liquid cannot flow out of the cartilage disc radially. Two small reservoirs, one on either side of the cartilage disc were directly connected to capillary tubes, each 1 mm in diameter. Ringer's solution was then added to both sides of the cartilage, so that both reservoirs were completely filled and both capillaries, partially filled. The capillaries were then arranged vertically, and the upper one was connected to a nitrogen cylinder, fitted with a pressure gauge. Thus, the Ringer's solution on the upper side of the cartilage slice, was subjected to a gauge pressure of one atmosphere. The flow rate of Ringer's solution (and therefore of water) through the slice was calculated by measuring the rate of movement of both[1] menisci.

(6) Calculations and presentation of results

From the measurements of liquid flow rate, the permeability coefficient K_h of a slice of cartilage at depth 'h' from the articular surface was calculated. The next problem was how to calculate the permeability of the entire thickness of an area of cartilage, from a knowledge of the permeability of individual slices; the solution which was adopted made the assump-

1. This acted as a check on any leakage radially.

ption that a permeability coefficient is essentially a measure of the resistance of a porous material to liquid flow. The lower is the resistance, the higher is the permeability coefficient. If the individual slices of cartilage were to be placed on top of each other in the correct order, then the total resistance of all the slices, and hence of the total thickness, would depend on the resistances, or permeabilities, of all the slices considered together. This was considered to be an analagous situation to electrical resistors arranged in series, in which case, if there are 'n' resistors, the effective resistance R, of the circuit is given by:

$$R = \sum_{i=1}^{n} R_i \qquad (10)$$

Equation (10) could have been used with permeability coefficients substituted for electrical resistances, to find the overall permeability of each area. However, it was already known that permeability varies continuously with depth from the articular surface, therefore on this basis graphs were plotted of the reciprocal of the permeability coefficient $^1/K_h$ versus depth from the articular surface. Fig. 42 (facing p. 96) shows a typical example of such a graph.

The permeability coefficient for the entire thickness of each area of cartilage was calculated from the equation:

$$\frac{1}{K} = \frac{1}{H}\int_o^H \left(\frac{1}{K_h}\right) \, dh$$

where 'h' is the distance of an individual slice below the articular surface,

H is the total thickness,

K_h is the permeability coefficient of a slice distance 'h' below the surface.

The integral was evaluated numerically from each graph of $^1/K_h$ versus 'h'.

As previously explained the results of this investigation are discussed, together with the results of the entire investigation in Chapter 9.

CHAPTER 9

RESULTS AND DISCUSSION

1. Qualitative description of an indentation versus time curve

Fig. 16 (facing p. 49) shows four indentation-time curves which are typical in shape, of all the curves recorded during the indentation surveys. The shape of the load phase agrees with the recorded curves of earlier investigators for example Gocke, Hirsch, Elmore and Sokoloff (see Chapter 1). In the present investigation however, complete recovery was recorded after the load had been removed. This observation agrees only with the results of Elmore and Sokoloff, since all previous investigators performed their experiments in air which led to incomplete recovery from loads within the physiological range.

Upon application of a static load via an indenter, cartilage displays essentially a two phase response.

Initially an 'instantaneous' or springlike indentation occurs. It has already been stated in Para. 5 of Chapter 1 (p. 9) that this indentation occurs in a few hundredths of a second, and yet it forms approximately one third of the total indentation measured at thirty minutes after load application. Sokoloff (1966) also recorded a similar figure.

Following on from the 'instantaneous' indentation is the creep, or time dependent phase of the indentation-time curve. In this phase the indentation continues to increase, but at a decreasing rate. This rate is initially high, the curve being continuous with the 'instantaneous' indentation, but at a time of thirty minutes after application of the load the gradient is approaching zero. However, the gradient does not actually reach zero, even at a time of one hour after application of the load, Sokoloff (1966). However, the increase in indentation which occurred between

thirty minutes and one hour, constituted approximately three per cent of the total indentation. With these figures in mind, the indentationsrecorded at thirty minutes after load application will be termed equilibrium values.

Removal of the load produces an 'instantaneous' recovery, followed thereafter by a time dependent recovery back to the original thickness, providing that the cartilage is immersed in isotonic saline solution, Elmore et al. (1963). The unload phase of an indentation-time curve for cartilage therefore displays similar features to the load phase, but in reverse.

(1.1) The influence of the structure of cartilage on the observed indentation-time curve

The indentation of cartilage may be considered in two sections:

1. The indentation and recovery of the matrix and,
2. The expression and resorption of extracellular water from, and by the matrix.

The indentation of the matrix accounts for the 'instantaneous' indentation and recovery, on application of the load. The expression and resorption of water, accounts for most of the time dependent phase of an indentation curve. However it is likely that some molecular rearrangement of the matrix also occurs during the creep phase which is superimposed on the liquid expression component.

The role of water expression and resorption will be considered first, because it constitutes the major part of both the indentation and recovery curves.

Benninghoff was the first investigator to suggest that the de-

formation and recovery of cartilage, were due largely to the expression and resorption of intercellular water. This suggestion has since been confirmed experimentally by other workers, and in particular by McCutchen (1962), Edwards (1964) and Linn and Sokoloff (1965).

In the report of the work of the two last-named authors it was pointed out that the protein-polysaccharides (mucopolysaccharides) of cartilage are hydrophilic colloids that acquire gel like stiffness, and bind water when associated with collagen fibrils (Schubert 1964). It was further explained, that the imbibition of water by hydrophilic gels depends largely upon the presence of fixed ionic groups that cannot diffuse into the surrounding solution. The partition of water and diffusible electrolytes between the matrix and the moveable interstitial fluid, is governed by Donnan osmotic effects-(West (1962) and more recently Maroudas (1968)). Linn and Sokoloff suggested that when a load is applied to cartilage, which exceeds the osmotic pressure of the colloids mentioned above, then fluid is expressed.

The same authors showed experimentally, that a marked similarity exists between the water expressed from cartilage and the deformation, with one important difference; the water expression proceeds continuously from zero with increasing time, whereas the deformation assumes a finite value upon load application, and thereafter increases as a function of time. These observations were partially explained by the fact that the apparatus used for squeezing water out of cartilage, restricted the flow on three of the four boundaries, whereas indentation tests allowed deformation and water flow from the sides of the tested area. This did not completely explain the difference in behaviour however, and Linn and Sokoloff finally concluded that cartilage possesses a rubber-like elasticity in addition to the water expression. This introduces the first aspect of the total deformation of

cartilage, that of deformation of the matrix.

It has been demonstrated previously, particularly by Maroudas (1968), that the matrix of cartilage consists largely of water and a network of linear polyamions with associated fixed negative charges. The molecular structure of the matrix neglecting the water is therefore fundamentally similar to that of a high polymer, and in particular to that of an elastomer, for example natural rubber. This predicted similarity is confirmed by the observed mechanical behaviour of rubbers [Turner Alfrey Jr. (1948)], which displays deformation-time curves similar in shape to those of cartilage, but with a much shorter time scale, since there is no liquid flow component.

The 'instantaneous' deformation of rubber, on application of a load, occurs as a result of an elastic deformation of the molecular network. The creep component of the deformation of rubber occurs as a result of a viscous molecular flow process, which may be described as a molecular diffusion. From the analoguepostulated above between rubber and cartilage, it seems likely that similar physical processes produce the 'instantaneous', and a part of the creep, indentation of cartilage.

An analoguewhich may serve to clarify the observed response of cartilage to a static indenting load is that of a balloon filled with water, which is loaded and punctured simultaneously. The rubber sheath of the balloon would deform elastically before any significant liquid flow could occur. Following on from the 'instantaneous' deformation would be the liquid flow component, which proceeds as a function of time. The balloon analogueloses its relevance at this stage because the rubber sheath cannot support any load on its own, whereas the matrix of cartilage can.

From what has been said, it appears highly probable that both components contributing to the indentation of cartilage, depend largely on the fixed negative charge density associated with the mucopolysaccharide moiety of the matrix. It appears unlikely, that the radially disposed collagen fibres impart a significant resistance to compression, but the fibres at and near to the articular surface, which are orientated predominantly parallel to the surface, almost certainly act as a stiffening layer, in the sense that they resist surface tension forces. This observation is discussed more fully in Chapter 10.

The combined investigations reported in Chapters 7 and 8, were performed to determine the relationship between the stiffness of cartilage and its structure. The results of these investigations, discussed in Paras. 4 and 5 of this Chapter confirm and extend the hypothesis that stiffness and the mucopolysaccharide content of cartilage are closely related.

It is interesting that Hirsch (1944) and Sokoloff (1966) both established a relationship between the stiffness of cartilage and the chondroitin sulphate content. This relationship however was only semi-quantitative since it was established mainly by reference to histological sections. In addition the importance of keratan sulphate and collagen were not determined.

(1.2) The shape of an indentation versus time curve expressed mathematically

It is common practice to explain the behaviour of visco-elastic materials such as rubber or cartilage, by first setting up a mechanical analogueto the physical system. The analogues most commonly used consist of coulomb friction, spring, and dashpot elements arranged in different combinations of series and parallel connections. The shape of a load-

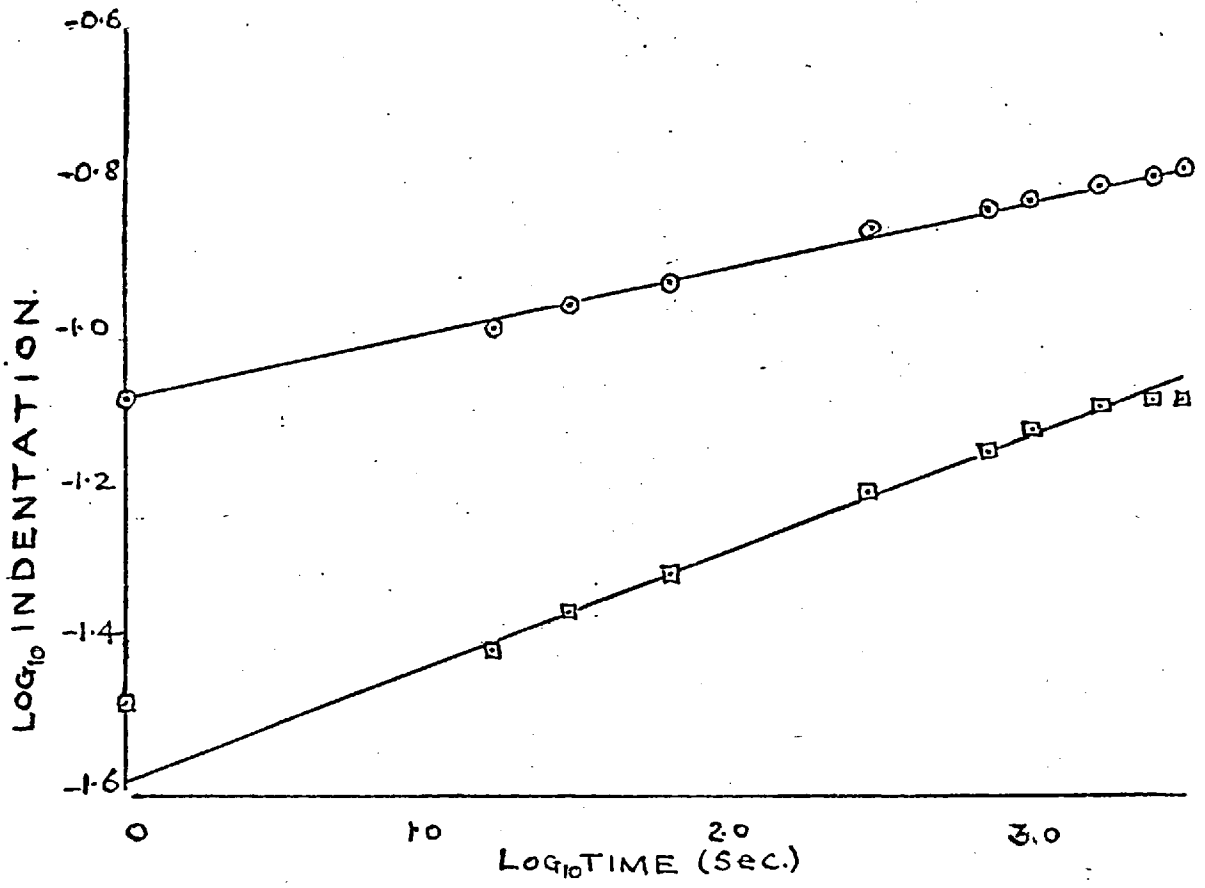


FIG. 19. INDENTATION–TIME CURVES PUBLISHED BY SOKOLOFF (1966), PLOTTED LOGARITHMICALLY

⊙ FIBRILLATED PATELLAR CARTILAGE.

⊡ VISIBLY NORMAL " "

deformation curve for the material, is fitted qualitatively by the appropriate arrangement of elements. The behavioural equation for the analogue is formulated, and by adjusting constant terms the real curve is fitted by the curve predicted from the characteristics of the analogue. Unfortunately the mathematical expressions required to describe visco-elastic materials are cumbersome, since the solution leads to a distribution of spring and dashpot elements each with its own mathematical expression. Abrahams (1964) however, succeeded in fitting an expression, containing three exponential terms to his observed data on the tensile properties of costal cartilage.

Fantuzzo and Graziatti (1967) have proposed a somewhat simplified arrangement of spring and dashpot elements for articular cartilage, the mathematical solution of which would involve a multi-exponential equation. The spring and dashpot analoguefor visco-elastic materials is intended to be a qualitative description of the response of a real system, and it is therefore not possible usually, to identify components of the real system, with elements of the analogy. It was discovered in the present investigation, that a much simpler mathematical expression fitted with close agreement, the indentation curves presented in Fig. 16.

The load phases of the curves shown in Fig. 16 (facing p. 49), together with the curves for six other areas of cartilage on two other femoral heads, were plotted logarithmically. These graphs are shown in Figs. 17 and 18 (facing p. 50). In addition two curves published by Sokoloff (1966) for cartilage on the patella were also plotted logarithmically. These graphs are presented in Fig. 19 (facing p. 81).

It can be seen in Figs. 17, 18 and 19 that all the logarithmic plots of indentation versus time were straight lines, with very little scatter,

within the period from two seconds to thirty minutes after load application.

Mathematically, a linear relationship between the logarithms of two quantities 'y' and 't' may be expressed by an equation of the form:

$$\log y = \log K + N. \log. t \qquad (12)$$

where in the present case:

y = indentation and,

t = time

K and N are constants.

Equation (12) implies a relationship of the form:

$$y = Kt^N \qquad (13)$$

Equation (13) dictates that at time t = 0, the indentation is also equal to zero, whereas in practice it has been shown that cartilage exhibits an 'instantaneous' indentation when the load is suddenly applied. Below two seconds therefore, equation (13) does not fit the experimental curves, but from two seconds up to thirty minutes, and in the case of Sokoloff (1966) up to sixty minutes, the equation describes the curves with good agreement.

The value of 'K' varies as the indentation of cartilage varies. The limits of 'K' which were measured from Figs. 17 and 18 were K = 0.044 to K = 0.066, with 'K' increasing as the indentation increased. The values of 'K' measured from the logarithmic plots of the results of Sokoloff (Fig. 19) are widely different. The lower value which relates to normal patellar cartilage, is within the range of values quoted above for cartilage on the femoral head; whereas the value of K relating to fibrillated patellar cartilage is approximately 0.079, which is considerably larger than the other values. This confirms the tendency seen in visibly normal cartilage on the femoral head, that the value of 'K' increases as the indentation of cartilage increases.

Equation (12) dictates that the gradient of a linear logarithmic plot of indentation versus time, gives the value of 'N' in equation (13). Measurement of the gradients of the lines in Figs. 17, 18 and 19 showed that the variation of 'N' for visibly normal cartilage on the femoral head is somewhat less than the corresponding variation of 'K'. However, there is a tendency for 'N' to increase as the indentation of the cartilage decreases. The values of 'N' relating to the upper and lower lines of Figs. 17 and 18 respectively, are 0.17, 0.21 and 0.15, 0.18 from which it can be seen that 'N' increases as the indentation decreases. The two values for patellar cartilage from the results of Sokoloff (1966), are N = 0.15 for visibly normal cartilage, and N = 0.06 for fibrillated cartilage. These two figures show that visibly normal patellar cartilage is very similar to visibly normal cartilage on the femoral head. However, the value of 'N' for fibrillated cartilage is approximately one third that of visibly normal cartilage.

(2) The pattern of variation of indentation over the femoral head

The indentations at two seconds which were extracted from indentation versus time curves for each indented area are presented in three ways:

1. Firstly in column B of Tables 18 and 19 of Appendix 1. (Table 18 refers to Series 1 tests using the plane-ended indenter and a load of 5 lbf; Table 19 refers to Series 2 tests using the hemispherically-ended indenter, and a load of 2 lbf).
2. As layered maps of the variation of cartilage indentation, over each femoral head. These maps are presented in Figs. 21 and 23, (between pp. 83 and 84).
3. As histograms of indentation for each femoral head, with the cor-

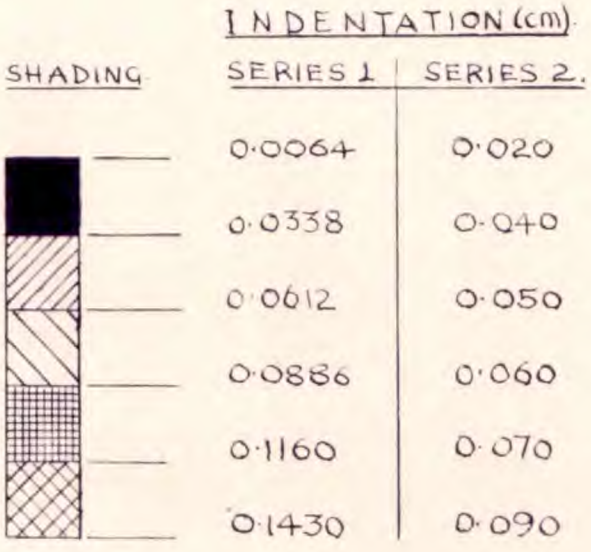


FIG. A. KEY TO SHADINGS USED IN INDENTATION MAPS AND HISTOGRAMS SHOWN IN FIGS. 21 TO 24.

FIG 21 INDENTATION MAPS - SERIES 1.



♀66, SPEC. 9: N.



♂61, SPEC. 10: N.



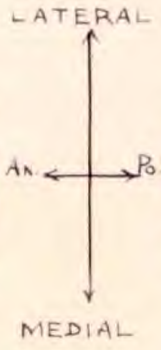
♂46, SPEC 2: GRADE 1



♂73, SPEC. 8; GRADE 1.



♀19, SPEC 11: GRADE 1





♀30, SPEC: 3: GRADE 1-2.



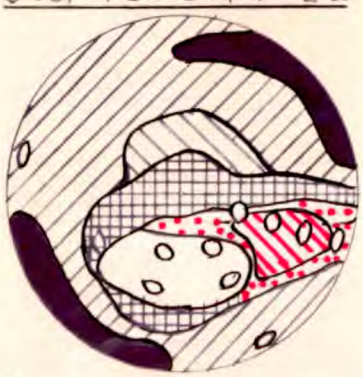
♀68, SPEC. 7: GRADE 2.



♂75, SPEC. 6: GRADE 2.



♀55, SPEC. 4: GRADE 2.



♀59, SPEC. 5: GRADE 3.

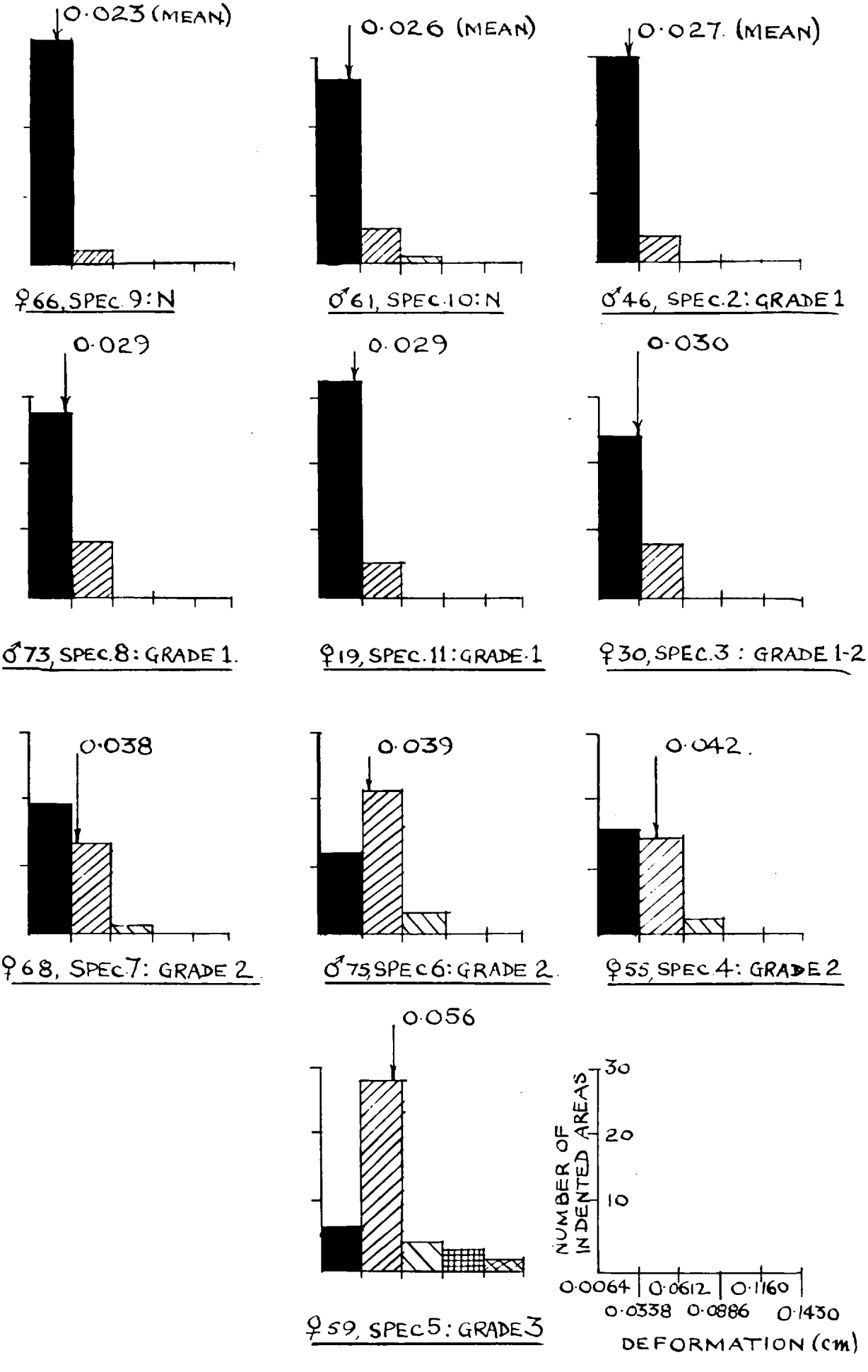


FIG. 22. HISTOGRAMS OF INDENTATIONS: SERIES 1.

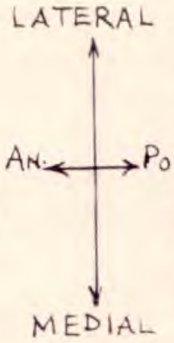


♂33 SPEC. 20: N



♂67 SPEC 21: N.







♂55 SPEC 35: GRADE 2

♀67, SPEC 22: GRADE 2-3

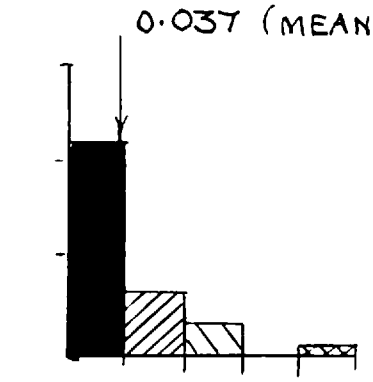


♀82, SPEC. 34: GRADE 3.

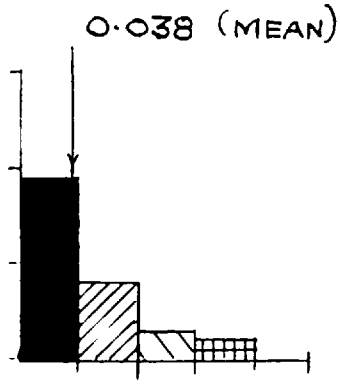


♂69 SPEC. 57: GRADE 3.

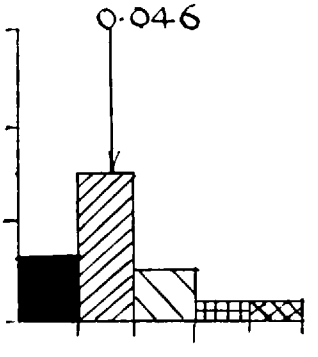
FIG. 23 INDENTATION MAPS; SERIES 2.



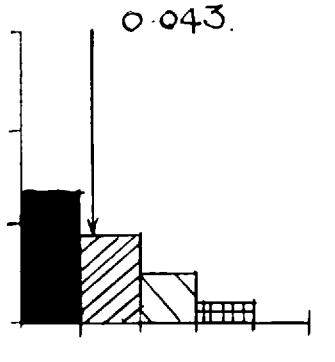
♂33, SPEC. 20 : N



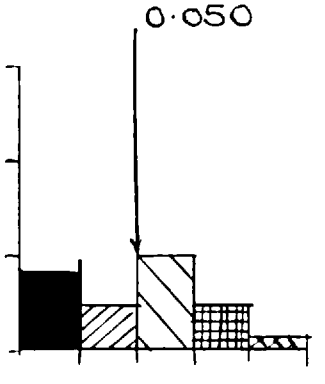
♂67, SPEC. 21: N



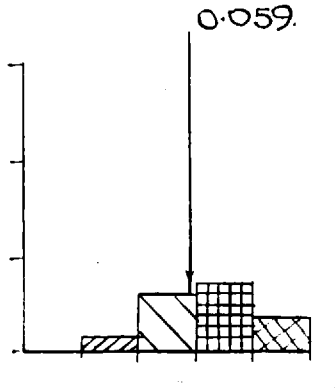
♂55, SPEC. 35: GRADE 1.



♀67, SPEC 22: GRADE 2-3.



♀82, SPEC. 34: GRADE 3



♂69, SPEC. 57: GRADE 3.

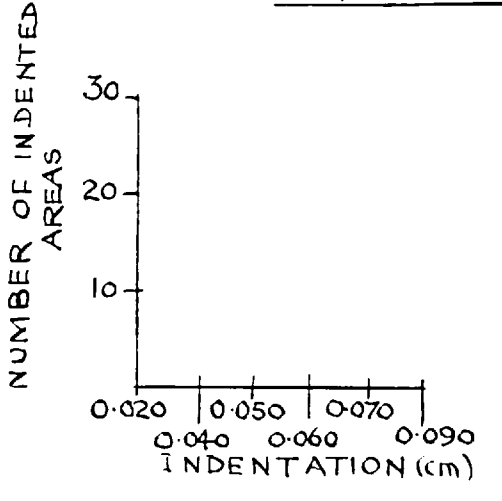


FIG. 24. HISTOGRAMS OF INDENTATIONS: SERIES 2

responding mean indentation. These appear in Figs. 22 and 24 (between pp. 83 and 84).

(2.1) Results

Referring to the layered maps and histograms of Figs. 21 to 24, the following observations may be made:

1. There is a regional variation of the indentation of cartilage on the femoral head. This variation increases as the grade number of a femoral head increases, that is, as the severity of localised degeneration in untested areas inccreases. The observation that the indentation varies locally confirms the results of Hirsch (1944), Elmore et al. (1963) and Sokoloff (1966), which relate to patellar cartilage.

2. The variation of indentation over the femoral head is systematic, and forms a pattern which is reproducible from specimen to specimen, within experimental limits. Figs. 21 and 23 (between pp. 83 and 84) show, that the cartilage in the lowest indentation range (the dark shaded area; see Fig. A (after p. 83) is situated mainly on the superior aspect of a femoral head, but it also extends around to the anterior and posterior facets, and in some cases even to the inferior surface (inferior to the fovea).

   Although the extent of this band of cartilage of low deformability varies, it does not in general include the area adjacent to the margin of the fovea (see Fig. A for Key). In this region as Figs. 21 and 23 (between pp. 83 and 84), show, the indentation is invariably a maximum for each femoral head. It is interesting to note that the area near the margin of the fovea is also a common site of early cartilage degeneration.

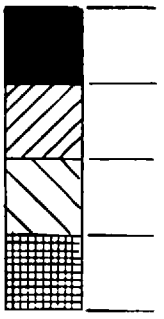
(3) Both Figs. 21 and 23, together with the histograms of Figs. 22 and 24 show clearly, that an increase in the overall indentation of cartilage on the femoral head occurs, as the grade number of the head increases. In the layered maps of Figs. 21 and 23, this is most clearly seen by the gradual disappearance of the dark shaded (low indentation range) area, as the grade number of each femoral head increases. The indentation of the area near to the margin of the fovea also increases. The histograms of Figs. 22 and 24 show how the distribution of indented areas in the indentation ranges, changes from being biassed towards the low indentation end of the spectrum (dark end), to the high indentation end (criss-cross hatching). The mean indentation figures confirm this observation, since in the tests of Series 1, the mean indentations for the visibly normal femoral heads are 0.023 cm. and 0.026 cm. whereas the mean indentation for a grade 3 femoral head is 0.056 cm. The corresponding figures for Series 2 tests are 0.037 cm, and 0.038cm, and 0.059 cm. respectively.

This last observation means that visibly normal cartilage, on femoral heads displaying localised cartilage degeneration, is more deformable than corresponding areas of cartilage on visibly normal femoral heads.

(3) The pattern of variation of creep modulus at two seconds, for cartilage over the femoral head

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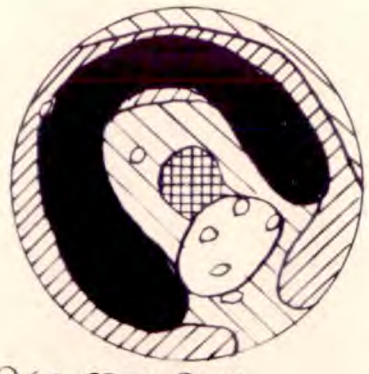
In Para. 5 of Chapter 5 it was shown that the indentation of cartilage is thickness dependent, and that the correlation coefficient between the indentation at two seconds after load application, and the

SHADING.	CREEP MODULUS AT 2 SECONDS (kg/cm^2). SERIES 1	SERIES 2.
	100 (PLUS)	100 (PLUS)
	80	90
	60	70
	40	50
	0	0

OTHER SHADINGS ARE THE SAME AS THOSE GIVEN IN FIG. A. FOR INDENTATION MAPS AND HISTOGRAMS.

FIG. B. KEY TO SHADINGS USED IN MAPS AND HISTOGRAMS OF CREEP MODULUS AT 2 SECONDS SHOWN IN FIGS. 29 TO 32.

FIG 29. MAPS OF CREEP MODULUS: SERIES 1.



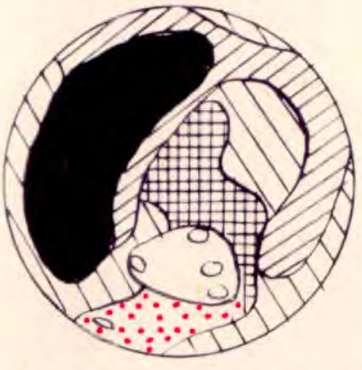
♀66, SPEC 9: N



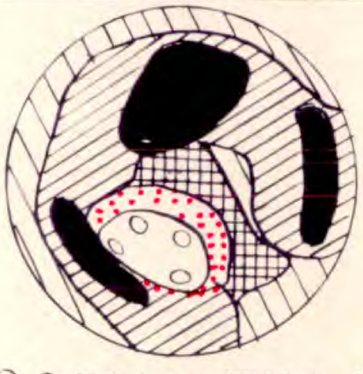
♂61, SPEC 10: N



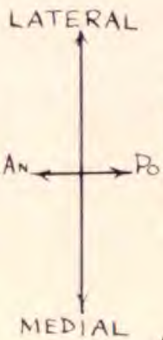
♂46, SPEC. 2: GRADE 1



♂73, SPEC. 8: GRADE 1



♀19, SPEC. 11: GRADE 1

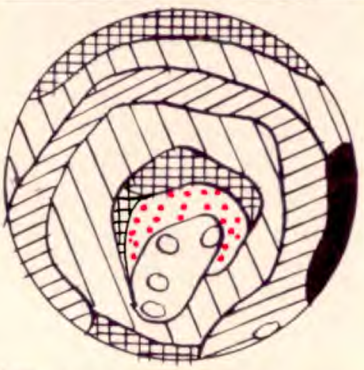




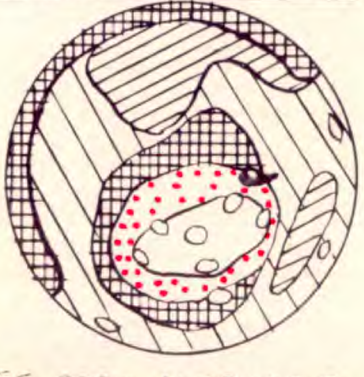
♀30, SPEC. 3: GRADE 1-2



♀68, SPEC. 7: GRADE 2



♂75, SPEC. 6: GRADE 2



♀55, SPEC. 4: GRADE 2



♀59, SPEC. 5: GRADE 3

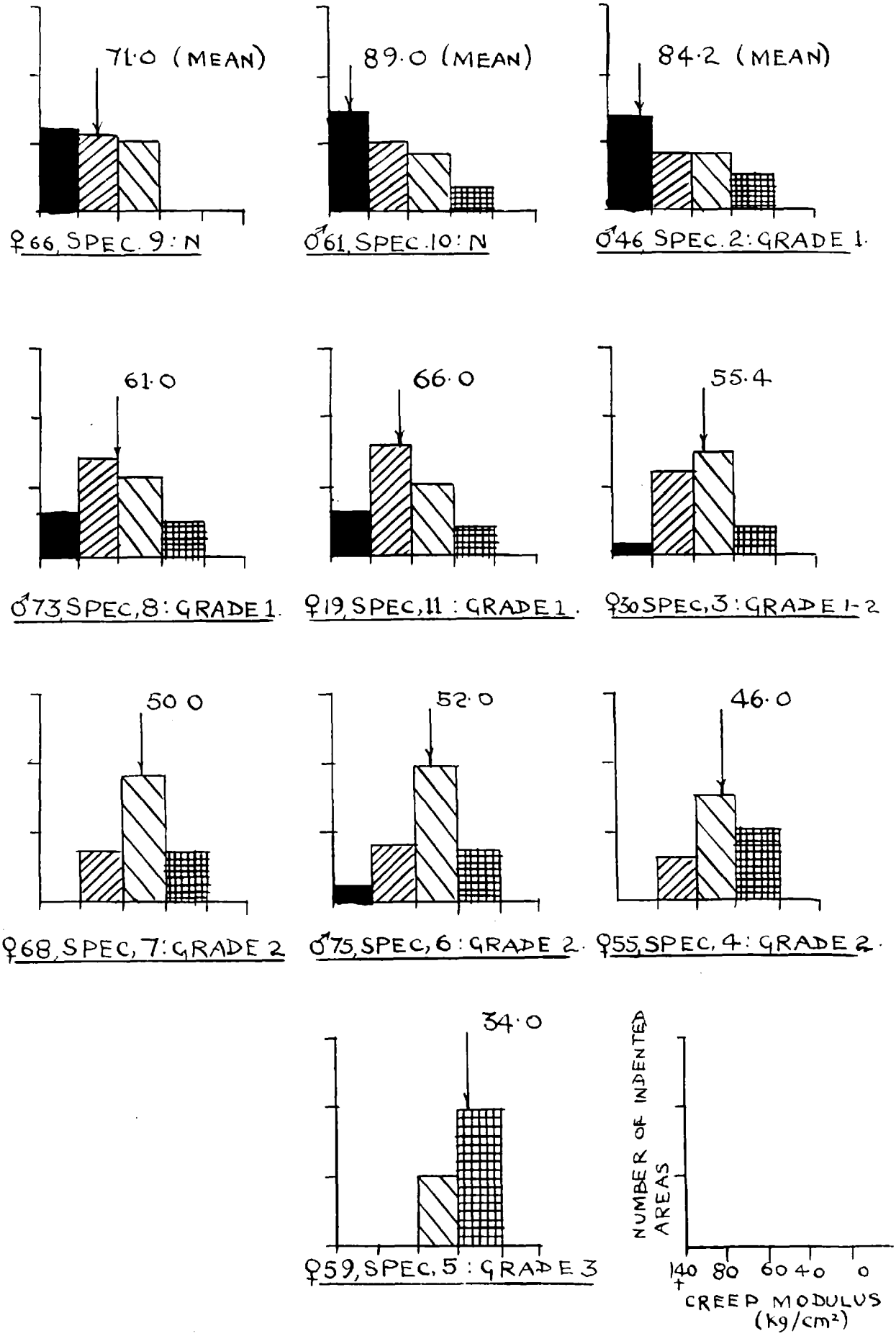


FIG. 30. HISTOGRAMS OF CREEP MODULUS: SERIES 1.



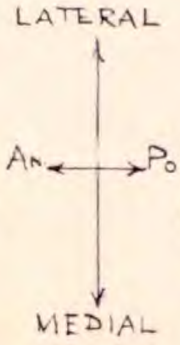
♂33. SPEC. 20: N

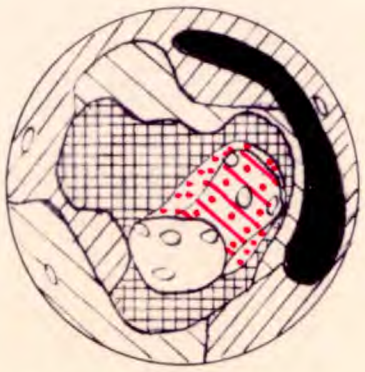


♂67 SPEC 21: N

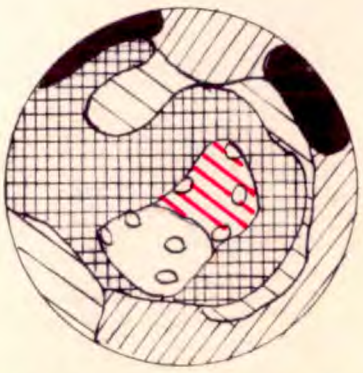


♂55 SPEC. 35: GRADE 2.





♀67. SPEC 22 GRADE 2-3.



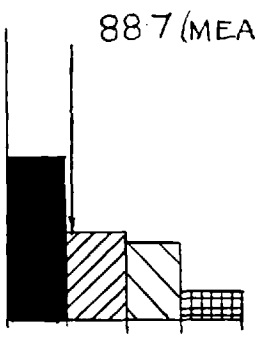
♀82 SPEC. 34 GRADE 3.



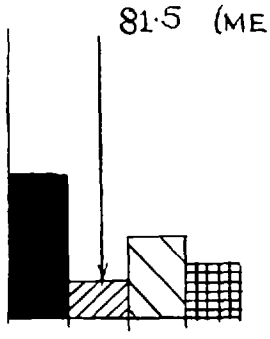
♂69 SPEC. 57 GRADE 3.

FIG 31 MAPS OF CREEP MODULUS

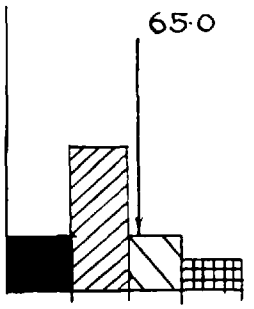
SERIES 2



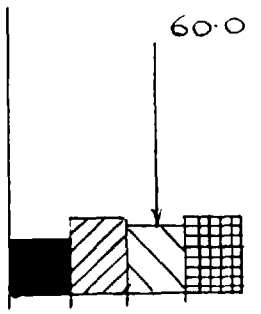
♂33 SPEC. 20. N



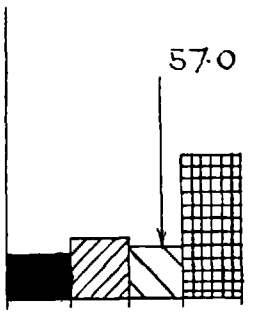
♂67, SPEC. 21 N.



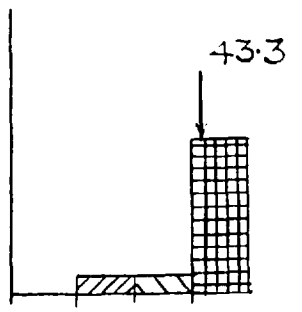
♂55, SPEC. 35 GRADE 2.



♀67, SPEC 22 GRADE 2-3



♀82, SPEC 34 GRADE 3



♂69 SPEC. 57 GRADE 3.

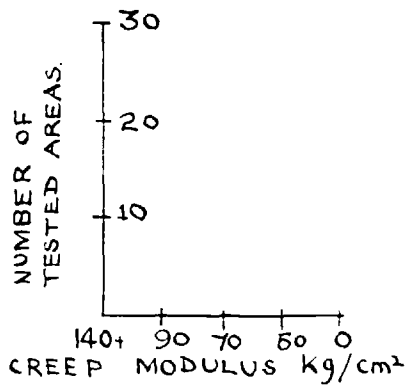


FIG 32. HISTOGRAMS OF CREEP MODULUS: SERIES 2.

thickness of cartilage on the femoral head, is statistically significant. The indentation and thickness of each area of cartilage, were then used in equations (6) and (7), in Para 3 of Chapter 6 (p. 60) to calculate the creep modulus at two seconds, which is a measure of the stiffness of cartilage alone. The results of the indentation surveys in the form of creep modulus values are presented in an identical manner to the indentation results:

1. In column C of Tables 18 and 19, (See Appendix 1),
2. As layered maps of the variation of creep modulus in Figs. 29 and 31 (between pp. 85 and 86).
3. Finally, as histograms of creep modulus, see Figs. 30 and 32 also (between pp. 85 and 86), with the mean creep modulus for each femoral head.

The following observations were made about Figs. 29 to 32:

1. The stiffness of cartilage on the femoral head represented by the creep modulus varies regionally, in a similar manner to the variation of indentation. However, the stiffness varies more widely than indentation, on any one femoral head.
2. The variation of creep modulus over the femoral head displays a systematic pattern , which is similar to that displayed by the variation of indentation. In Figs. 29 and 31, the band of stiffest cartilage (the dark shaded area) is more limited in extent than the area of minimum indentation shown in Figs. 21 and 23, nevertheless it also extends over the superior aspect of the femoral head, and around to the anterior and posterior facets. The area of softest cartilage (lowest creep modulus) on a femoral head is situated near to the margin of the fovea. However, when the results are presented

as the creep modulus values, it can be seen that this area is considerably softer than the stiffest band of cartilage, in fact these two areas occupy the two ranges at the extremes of the creep modulus spectrum.

3. The overall softening of visibly normal cartilage as the grade number of a femoral head increases, is perhaps even more clearly illustrated on the maps of creep modulus, than it was on the maps of indentation. It can be seen particularly in Figs. 29, that the band of stiffest cartilage (the dark band) see Fig. B after p. 85 gradually disappears as the grade number of the femoral heads increases, until it is replaced by cartilage in the next lower stiffness range. Finally, the grade 3 or ulcerated femoral head is covered almost entirely by cartilage in the lowest creep modulus range (the criss-cross hatching). There is still a small band of stiffer cartilage within the limits of the band discussed previously, but this now occupies the creep modulus range adjacent to the lowest range.

The results described above were found to be independent of sex and of the age of a specimen, except in so far as older people are more likely to display severe localised cartilage degeneration on the femoral head, than younger people.

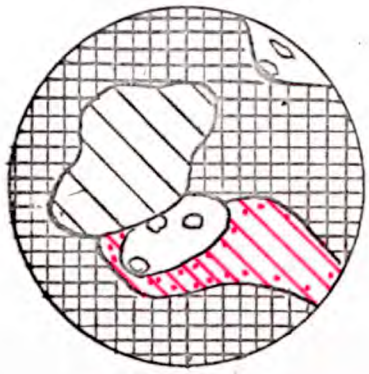
(4) Discussion

The pattern of variation of the stiffness of cartilage on the human femoral head, is significantly similar to the contact area between the femoral head, and the acetabulum in a hip joint. This is shown by the results of Greenwald (personal communication) in the University of Oxford, who has plotted the contact area between the cartilage of the femoral head and ace-

tabulum using a dye technique. The contact area which he observed for a compressive stress of approximately 400 lbf/in^2, which is approximately equal to the nominal stress applied in the indentation surveys of the present investigation, was very similar to the band of stiffest cartilage on the femoral heads shown in Figs. 29 and 31 (after p. 85), and discussed in Para. 3 of this Chapter. In addition, the area adjacent to the margin of the fovea is an area of cartilage on the femoral head which does not contact the cartilage in the acetabulum, according to Greenwald. Although the tests performed by Greenwald were static, in the sense that the joints were not moved, nevertheless the similarity between the contact and non-contact regions, and the stiff and soft cartilage on the femoral head is striking. It is hoped in the future to perform a combined investigation with Greenwald in which femoral heads will be indented by the author, and the contact zones mapped by Greenwald, this time applying movement to the hip joint.

It is tempting on the basis of these observations, to suggest that the stiffness of cartilage on the femoral head is dependent on the load distribution on the joint; the stiffest cartilage occupying the area of maximum load and the softest cartilage occupying the regions of minimum load. Indeed, Bullough et al. (1968) produced evidence, which suggested that the area of the head of the radius in the elbow joint, which consistently shows the earliest signs of cartilage degeneration is situated so that it seldom if ever, contacts the opposing articular cartilage. Bullough et al. (1968) also showed early signs of degeneration on the femoral head near to the fovea (see also Figs. 29 and 31), and argued speculatively that this was a seldom, if ever, loaded region.

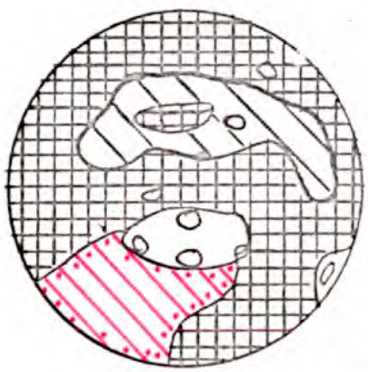
The relative softness, and early degeneration of cartilage in an area of minimum load, may be due to either a nutritional malfunction, mech-



♂40: GRADE 3.



♀82: GRADE 3-4



♀84: GRADE 3



♀86: GRADE 4 (O/A)



♀75: GRADE 4. (O/A).

FIG. 56. MAPS OF CREEP MODULUS FOR GRADE 3 AND OSTEO-ARTHROSIC FEMORAL HEADS.

(REPRODUCED BY PERMISSION OF Dr. C.J. SPIVEY.)

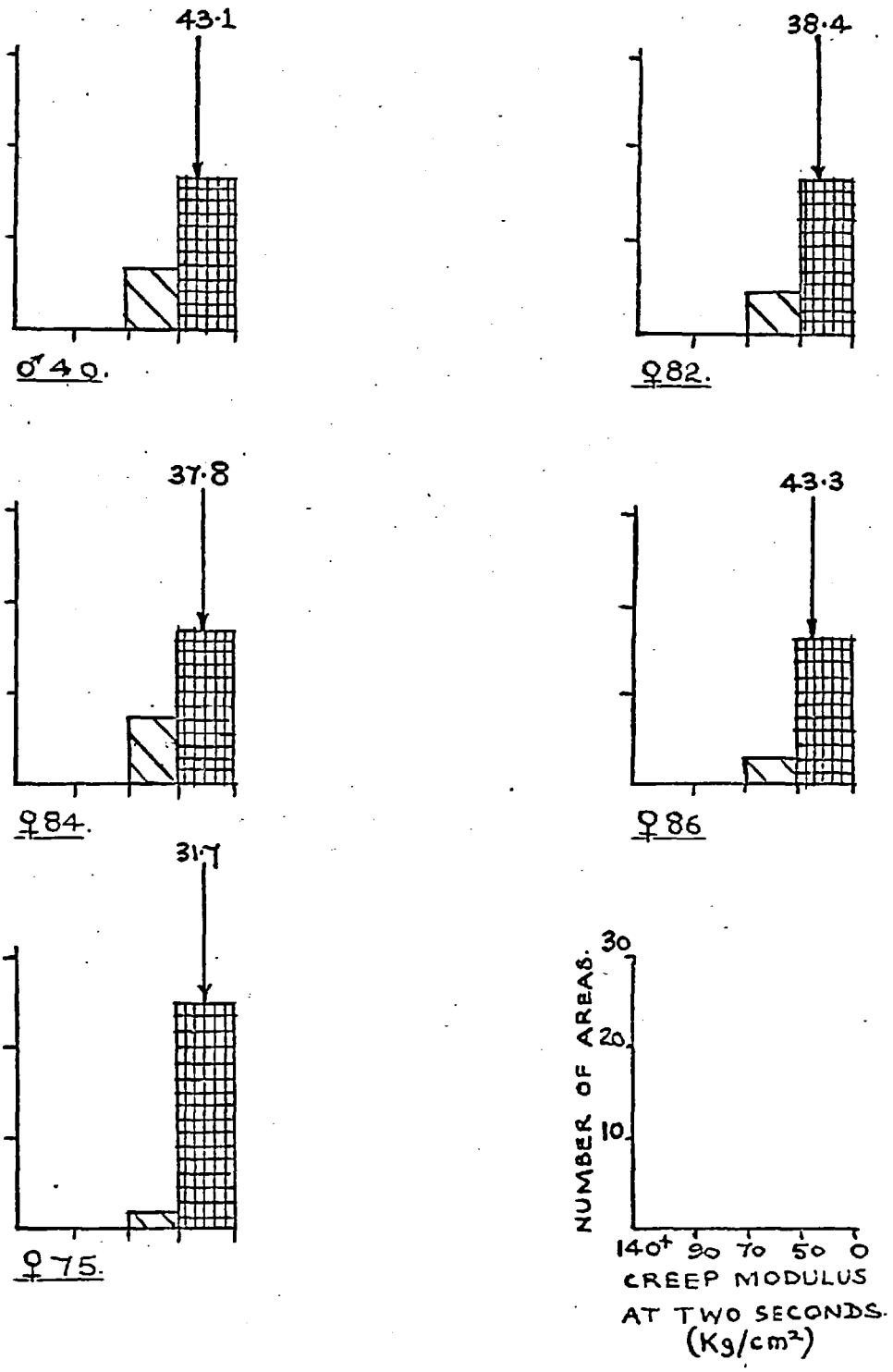


FIG. 57. HISTOGRAMS OF CREEP MODULUS FOR GRADE 3 AND OSTEOARTHROSIC FEMORAL HEADS. (BY PERMISSION OF C.J. SPIVEY)

anical damage due to an articular edge, or both. Future work by the author is planned to investigate the latter hypothesis.

The observed overall softening of visibly normal cartilage as the grade number of a femoral head increases, that is as the severity of localised degeneration increases, is possibly of importance in the aetiology of osteoarthrosis. It will be shown in Para. 5 of this Chapter, that stiffness is closely related to the mucopolysaccharide content of cartilage, and thus as the content of this component of the matrix decreases, so does the stiffness. A depletion of the mucopolysaccharide content is a feature of degenerate cartilage, as is extreme softness, therefore by implication it appears that the results described above indicate, that the process of degeneration which is clearly visible in localised areas, is not restricted to these areas but that it also affects the visibly normal cartilage. The results show, that the stiffness of this cartilage may be reduced by a factor of three or more in the case of a femoral head displaying areas of ulceration. This observation is confirmed by the work of Spivey, who, using the apparatus and methods of the author, indented visibly normal cartilage on five femoral heads with grades three and four degenerative changes. The results of these tests are presented as layered maps and histograms of creep modulus, in Figs. 56 and 57, (between pp. 88 and 89).

The importance of some of these results in the aetiology of osteoarthrosis may be explained in the following way: the earliest signs of cartilage degeneration are frequently observed near to the margin of the fovea, in an area which from the results of Greenwald appears to be lightly loaded compared to the superior surface and posterior and anterior facets. This area may remain the only area on the femoral to display visible degeneration, even though the latter may have reached grade three, or the ulcertaion stage. Many cadaveric

femoral heads have been observed by the author, from people within the last two decades of life, on which visible degeneration was restricted to a localised area of ulceration on the superior/anterior margin of the fovea. The remainder of the joint surface was visibly normal, and it is unlikely that joint mobility was significantly affected.

If however, the softening process which has been observed in this investigation, reaches the stage where the stiffness of the cartilage in the area of maximum load occupies the minimum creep modulus range, then it is possible that the cartilage could be damaged mechanically. This would even be more likely if the load distribution in the joint should change, which could happen if and when marginal osteophytes attained proportions sufficient to alter the location of the femoral head in the acetabulum. If as a result of a change in the load distribution, stress concentrations occurred, then it is easy to see how this, together with the observed softening of cartilage in the loaded region, could quite conceivably lead to mechanical damage of the cartilage and thence to osteoarthrosis.

Although the foregoing is somewhat speculative in the absence of a detailed knowledge of the load distribution in the hip, or how it may change, still there is some evidence to suggest that this process can occur. There are cases on record of undiagnosed congenital dislocation of the hip causing premature osteoarthrosis in relatively young people. In addition, although perhaps not directly linked to the argument, Salter and Field (1960) and Thaxter et al. (1965) have reported cartilage necrosis in experimental animals, when the joints in question were immobilised and subjected to continuous compression.

(5) The results of indentation tests and chemical analysis

(5.1) Method of presentation

The results of this combined investigation are presented in two ways:

GRADIENT TO 'Y' AXIS	INTERCEPT ON 'X' AXIS	PARTIAL CORRELATION COEFFICIENT 'r'	LEVEL OF SIGNIFICANCE
0·517	57·292	0·854 TOTAL 0·849	WHEN 'r' = 0·457 P = 0·001 ∴ P < 0·001

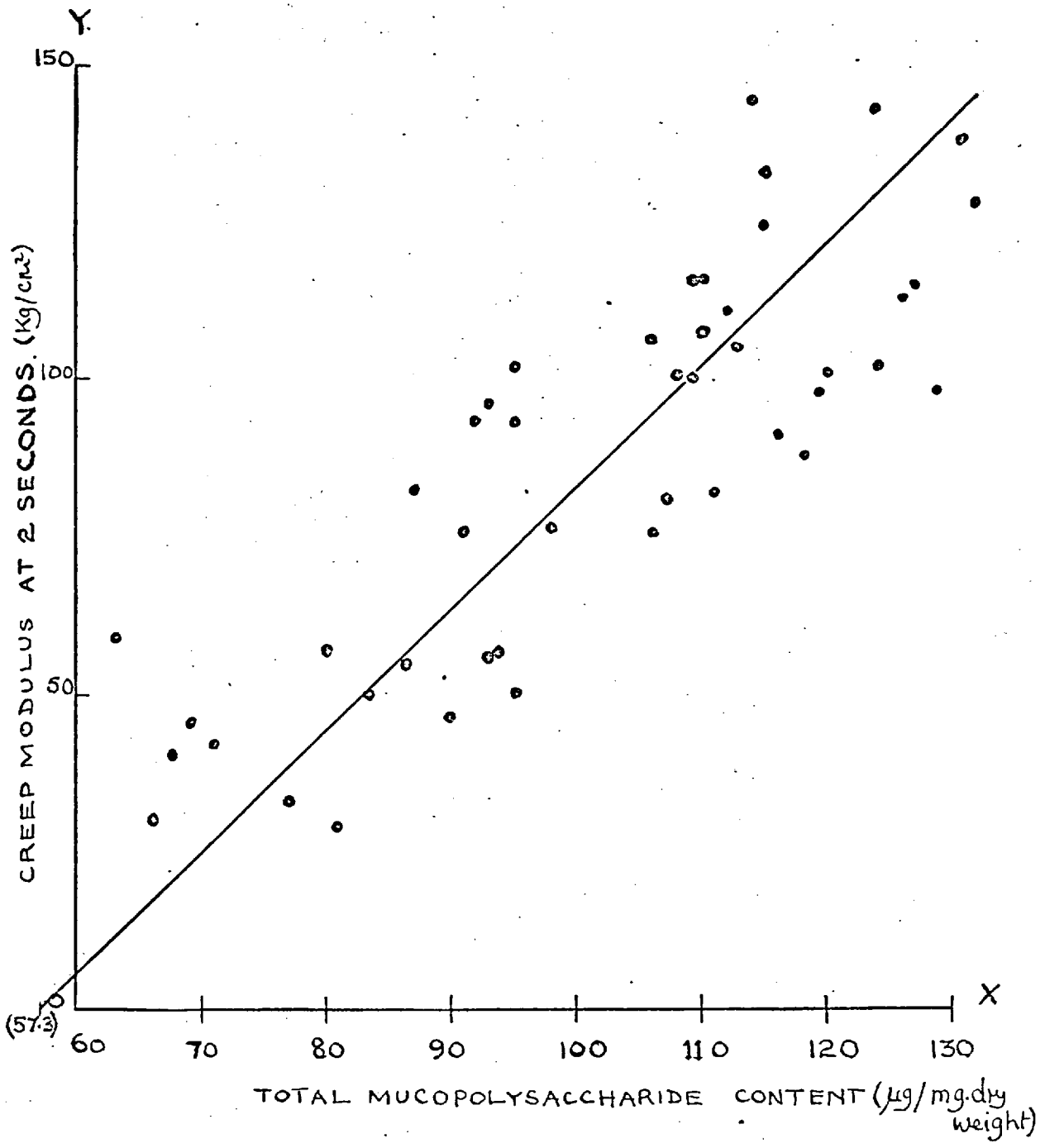


FIG. 37. CREEP MODULUS AT 2 SECONDS vs. TOTAL MUCOPOLYSACCHARIDE CONTENT

1. Firstly, in Table 27 of Appendix 1 (pp. 158 to 159).

2. Secondly, as graphs of creep modulus versus the chemical components listed in Para. 1 of Chapter 7 (p. 66). To each graph was fitted the best straight line according to the least squares analysis. The partial correlation coefficient was also calculated for each set of experimental data, together with its statistical probability.

(5.2) Results

Initially, the relationships between creep modulus and total mucopolysaccharide content, and creep modulus and collagen content were determined. The graphs relating to these two sets of data are presented in Figs. 37 and 40 (facing pp. 91 and 92). It can be seen from these two graphs, that the correlation between creep modulus and total mucopolysaccharide content is highly significant, ($r=0.854$; $p<0.001$). There is a positive increase in creep modulus as the total mucopolysaccharide content increases. The relationship between the creep modulus of cartilage and the collagen content is not so significant ($r=0,233$, $P>0.1$). Considering the structure of cartilage as shown in Fig. 3 (facing p. 6) it can be seen that the collagen fibres appear to form a bracing system. Collagen fibres themselves are not rigid, and therefore they rely upon the rest of the matrix to support them. When a compressive load is applied normally to the articular surface it is most probable that the collagen fibres orientated parallel to the surface, at and near to the latter, act as a stiffening layer (see Chapter 10 of this thesis on tension tests parallel to the articular surface). It is unlikely however that the fibres in the deeper zones, which are orientated predominantly radially, have a significant role in supporting a compression load. The results quoted above appear to confirm quantitavely, the relative contributions of the total mucopolysaccharide and collagen moieties which have been deduced

GRADIENT TO 'Y'-AXIS	INTERCEPT ON 'X'-AXIS	PARTIAL CORRELATION COEFFICIENT	LEVEL OF SIGNIFICANCE
0·027	55·038	0·233	WHEN T=0.251 P=0.10 ∴ P > 0.10

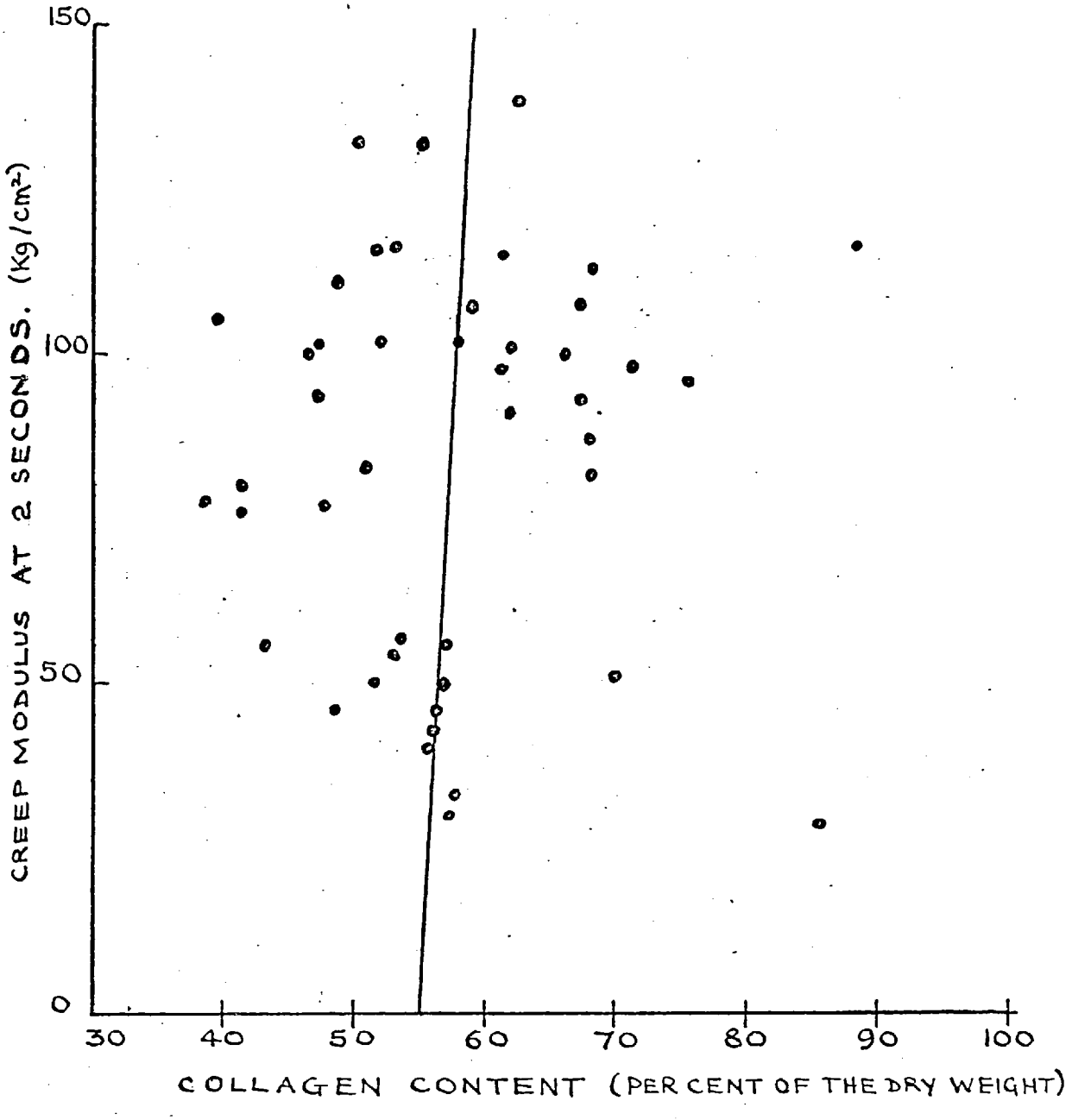


FIG.40. CREEP MODULUS AT 2 SECONDS vs. COLLAGEN CONTENT.

qualitatively.

The total mucopolysaccharide content of articular cartilage has two main components:

1. Chondroitin sulphate and,

2. Keratan sulphate.

Hitherto, previous investigations have concentrated mainly on the first of these two components. In this investigation however, the amounts of both components were calculated, for the indented areas of cartilage. Figs. 38 and 39 (facing p. 93) show the graphs of creep modulus versus the amount of each mucopolysaccharide. It can be seen, that there is a statistically significant correlation between the two variables in both graphs. In fact, for creep modulus versus chondroitin sulphate, the partial correlation coefficient $r = 0.810$ ($P < 0.001$), and for creep modulus versus keratan sulphate $r = 0.800$ ($P < 0.001$). Thus it appears that the roles of each of the component mucopolysaccharides towards the stiffness of cartilage in compression, are equally important. This is an observation which has not been made previously, because as mentioned earlier the role of keratan sulphate has been overlooked in previous investigations.

(6) Observations from histological sections of the indented areas of cartilage

Histological sections of the indented areas were prepared by Bullough then of the Professorial Unit, Nuffield Orthopaedic Centre, now of the Hospital for Special Surgery, New York. Full depth sections were stained in three ways:

1. With Alcian Blue which stains the total mucopolysaccharide ground substance.

2. With Alcian Blue in 0.9 molar magnesium chloride which stains specifically for keratan sulphate and,

GRADIENT TO 'Y' AXIS	INTERCEPT ON 'X' AXIS	PARTIAL CORRELATION COEFFICIENT 'r'	LEVEL OF SIGNIFICANCE
0·313	47·326	0·810	P < 0·001
		TOTAL 0·620	

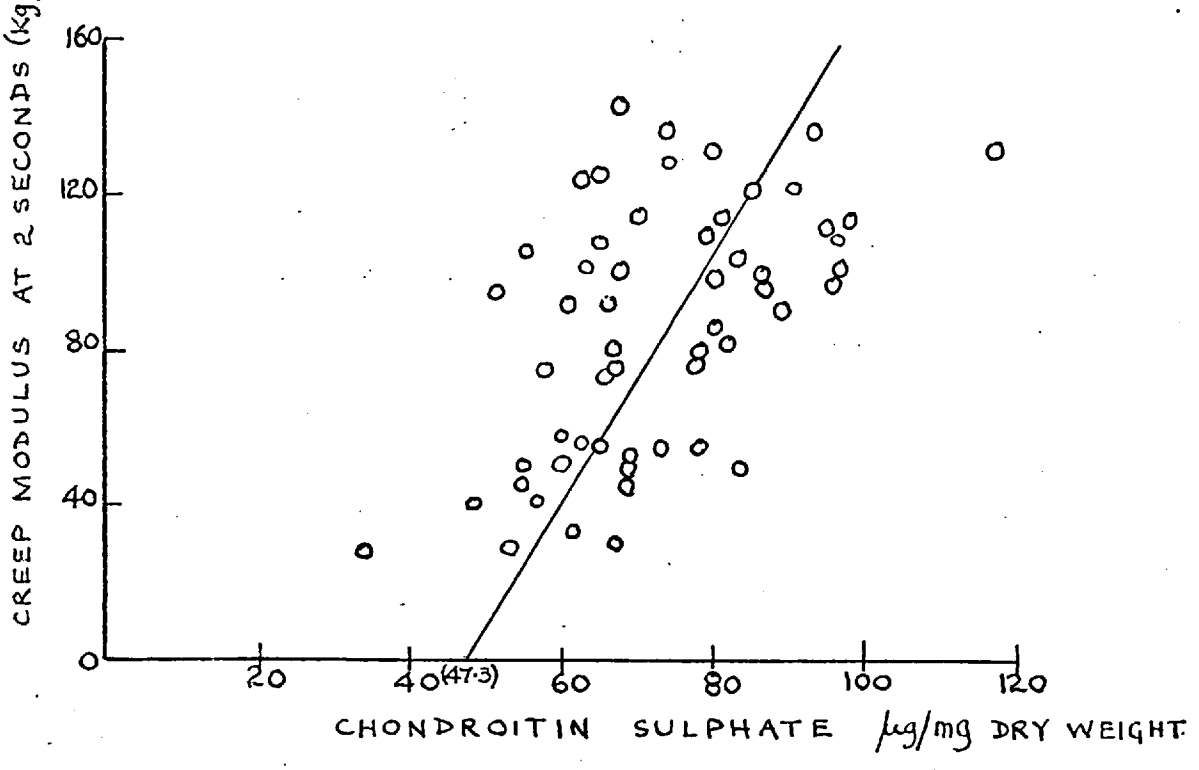


FIG. 38. CREEP MODULUS AT 2 SECONDS VS CHONDROITIN SULPHATE CONTENT.

GRADIENT TO 'Y' AXIS	INTERCEPT ON 'X' AXIS	PARTIAL CORRELATION COEFFICIENT 'r'	LEVEL OF SIGNIFICANCE
0·237	8·245	0·800	P < 0·001
		TOTAL 0·610	

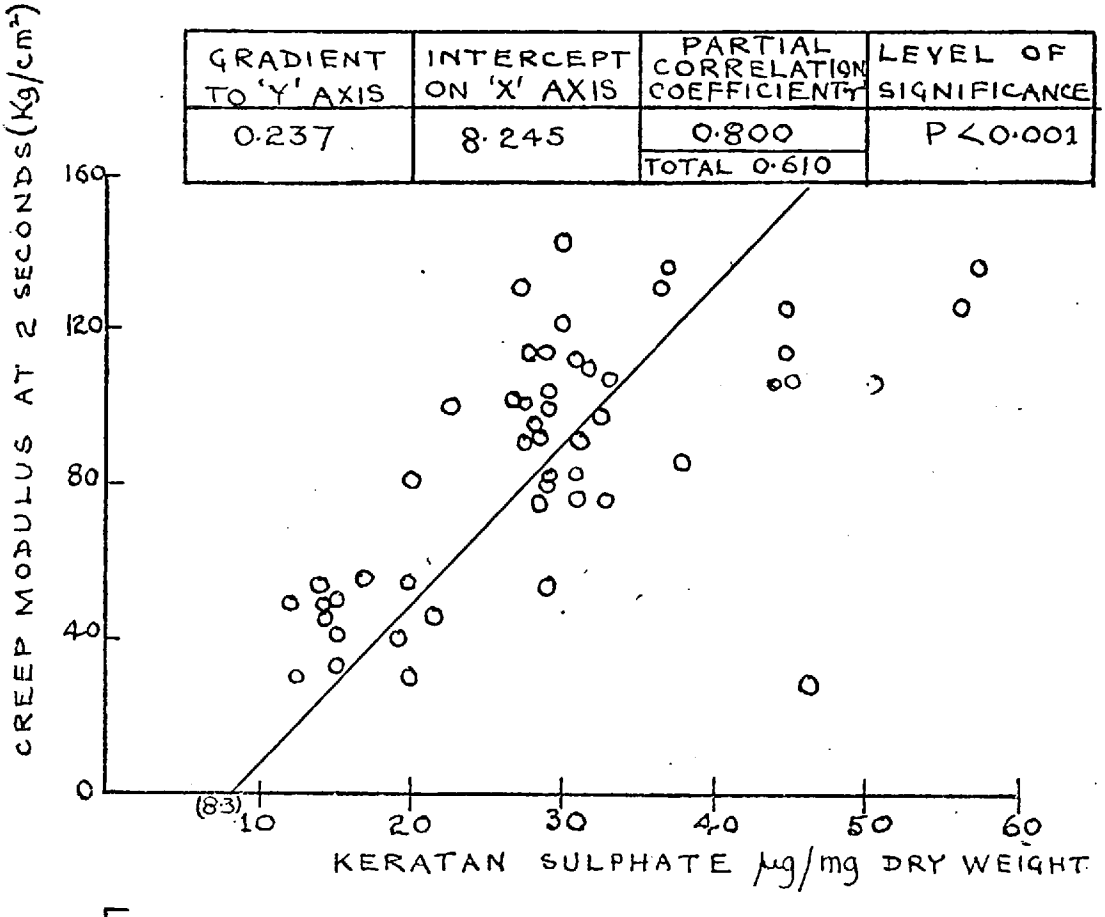


FIG 39: CREEP MODULUS AT TWO SECONDS VS KERATAN SULPHATE CONTENT.

3. With Alcian Blue in 0.4 molar magnesium chloride which stains specifically for chondroitin sulphate. The intensity of staining was not taken as an accurate measure of the amount of each of the quantities listed above, because it is usually accepted, that the intensity of stain is only semi-quantitative, particularly if the sections are prepared in different batches, as they were in the present investigation. In addition, chemical analysis provided the quantitative measurements far more accurately than histology could. However, the sections stained for total mucopolysaccharide in particular did stain much more intensely in areas of stiff cartilage than soft areas. In fact, in very soft areas, the staining intensity was extremely weak.

The main purpose of staining histological sections of the tested areas, was therefore to determine the distribution of staining intensity and how it changed with cartilage stiffness. The following general observations serve to illustrate the main differences between cartilage at the two extremes of the stiffness range:

1. The surface of areas of stiff cartilage was invariably intact, and appeared to be smooth when viewed under the ordinary light microscope. The surface of areas of soft cartilage however, was invariably uneven and in many cases it was slightly fragmented, even though it appeared to be smooth to the naked eye.

2. The most significant difference between the areas of stiff and soft cartilage, was the distance below the articular surface to which loss of staining occurred. In the case of the stiffest areas of cartilage, this zone was approximately one eighth of the total thickness of a section at most, whereas in the soft areas, loss of staining extended from at least one quarter of the total thickness to almost the full thickness of a section.

3. One other difference between the areas of stiff and soft cartilage was the shape of the chondrocytes, and the distribution of staining intensity around them. Considering firstly the stiff areas of cartilage; the chondrocytes were usually relatively small and arranged in clusters. The nuclei stained very intensely, as did the rims of the cells, extending to at least two cell diameters into the intercellular matrix. In many cases these intensely staining areas joined together to form a network effect. In contrast, the chondrocytes in the soft areas of cartilage were frequently large and irregular in shape. The nuclei did not stain intensely, neither did the areas around the rims of the chondrocytes. In fact, a non-staining zone was frequently observed, which separated the rim of a cell from the stained areas of the matrix, which themselves were fragmentary and streaked in appearance.

Because of the observation of Meachim and Collins (1962), that the rate of production[1] of mucopolysaccharide by the chondrocytes is higher in degenerate areas of cartilage, than in visibly normal areas, it appears that the overall decrease in mucopolysaccharide content in these degenerate areas is due possibly to either:

1. The mucopolysaccharides moving out of cartilage at a faster rate than the chondrocytes can replenish them or,
2. A lack of correct incorporation of the mucopolysaccharides into the matrix.

The possibility exists that both phenomena occur together. It is to be emphasised that these are hypotheses since no investigation has been published to confirm or deny either. The observations from the histological

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1. Measured by the uptake of $^{35}SO_4$ in vitro.

sections in the present investigation indicate, that the loss of mucopolysaccharides in cartilage extends to a greater depth in soft areas of cartilage, than it does in stiff areas. The surface of a soft area is frequently fragmented, whereas that of a relatively stiff area is usually intact. The possibility exists therefore, that there is a net outflow of mucopolysaccharides from areas of very soft cartilage, because the permeability of the surface zone is increased. Maroudas (1968) has shown that the permeability of cartilage for most of its thickness is closely related to the mucopolysaccharide content, the higher the latter the lower is the permeability. However, at the articular surface the permeability shows a significant decrease, despite the fact that there is comparatively little mucopolysaccharide in this region. It is speculated that the relatively tightly packed mesh of collagen fibres at the articular surface forms a physical barrier to the outflow of mucopolysaccharides, but when these fibres are fractured the rate of outflow increases.

The suggestions made in this paragraph are somewhat speculative, in an attempt to interpret the observations from the histological sections. The intensity of staining was not used as a measure of mucopolysaccharide content, as previous investigators have tended to do. Chemical analysis performed this more accurately. Instead, observations were made on the surface smoothness and integrity, the depth below the articular surface to which the non-staining zone extended, and the shapes of the chondrocytes and the distribution of staining around them. These observations were related to the relative stiffness of the areas of cartilage considered.

7. The results of indentation tests and permeability measurements

It was explained in Chapter 8, how permeability measurements were

GRADIENT TO 'Y' AXIS	INTERCEPT ON 'X' AXIS	CORRELATION COEFFICIENT	LEVEL OF SIGNIFICANCE
-0·018	3·948	-0·772	P< 0·001

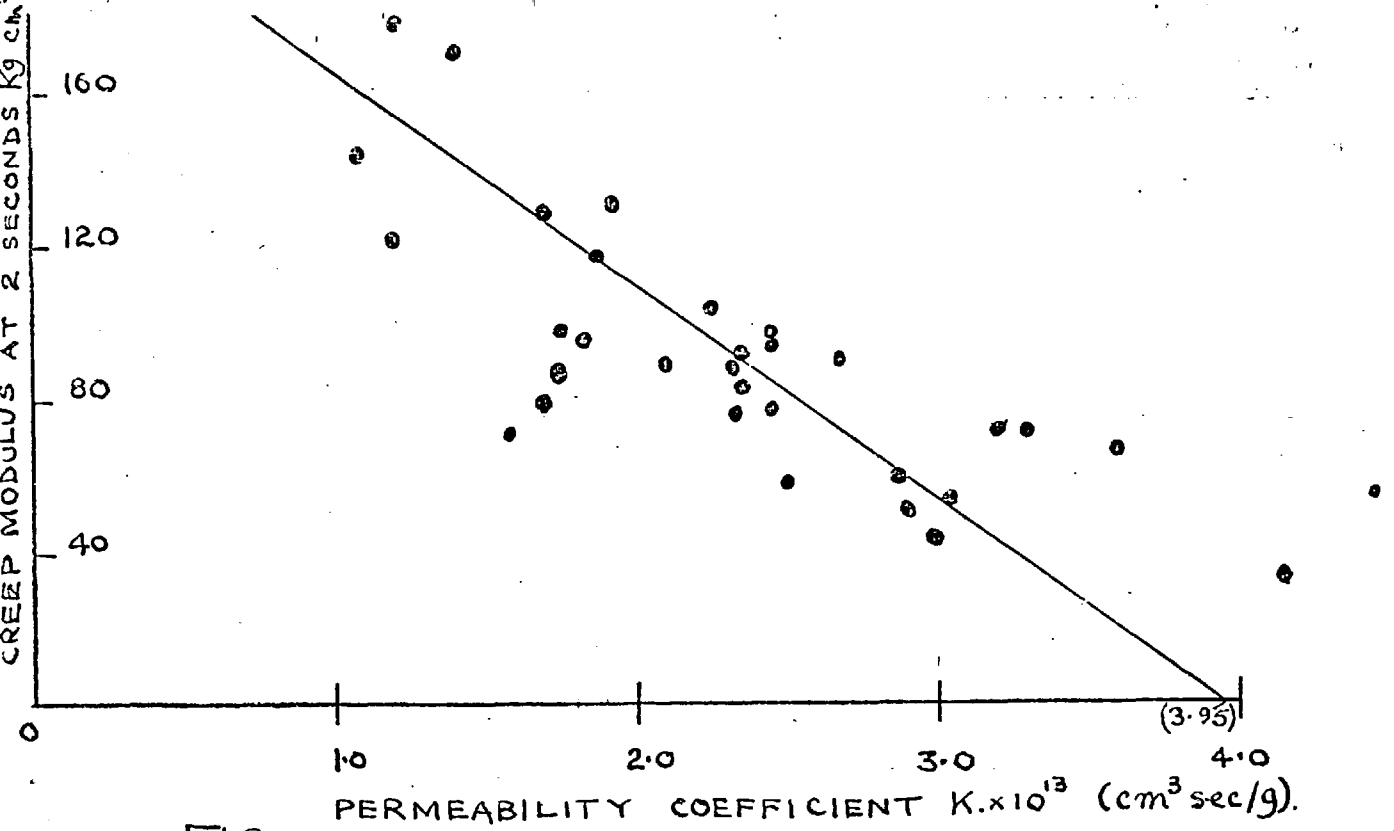


FIG.43, CREEP MODULUS vs. PERMEABILITY COEFFICIENT.

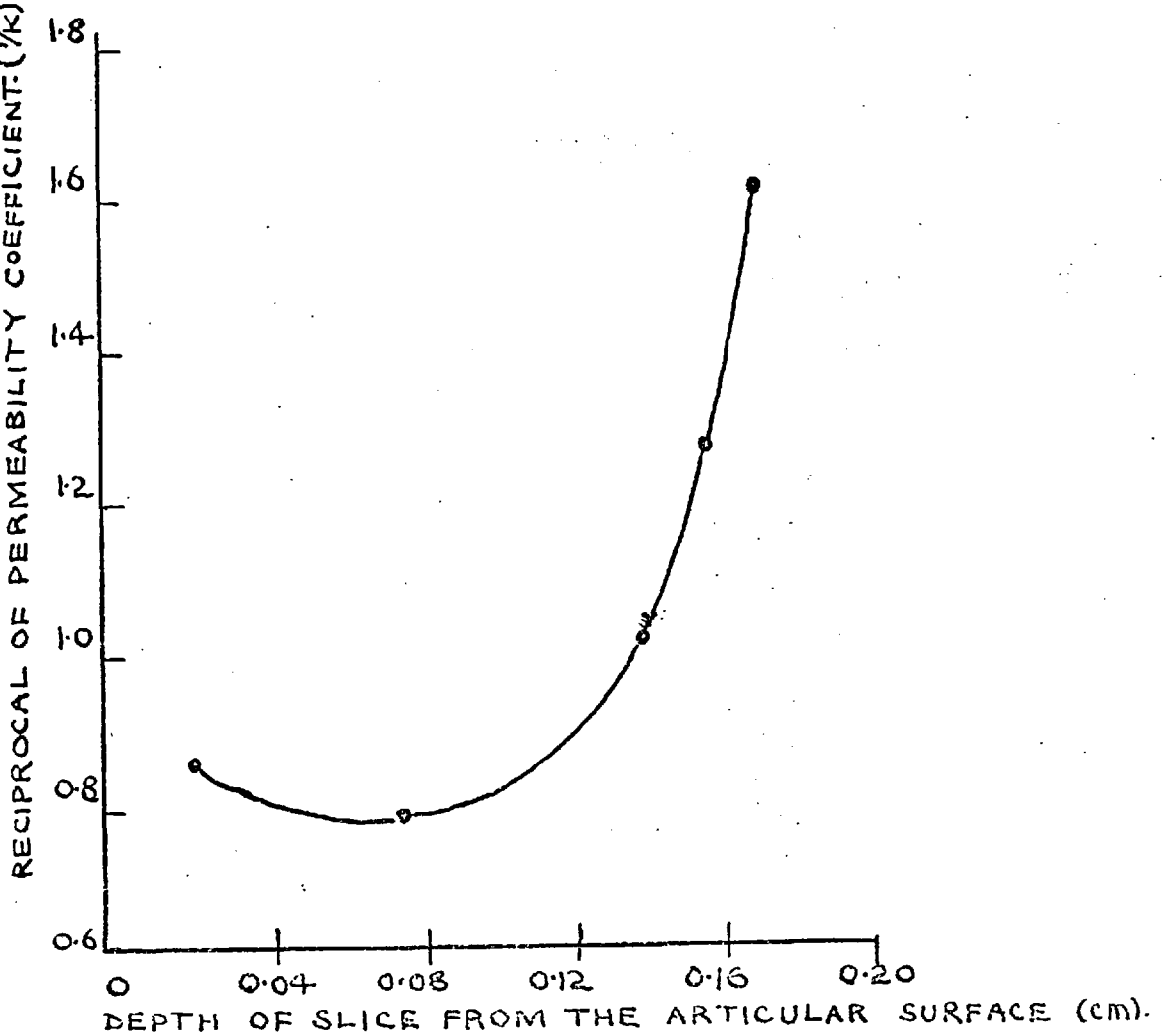


FIG.42. TYPICAL CURVE OF RECIPROCAL PERMEABILITY vs. DEPTH OF SLICE FROM THE ARTICULAR SURFACE.

performed on areas of cartilage after they had been indented. The results of the permeability measurements, are shown in a typical curve of the reciprocal of the permeability coefficient ($^{1}/K_{h}$) versus depth 'h' from the articular surface. This curve is presented in Fig. 42 (facing p. 96). The measurements were made by Read, using the apparatus and techniques developed by Maroudas (1968). Fig. 43 (facing p. 96) shows the experimental points of creep modulus and permeability coefficient, calculated for all the areas of cartilage tested.

It can be seen, that there is a significant correlation between the two quantities ($r = -0.77$, $P < 0.001$). The graph shows that as the creep modulus increases, so the permeability coefficient decreases.

It was stated in Para. 1.1 of this Chapter that the mucopolysaccharide moiety of the matrix of cartilage, has associated fixed negative charges. The density of these charges largely determines the permeability of cartilage to water. The greater the fixed charge density the lower is the permeability, Maroudas (1968).

The conclusion therefore which may be drawn from the results at this stage of the investigation is, that they confirm the results of combined indentation tests and chemical analysis, in which it was shown that a significant partial correlation exists between the creep modulus and total mucopolysaccharide content of cartilage on the femoral head.

SECTION 3

Having completed the investigation of the compressive properties of articular cartilage, a preliminary investigation was performed to determine the tensile properties of cartilage, parallel to the articular surface, and at different distances below the latter. This section reports the method used, and the results obtained from these tests.

It is to be emphasised, that at this stage, this is a preliminary investigation, and the number of joint surfaces studied so far is limited to five. Work planned for the immediate future is intended to extend these tension tests on articular cartilage.

CHAPTER 10

THE TENSILE PROPERTIES OF CARTILAGE PARALLEL TO THE ARTICULAR CARTILAGE

(1) Introduction

The arrangement of collagen fibrils and fibres in articular cartilage, has interested investigators in this subject for well over a century. Observations relating to the collagen fibre orientation have been made, using several different techniques, for example: surface pricking by Hultkrantz (1898), polarised light microscopy by Bullough and Goodfellow (1968), electron microscopy by Weiss et al. (1968), Maroudas and Bullough (1968) and McCall (personal communication) and X-ray diffraction by Little et al. (1958). It is now generally accepted, that when viewed normal to the articular surface, the collagen fibres at and near to the latter, are arranged in parallel bundles, which are orientated predominantly parallel to the surface. In the deeper zones however, the fibre orientation is not so well defined,but there does appear to be a tendency for the fibres to be orientated predominantly radially. This is however still a point of controversy, McCall (personal communication[1]). The diameter of individual fibres appears to increase with depth from the articular surface. Concerning the packing density however there is another disagreement; Maroudas and Bullough (1968) observed a decrease in the packing density with depth whereas McCall (personal communication) claims that the packing density increases.

The functional significance of the collagen fibre orientation in cartilage has still yet to be explained fully. Some investigators have claimed, that the collagen fibres are aligned mainly in the direction of the principal tensile stress trajectories, whilst others claim that the orien-

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1. See Bibliography.

tation, at least at the surface, is related more to the joint geometry.

Surprisingly, no report could be found in the literature of tension tests performed on articular cartilage, either to determine the characteristics of the tissue in tension, or from them to confirm or deny the orientation of the collagen fibres as revealed by the techniques mentioned above. The purpose of this investigation was therefore to supply this information by performing tension tests on cartilage, parallel to the articular surface, and at different distances below the latter.

(2) Review of the literature

Probably the earliest recorded observation concerning the collagen fibre orientation in articular cartilage, is that of Hunter (1743), who observed that the fibres extended from the sub-chondral bone up to the articular surface. This view was not generally supported however. Van der Stricht (1885), using more refined techniques demonstrated, that the matrix of cartilage consists of a network of fine fibres embedded in a non-structural gelatinous material.

Hultkrantz (1898) using the surface puncture technique developed by Langer for skin, was the first worker to produce cleavage line patterns in the surface of articular cartilage. Hultkrantz observed, that when the surface of cartilage was punctured by a conical pointed awl and the punctures then stained, cleavage lines were obtained instead of round holes. When an entire joint surface was pricked in this manner the cleavage lines formed a pattern, which was reproducible on the corresponding joint surface, from specimen to specimen. Hultkrantz concluded, that the cleavage line pattern indicated the predominant surface collagen orientation, which was orientated in the direction of the maximum tension strains. These strains, it was argued, were probably produced both by friction, in which case the

fibres would be aligned in the direction of motion; and by pressure, in which case they would probably follow the direction of maximum concavity of the joint surface. Roux (1898) recorded that when an elastic body is loaded in compression, it becomes smaller in the direction of compression and larger, perpendicular to the latter. Roux therefore maintained, that tension strains were produced in the latter direction.

Triepel (1902) supported the trajectorial view of Hultkrantz, but Fick (1910) then pointed out that the strains due to friction are probably very small, when compared to the strains caused by compressing the two surfaces.

Benninghoff (1924, 1924 and 1939) did not agree that the collagen fibres had a trajectorial orientation. In addition he pointed out, that there is frequently a significant difference between the cleavage line pattern and the direction of maximum concavity. It was Benninghoff's view that 'the cleavage lines connect by the shortest route, and with the least curvature, the edges of the articular hyaline cartilage, they radiate vertically towards the edges and do not interact. He proposed as a model a rectangular section of cartilage loaded in compression. The edges would be relatively stiff so that they would flex but not elongate. The flexion would be greater at the centres of the longest edges, and it was argued that the collagen fibres spanned the articular edges with the observed orientation, in order to withstand this flexion.

Pauwels (1959) studied the tangential fibrous layer of the cartilage in the scapular glenoid cavity. In addition he made gelatin models of the cavity, and used a photoelastic technique to determine the stress distribution under a load system similar to that found under physiological conditions. Pauwels demonstrated that the cleavage line pattern was orien-

tated in the direction of the maximum tension strains. He stated that an optimum amount of collagen existed because each fibril experienced its maximum permissible tensile strain; although it is not obvious how this conclusion was reached. If the surface collagen orientation is trajectorial, it is interesting to compare this conclusion with the observation of Little et al. (1958), that the parallel fibre arrangement at the surface exists even in the cartilage of a foetus and a newborn child. If this implies that a cleavage line pattern exists on the joint surface at this stage of development, then it is difficult to see how the stress and strain trajectories in adult joints could be similar to those of a foetus or newborn child.

MacConaill (1960) did not agree with the view of Benninghoff, that the fibres in the deeper zones of cartilage are orientated predominantly radially. MacConaill held the view that the collagen fibres are arranged obliquely throughout the thickness of cartilage, except at the articular surface. He concluded that this orientation was much more appropriate for carrying the loads experienced by cartilage. Zarek and Edwards (1963) however, could not confirm that either of the collagen orientations proposed by Benninghoff or MacConaill, was more adapted to the stress distribution. The latter was determined for an idealised system of two spherical joint surfaces, one convex and the other concave.

More recently Bullough and Goodfellow (1968), using polarised light microscopy, confirmed the parallel orientation of collagen at the articular surface, and the predominantly radial orientation in the deeper zones. They also showed that the cleavage line pattern occurs at and near the surface layer only, and it is aligned along the principal direction of the collagen fibres. Thus it appears that the cleavage line pattern over

a joint surface, indicates the main collagen fibre orientation at and near to the surface. By way of an analogy, when a round nail is driven into a piece of wood, the latter splits along the grain lines, which are thus revealed by the splits.

Weiss et al. (1968) have shown, using electron microscopy, that the thickness of the parallel fibre layer at and near to the articular surface is variable. Sometimes it forms a considerable proportion of the total thickness of cartilage.

Finally, what is probably the most recent electron microscopical study of cartilage by McCall (personal communication), shows that the collagen fibres in the transition zone (see Fig. 3, facing p. 6) are arranged in 'S' shaped coils. This observation does not agree with previous studies. McCall also observed that the diameter of individual collagen fibres increases with distance below the articular surface, and the distance between adjacent fibres decreases. This last conclusion does not agree with the results of Maroudas and Bullough (1968) or Weiss et al. (1968) who both observed that the distance between adjacent fibres increases with distance below articular surface.

A review of the literature has shown,that a variety of techniques have been used by previous investigators to determine the orientation and distribution of collagen fibres in cartilage. The only method of which no report could be found is tension testing parallel to the articular surface. No tension tests appear to have been performed at:

1. Different orientations to the surface cleavage line pattern or,
2. Different distances below the articular surface,

in an attempt to confirm or deny by mechanical tests,the collagen orientation

within cartilage, as observed by other techniques.

(3) Objects of the present investigation

The objects of the present investigation were, to determine the tensile properties of human articular cartilage parallel to the articular surface at:

1. 0° and 90° to the surface cleavage line pattern and,
2. At four equi-spaced distances below the articular surface.

The aims of these tests were twofold:

1. To determine whether the tensile properties reflect the structure of cartilage which has been observed by other methods and,
2. To observe and measure the tensile properties of cartilage at the two orientations, and four distances below the surface, as previously explained.

(4) Materials

Specimens of cartilage were obtained from the femoral condyles of knees from human cadavers. All the specimens were obtained from routine post mortem examinations, and any specimens of which the cause of death might have affected the cartilage or sub-chondral bone were rejected. The method of storage was identical to that described in Chapter 2 (p. 15).

To date, ten rectangular areas of full thickness cartilage were excised from the femoral condyles of five knees within the age range of 50 to 67 years. Each area of cartilage was then sliced parallel to the articular surface into four layers of equal thickness, which in all cases were approximately 400 μ thick.

(5) Experimental method

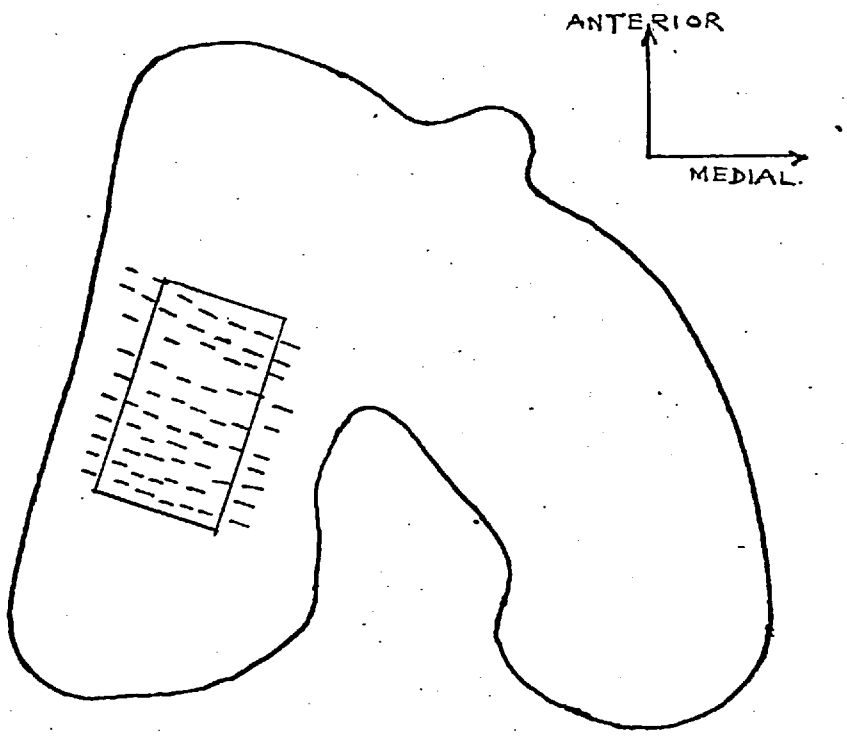
Initially, the cleavage line pattern was determined on the femoral condyles of several knees, by using the surface pricking technique developed



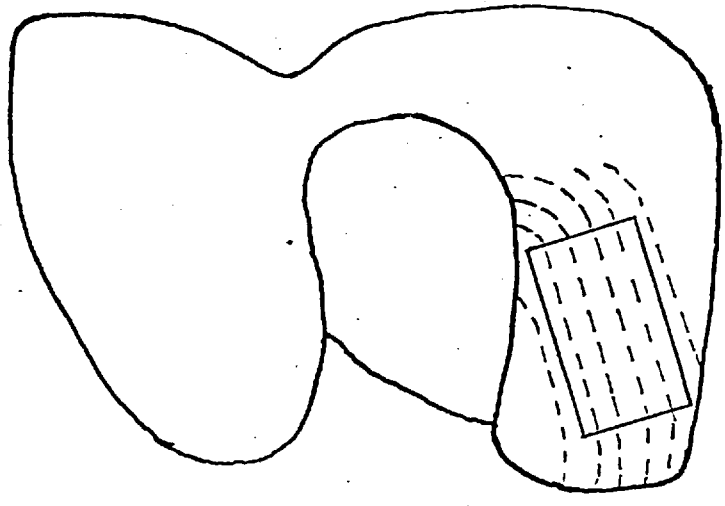
Fig. 45. Cleavage Line Pattern on the Surface of Human Femoral Condyles

by Hultkrantz (1898), described in Para 2 of this Chapter (p. 99) , Fig. 45 (facing p. 104) shows the pattern obtained. This pattern was used as a guide, from which the rectangular areas of cartilage were selected, either parallel to or perpendicular to the cleavage lines. Fig. 46 (facing p. 105) shows a diagram of two femoral condyles, in which it is shown how specimens, which were parallel to the cleavage lines,were selected from the front of the medial femoral condyle, and specimens perpendicular to the cleavage pattern were selected from the middle of the lateral condyle. It was necessary to determine the cleavage pattern on untested femoral condyles, because specimens to be tested were not pricked until after the termination of a test. Thisprecaution was observed to avoid inducing failure by propagating cracks from the cleavage lines.

Full thickness rectangular sections of cartilage, approximately 11mm. by 20mm, were then excised from the condyles in the areas indicated in Fig. 46, using a very sharp scalpel. These sections were then placed with the articular surface downwards, on to the cutting platform of a Leitz Thermal Module freezing microtome, the uppermost excised face (nearest to the sub-chondral bone) was sliced, until it was parallel to the surface. The specimen was then thawed, and the thickness was measured in several places using the special micrometer screw gauge described in Para. 1 of Chapter 4 (p. 31) The cartilage section was then replaced on to the microtome platform surface downwards, and four layers of equal thickness (approximately 400μ) were then carefully sliced. Finally, using the specially prepared cutter shown in Fig. 47 (facing p. 106), each layer of cartilage was then cut into a dumbell shaped tension specimen. The load axis of the specimen was aligned parallel to the long edge of the rectangular section, and this in turn



FEMORAL CONDYLES VIEWED FROM BELOW. SECTION OF CARTILAGE CUT ACROSS CLEAVAGE LINES.



FEMORAL CONDYLES VIEWED FROM POSTERIOR DIRECTION. SECTION OF CARTILAGE CUT PARALLEL TO CLEAVAGE LINES.

FIG. 46. SHOWING LOCATION ON FEMORAL CONDYLES FROM WHICH SPECIMENS WERE TAKEN.

had already been aligned, at either 0° or 90° to the cleavage line pattern. In this way the load axis for each specimen was aligned at either 0° or 90° to the cleavage line pattern.

Each specimen was then placed over a grid of fine lines, one millimetre apart, and two points were marked with Indian ink, two millimetres apart, symmetrically placed about the centre point of the specimen.

The distance between the closest edges of the two marks (gauge length); the width across the centre of the specimen; and the thickness of each specimen was then measured with a travelling microscope and the micrometer screw gauge.

Each specimen was mounted in turn in the grips shown in Fig. 48 (facing p. 106), the surfaces of which had been shot-blasted to give the necessary surface roughness to grip the specimens, without tearing the cartilage. The specimen and grips were then mounted in a Tensometer E Type materials testing machine, see Fig. 49 (facing p. 107) and Fig. 50 (facing p. 107). A camera was positioned in such a way as to obtain almost a full frame image of the specimen. An increasing tensile load was applied to each specimen at a constant rate of cross-head movement of 4.3 mm per minute, and a continuous running plot of load versus cross-head movement was recorded. During each test, Ringer's solution was added frequently to the specimen, and photographs were taken at convenient intervals of elongation. The negatives were projected onto a screen from a 35mm slide projector, the distance between the screen and the projector lens being such as to give a magnification of approximately 20:1 between the specimen dimensions and the projected image. The distance between the two marks representing the gauge length was measured on the projected image, which enabled the



Fig. 47. Cutter Used for Preparing Tension Specimens





Fig. 48. Grips with Shot-Blasted Surfaces. Used for Tension Tests

extension of a specimen to be determined. A mark was made on the curve of load versus cross head movement, when each photograph was taken, so that the load corresponding to each extension was known. From these figures a graph of nominal stress (load ÷ original central cross-sectional area) versus extension, was plotted for each specimen.

(5.1) Specimen profile

It is common practice, when performing tension tests, to use a specimen with a parallel sided central section. Specimens of cartilage were initially cut to this profile, but they invariably failed near to one end, outside the gauge length and at the curved section. It was eventually concluded that the blades of the cutter possessed small imperfections at the section in question, and these in turn caused flaws in the edge of the cartilage specimen which produced premature failure by tearing.

The specimen profile which was used in this investigation , shown in Fig. 51(facing p. 108) was that of an arc of a circle, this being a common profile employed in tension tests on rubber, when similar problems are encountered. The cutter blades were sharpened without introducing imperfections, and the result was, that all the specimens failed within the gauge length.

Some new parallel-sided cutters have since been made which are satisfactory. It is intended that these will be used in future tests.

(6) Results and discussion

(6.1) Variation of the nominal stress versus extension curve with distance below the articular surface

Figs. 52 and 53 (facing pp.109 ,110) show curves of nominal stress versus extension, for the four layers taken from the same area of cartilage.

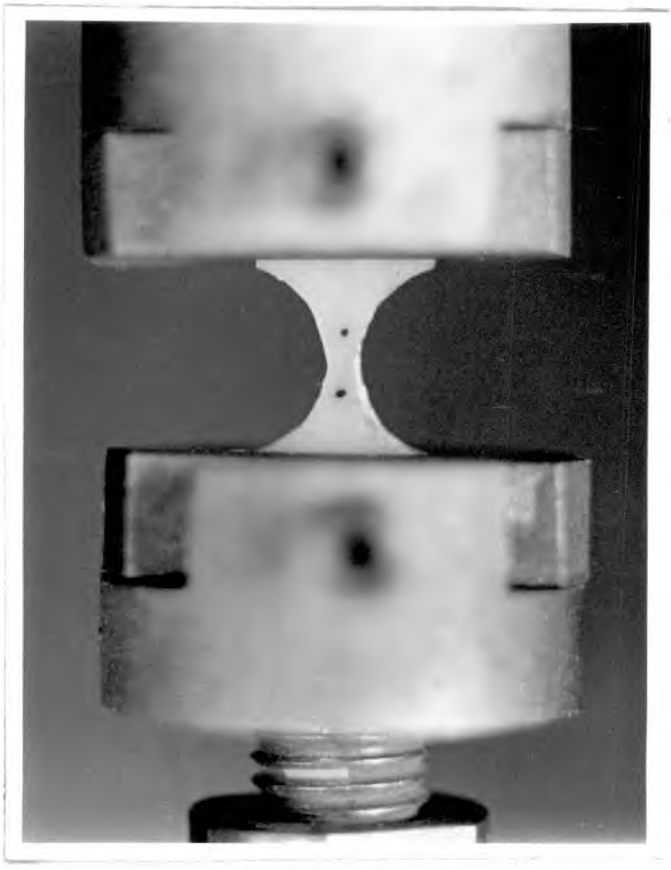


Fig. 49. Tension Specimen Mounted in Grips with Gauge Length Marked



Fig. 50. View of the Tensometer Testing Machine with Specimen Mounted Between Grips

It can be seen that the stiffness of each specimen, which is represented by the slope of a curve, decreases with distance below the articular surface.

The stress at failure does not show a systematic variation. The reason for this seems to be indicated by the manner in which a specimen failed. Some specimens failed by tearing, whereas others failed suddenly. The latter specimens usually failed at a higher stress. It appears that failure by tearing, was the result of an imperfection at the edge of a specimen, from which a crack was propagated.

(6.2) Variation of the nominal stress versus extension curve with orientation to the cleavage line pattern

Fig. 54 (facing p. 111) shows the nominal stress versus extension curves for the surface layers of two pairs of specimens cut parallel to, and perpendicular to the cleavage line pattern.

It can be seen that the specimens cut parallel to the cleavage lines were stiffer (steeper gradient), and failed at a higher stress, than specimens cut perpendicularly to the cleavage lines.

Table 29 (facing p.112) shows the stiffness and the nominal stress at failure (U.T.S.) for the surface layers of all the specimens tested. The data shown in this table confirms the results shown in Fig. 54, that the stiffness and strength of specimens cut parallel to the cleavage lines, are both significantly greater than the stiffness and strength of specimens cut perpendicular to the cleavage lines.

(6.3) The effect of localised degeneration

In section 1 of this thesis it was shown how the indentation stiffness of visibly normal cartilage covering the human femoral head decreases, as the severity of localised degeneration increases. A similar tendency was ob-

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ALL MEASUREMENTS IN INCHES.

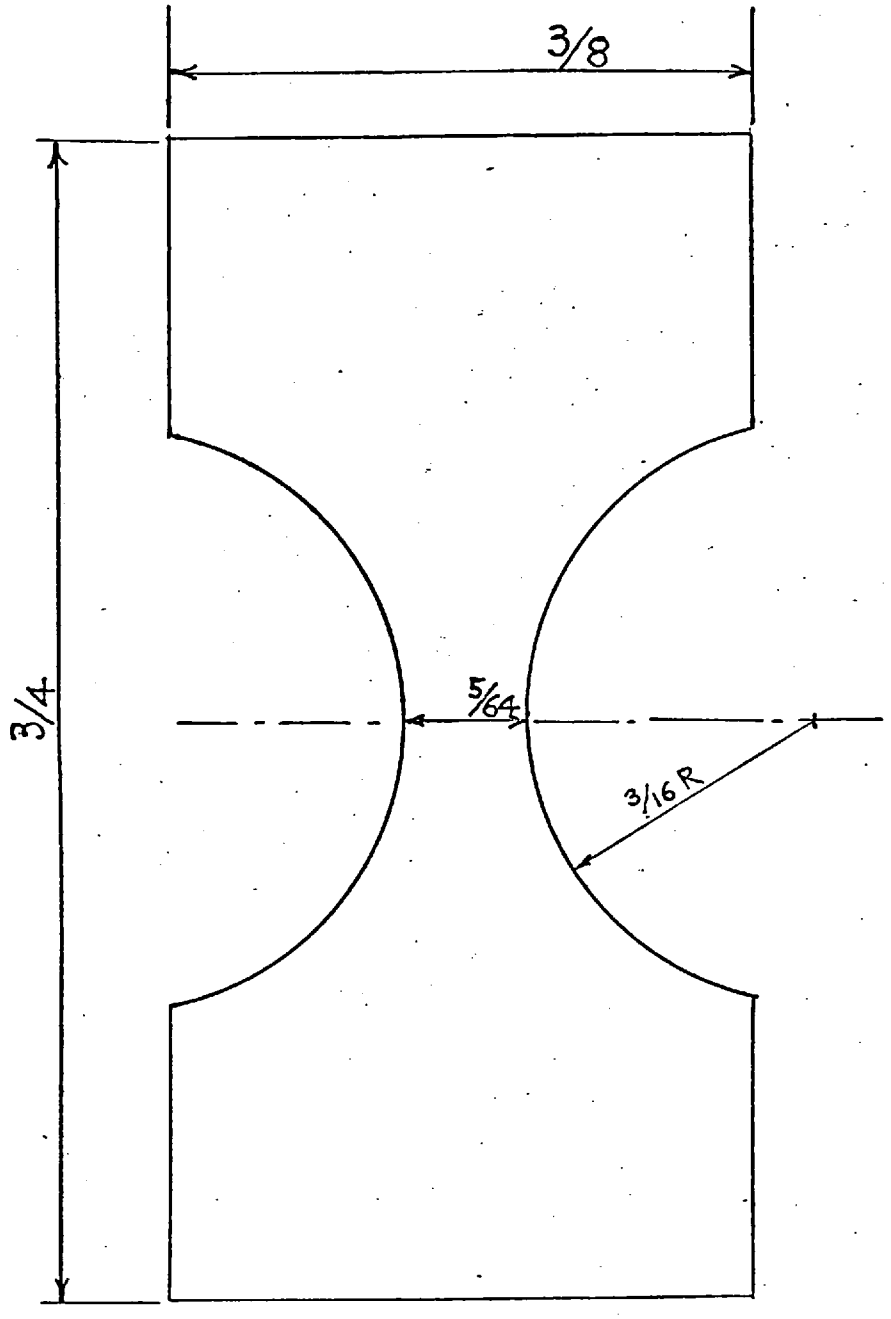


FIG. 51. SPECIMEN DIMENSIONS

served in the tension test results.

With reference to Table 29, it can be seen that specimens (1) and (3) displayed some localised areas of degeneration, denoted by the letter F which implies fibrillation. The surface layer of specimen (1), which was cut perpendicular to the cleavage lines, is significantly softer and weaker than corresponding layers of the other specimens. The layer cut parallel to the cleavage lines in specimen (1) however, does not show any significant difference from the corresponding layers of the other specimen. On the other hand, the surface layer of specimen (3) cut parallel to the cleavage lines is significantly softer and weaker than the corresponding layers of the other specimens, whereas the layer cut perpendicular to the cleavage lines shows no similar reduction of stiffness or strength.

It is not possible to state with certainty on the basis of the results to date, that the severity of localised degeneration influences the tensile strength of visibly normal cartilage, from elsewhere on the same joint surface. However, a tendency has been observed, which will be either confirmed or denied by future experiments.

(7) Discussion and conclusions

The results of tension tests parallel to the articular surface performed on forty specimens from ten human femoral condyles, show that the stiffness and strength of visibly normal cartilage depends on two factors and possibly three;

1. The orientation of the surface cleavage line pattern with respect to the axis of tension,
2. The distance below the articular surface and,
3. The severity of localised degeneration in untested areas.

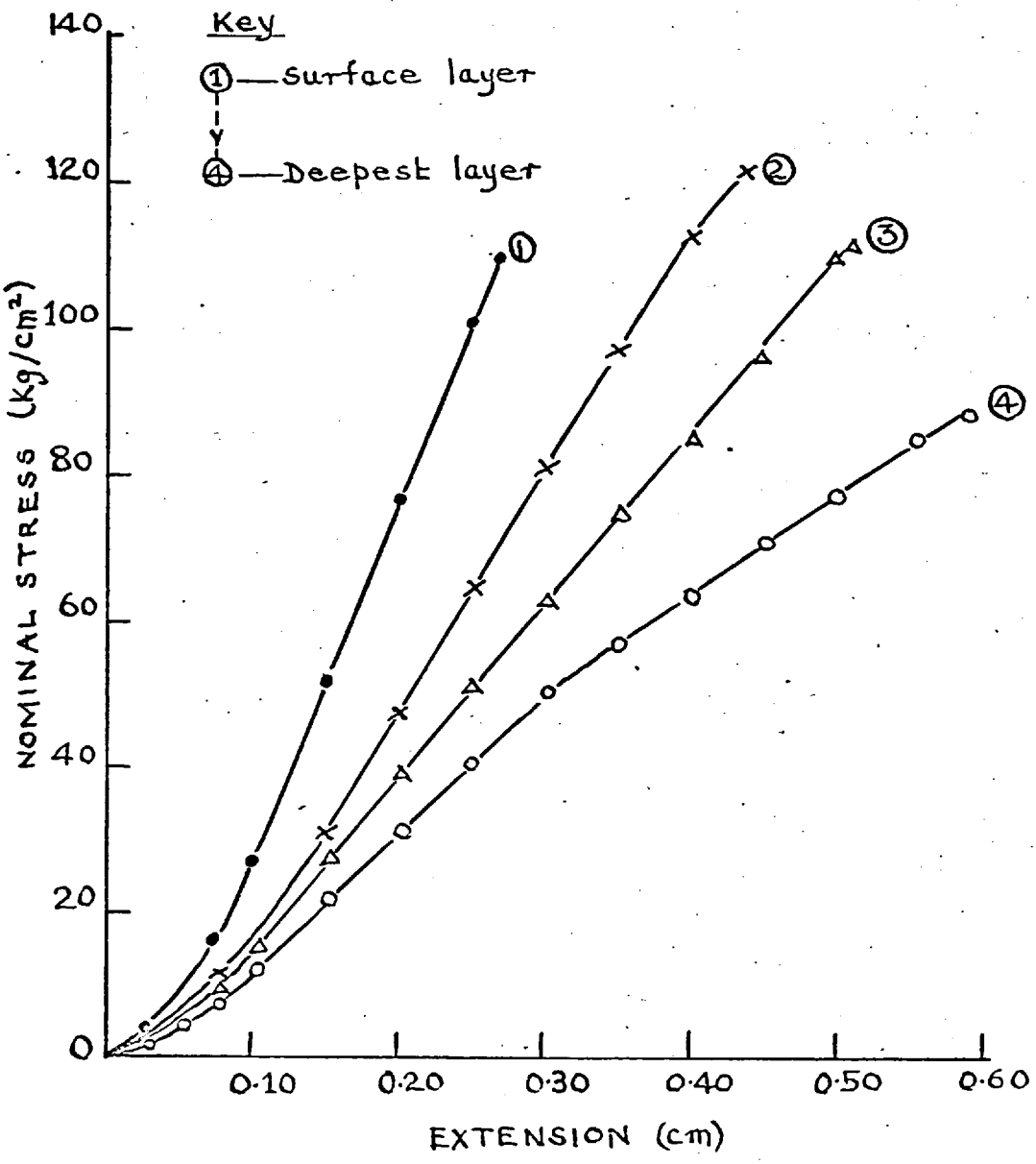


FIG.52 CURVES OF NOMINAL STRESS (load ÷ original cross-sectional area) VERSUS EXTENSION FOR ALL FOUR LAYERS OF A SPECIMEN TAKEN FROM A 60 YEAR OLD MALE. CLEAVAGE LINES PARALLEL TO THE LOAD AXIS.

The observation that the surface layers of specimens cut parallel to the surface cleavage lines, are both stiffer and stronger than the surface layers of specimens from the same condyles cut perpendicular to the cleavage lines, shows that the cleavage line pattern most probably indicates the main collagen fibre orientation at and near to the articular surface. This may be deduced because collagen appears to be the main tension resistant fibre of connective tissues. As such, it is both stiff and strong. Therefore it would be expected, that tensile tests on specimens parallel to the collagen fibre orientation in a fibre reinforced material, would yield results indicating a stiffer and stronger configuration, than tests performed perpendicular to the main fibre orientation. This has been observed in cartilage and the conclusion mentioned above, was drawn. Thus the results of tension tests for the surface layer of cartilage, confirm the observations of Bullough and Goodfellow (1968) using the technique of polarised microscopy.

In addition it has been shown that the stiffness of cartilage decreases with distance below the articular surface. This observation would be expected, if the predominant orientation of the tension resistant collagen fibres changed, from being parallel to the surface, to a predominantly radial configuration. Thus the results agree with the change of collagen fibre orientation, which has been observed using other techniques, see Para. 1 of this Chapter (p. 98).

Finally it has been observed that there is a tendency for localised areas of degeneration to influence the stiffness and strength of visibly normal cartilage, from the same joint surface. The results of chemical analyses performed on indented areas of cartilage from human femoral heads

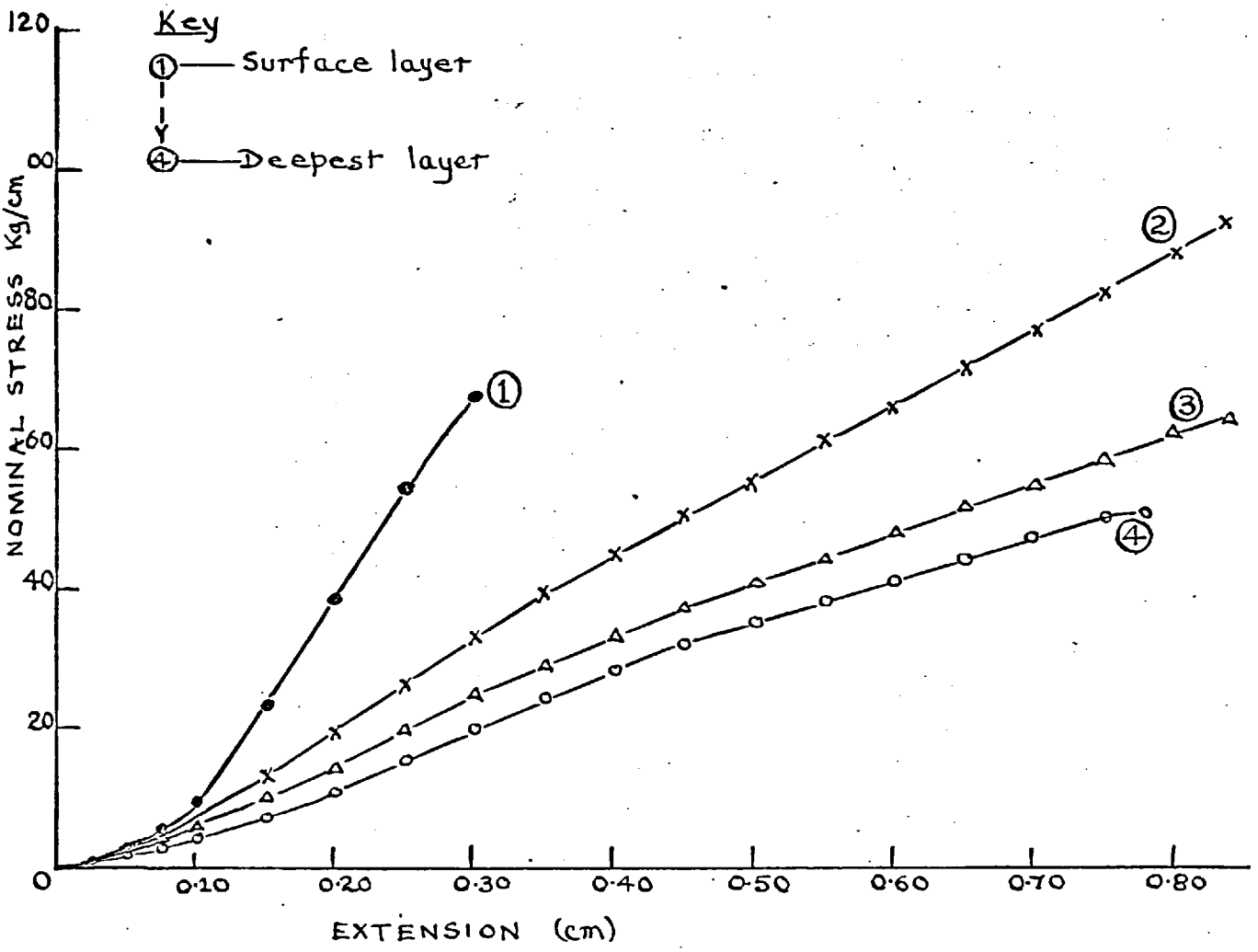


FIG. 53. CURVES OF NOMINAL STRESS VS. EXTENSION FOR ALL FOUR LAYERS OF A SPECIMEN TAKEN FROM A 51 YEAR OLD FEMALE. CLEAVAGE LINES PERPENDICULAR TO THE LOAD AXIS.

showed, that the mucopolysaccharide content of visibly normal cartilage from femoral heads displaying localised areas of degeneration, decreased as the severity of the degeneration increased. If similar changes occur on the femoral condyles it would be expected that they would be confirmed by the results of tension tests, since in a fibre reinforced material, the transfer of stress between adjacent fibres, depends largely on the manner in which shear stresses are transferred from the fibres to the matrix and thence to an adjacent fibre. If the matrix is depleted or becomes softer, then it would be expected that this would also weaken the overall structure. In cartilage the situation is complicated by the possible existence of cross links between adjacent collagen fibres, which would aosl contribute to the transfer of the stresses between the main collagen fibres. It is hoped that work planned for the future will determine the relative importance of the matrix and collagen fibre cross links, in the tensile properties of articular cartilage.

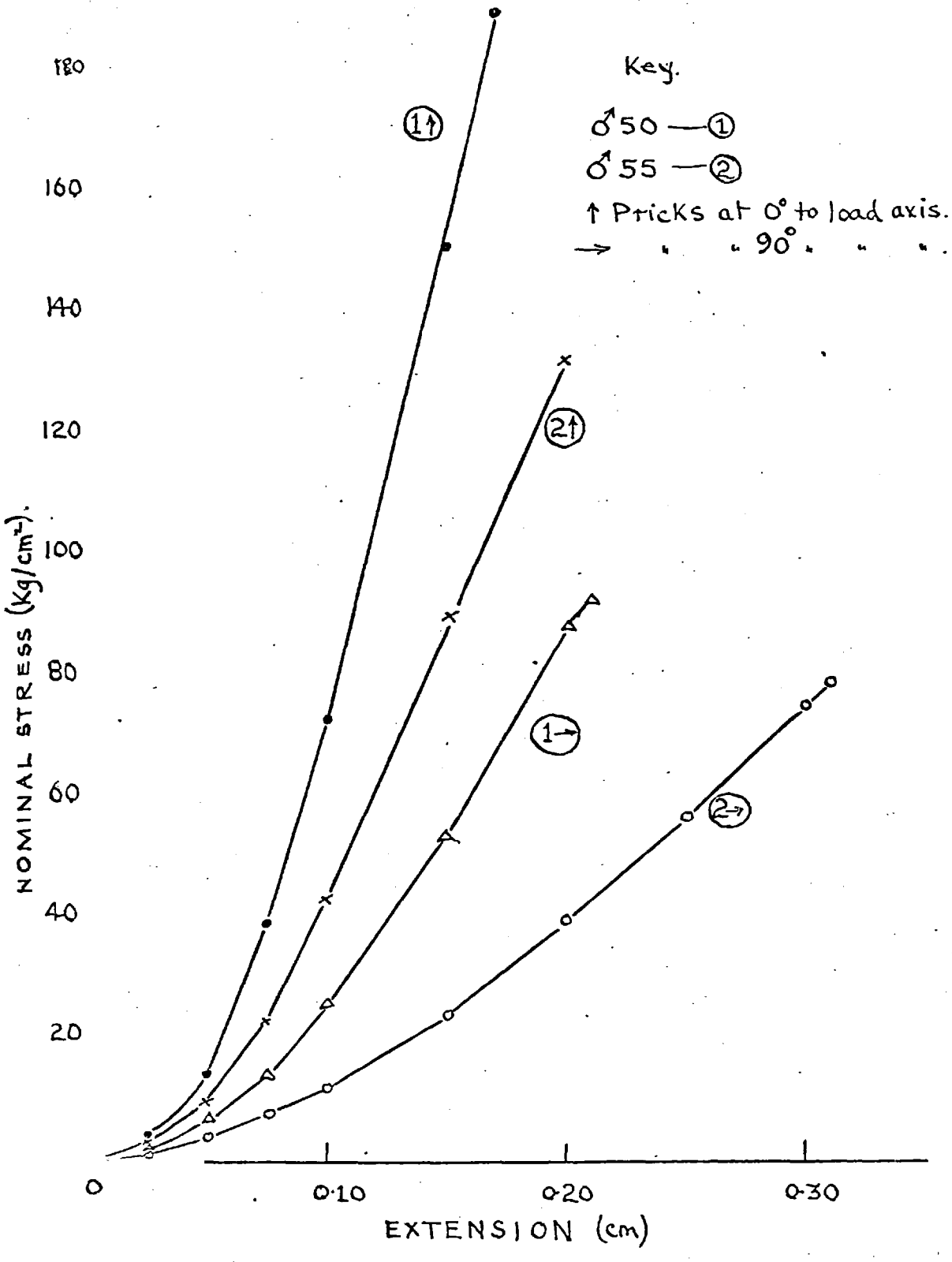


FIG. 54. CURVES FOR THE SURFACE LAYERS OF TWO SPECIMENS TAKEN FROM EACH OF TWO DIFFERENT FEMORAL CONDYLES. THEY ARE CUT AT 0° AND 90° TO THE PRICK LINES.

CHAPTER 11

SUMMARY OF CONCLUSIONS

This complete investigation may be considered in three sections:

(1) Indentation survey of the cartilage of human femoral heads in vitro,

(2) Relationship between cartilage stiffness and;

(a) The chemical components of cartilage,

(b) The permeability of cartilage to water.

(3) Tension tests parallel to the articular surface on cartilage from human femoral condyles in vitro.

Considering each section in order, the main results and conclusions may be summarised as follows, with emphasis laid on the advances made by this work.

(1) The Indentation Surveys

Sixteen femoral heads were studied within the age range of 19 to 82 years. A total of five hundred and ten areas of cartilage were indented. The tests were divided into two series: Series 1 employed a plane-ended cylindrical indenter, 0.125 in. in diameter with a load of 5 lbf; Series 2 tests employed a hemispherically-ended cylindrical indenter of the same diameter, with a load of 2 lbf. The observations were recorded as continuous running plots of indentation versus time after load application. The indentations at two seconds after load application were then extracted and presented in two ways:

(a) As layered maps of the variation of indentation over each femoral head, and

(b) As histograms of indentation with the mean indentation for each femoral head.

The observations showed that:

SPECIMEN	PARALLEL		ACROSS		
	U.T.S. (Kg/cm^2)	STIFFNESS (Kg/cm)	U.T.S. (Kg/cm^2)	STIFFNESS (Kg/cm)	
(1) Female 67	160	860	47	226	F.
(2) " 51	160	750	68	300	N
(3) Male 60	111	500	74	204	F
(4) " 55	133	900	81	370	N
(5) " 50	190	1700	94	550	N

TABLE 29. ULTIMATE TENSILE STRESS AND STIFFNESS OF THE SURFACE LAYERS OF ALL THE SPECIMENS. TWO ORIENTATIONS TO THE CLEAVAGE LINES.

N : VISIBLY NORMAL JOINT SURFACE.

F : LOCALISED AREAS OF FIBRILLATION.

1. The indentation versus time curve for cartilage, possessed a typical shape, irrespective of the shape of indenter used. This curve shape agreed qualitatively with the results of previous workers for the load phase, but with only the results of Elmore et al. (1963) and Sokoloff (1966) in the unload phase. This latter observation was explained by the fact that all other workers had performed their experiments in air, with consequent drying of the cartilage, and thus incomplete recovery of the indentation when the load was removed. In this investigation and those of Elmore et al. (1963) and Sokoloff (1966), the tests were performed whilst the femoral head was surrounded by Ringer's saline solution at 37°C. Recovery from the loaded state was consequently complete.

2. The shape of an indentation versus time curve was explained, in the light of the present state of knowledge of the structure and properties of cartilage, as a material.

In addition, the shape of an indentation versus time curve between 2 seconds and thirty minutes after load application was expressed mathematically, by the relatively simple equation:

$$y = Kt^N$$

where y = indentation

t = time after load application

K and N are constants.

It was found that this equation also fitted two curves published by Sokoloff (1966) for cartilage on the human patella. The value of 'K' was found to increase as the indentation increased, whereas 'N' was found to decrease as the indentation increased.

(1.1) Observations from the indentation surveys

From the layered maps and histograms of indentation on the femoral heads tested, the following observations were made:

1. The indentation of visibly normal cartilage showed considerable localised variation;

2. The variation of indentation over each femoral head showed a systematic pattern in which;

   (a) The area in which the largest indentations occurred was invariably situated at or near to the margin of the fovea and;

   (b) The area in which the lowest indentations occurred, was located in a band, which extended over the superior aspect of a femoral head and around to the anterior and posterior facets.

3. This pattern of variation of indentation over the femoral head coincides closely with the contact and apparent non-contact areas between the femoral head and acetabulum, as shown by Greenwald (personal communication). The areas in which the lowest indentations occurred coincides with the contact area, whilst the area in which the largest indentations occurred, near to the fovea, coincides with the apparent non-contact area between the femoral head and acetabulum.

4. The indentation of corresponding areas of visibly normal cartilage increases, as the severity of degeneration of localised untested areas increases. The pattern of distribution of indentation over any one femoral head remains essentially the same, but, as the histograms and mean indentation data, in particular, show, the visibly normal cartilage becomes increasingly deformable, as the localised areas of degeneration become more severe, from a slight roughening

of the cartilage surface, to fibrillation with partial thickness loss and finally ulceration with full thickness loss of cartilage in that area.

(1.2) Creep modulus as a measure of cartilage stiffness

The indentation of cartilage on the femoral head was shown to be thickness dependent, and so a method was derived for eliminating this variable thickness effect. The experimental results of indentation tests by Waters (1965), on relatively thin sheets of natural rubber were considered. It was shown that at two seconds after load application the results of Waters relating to rubber could be applied to cartilage. Thus a creep modulus was derived which represented the stiffness of cartilage irrespective of thickness. The two equations used to calculate E, the creep modulus, were:

1. Hemispherical indenter (load 2 lbf)

$$E = 1.28 \left[\frac{1 - \exp\left(-0.42\ {}^{t}/a_{t}\right)}{d_{t}}\right]^{3/2}$$

2. Plane-ended indenter (load 5 lbf)

$$E = 5.37\ \frac{\emptyset\ ({}^{t}/R)}{d_{t}}$$

where $\emptyset({}^{t}/R)$ was taken from the published results of Waters (1965).

The two equations were used to calculate the creep modulus of all the areas of cartilage tested in the indentation surveys in Both Series 1 and Series 2.

Layered maps and histograms of the variation of creep modulus were produced in a manner identical to that in which the maps and histograms of indentation were produced.

1. The pattern of variation of creep modulus of cartilage over the femoral head, was very similar to the pattern of variation of indentation.

2. The area of softest cartilage (lowest creep modulus) on each femoral head, was located at and near to the margin of the fovea, whereas the area of the stiffest cartilage (highest creep modulus) was located in a band of somewhat more limited dimensions than the band in which the lowest indentations occurred, which extended over the superior aspect of a femoral head and around to the anterior and posterior facets.

3. The overall softening of the visibly normal cartilage as the severity of localised untested areas of degeneration increased, was even more evident in the maps and histograms of creep modulus.

From a survey of the literature it was concluded that no systematic survey of the variation of mechanical properties of articular cartilage over the hip joint had been previously performed. It was not known how the stiffness of visibly normal cartilage varied on any one joint surface, or how it varied from specimen to specimen. This investigation in addition to showing that cartilage stiffness varies systematically over the femoral head, has indicated that the process of cartilage degeneration is not restricted to areas of visibly degenerate cartilage, but it also affects areas of visibly normal cartilage. The manner in which this process could contribute to the aeteology of osteoarthrosis, has been discussed.

(2) The Relationship between the stiffness of cartilage and its chemical components

In order to assess the relative contribution of each of the main chemical components of cartilage to the stiffness, chemical analysis was performed on forty seven areas of cartilage on eight femoral heads, which had previously been indented.

A highly significant correlation was shown to exist between the

creep modulus at two seconds after load application, and the total mucopolysaccharide content (partial correlation coefficient $r = 0.854$; $P < 0.001$). The partial correlation between the creep modulus and collagen content was not so significant (partial correlation coefficient $r = 0.233$; $p > 0.1$). This confirms the observation, that in general the collagen fibres in cartilage are orientated predominantly radially for most of the thickness, and thus it seems that they do not play an important role in supporting compressive stresses. The total mucopolysaccharide content was considered to be composed principally of; chondroitin sulphate and keratan sulphate. Chemical analysis was performed to determine the relative amounts of these two constituents in the tested areas of cartilage.

It was found that for creep modulus versus chondroitin sulphate the partial correlation coefficient $r = 0.810$ ($P < 0.001$), and for creep modulus versus keratan sulphate, $r = 0.800$ ($P < 0.001$). Thus it was shown that the contributions of the two component mucopolysaccharides towards the stiffness of articular cartilage are approximately equally important.

Before the present investigation was performed, comparison between the stiffness of cartilage and its chemical components had been performed mainly by comparing indentation measurements with stained histological sections. In addition, emphasis had been laid upon a comparison between visibly normal and degenerate cartilage. This investigation has provided the first detailed comparison between the stiffness of cartilage (creep modulus) and the main chemical components, determined by chemical analysis. The contributions of keratan sulphate and collagen to stiffness have been considered for the first time.

(3) Observations on a comparison between the stiffness of cartilage and stained histological sections (with Bullough)

Hitherto in the literature, stained histological sections have been used, mainly to measure the amount of chondroitin sulphate present in indented areas of cartilage. This was determined from the intensity of staining. In the present investigation chemical analysis provided a more precise method of measuring the amounts of the chemical components of cartilage. However histological sections of the tested areas studied and the following observations were made:

1. The surface of relatively stiff areas of cartilage was invariably intact and smooth, whereas the surface of soft areas of cartilage was invariably fragmented, even though this was not visible to the naked eye.

2. The loss of staining (for total mucopolysaccharide and chondroitin sulphate and keratan sulphate) extended down from the articular surface. In the areas of stiff cartilage this zone in which loss of staining occurred, occupied a maximum of approximately one eighth of the total thickness of the cartilage section. In the areas of soft cartilage however, this zone occupied in general, a minimum of one quarter of the total thickness of a section and in some cases almost full thickness loss of staining was observed.

3. The final difference which was observed histologically between areas of stiff and soft cartilage, was the shape of the chondrocytes and the distribution and intensity of staining immediately surrounding them. In the areas of stiff cartilage the chondrocytes were relatively small and arranged in clusters. The nuclei stained intensely, as did the rims of the cells; this area extending to at

least two cell diameters into the intercellular matrix. In many cases these areas of intense staining joined together to form a network throughout the matrix. In contrast, the chondrocytes in the areas of soft cartilage were relatively large, and irregular in shape. The nuclei did not stain intensely and neither did the rims of the cells. In many cases a zone in which staining was absent was observed, which separated the rims of the chondrocytes from the stained areas of the matrix.

Thus it was shown that the stiffness of cartilage depends not only on the total amount of mucopolysaccharide, but also it appears, on the distribution throughout the matrix.

(4) The relationship between the stiffness of cartilage and its permeability to water (with Read and Maroudas)

The permeability of areas of cartilage which had previously been indented, was measured.

It was shown that a highly significant negative correlation existed, between the creep modulus of cartilage and its permeability to water, $r = -0.77$, $(P < 0.001)$. This confirmed the results of experiments performed independently, that:

1. The permeability of cartilage is inversely related to the fixed negative charge density, and

2. The fixed negative charge density is directly proportional to the total mucopolysaccharide content. Thus the results obtained previously by chemical analysis were confirmed indirectly by physicochemical measurements of permeability.

The correlation coefficient of -0.77 was considered to be insuf-

ficiently significant to warrant the use of either indentation tests in vivo or permeability measurements on very small areas of cartilage, in the diagnosis of cartilage degeneration. However, it is hoped that future work may make this possible. From the literature survey it was concluded that combined indentation and physicochemical measurements of articular cartilage had not been performed previously.

(5) Tension Tests

The results of tension tests performed parallel to the articular surface and at:

(a) Increasing distance below the articular surface, and

(b) Orientations of 0° and 90° to the surface cleavage line pattern,

have shown that:

1. The stiffness of cartilage decreases with distance below the articular surface. The surface layer is considerably stiffer than the deeper layers by a factor of approximately 2:1, or more.
2. The stiffness and stress at failure of the surface layer, are greater for specimens in which the cleavage lines are parallel to the load axis, than specimens in which the cleavage lines are perpendicular to the load axis. This result agrees with the observation of workers using different techniques, that the cleavage line pattern of cartilage indicates the predominant direction of the parallel collagen fibres, at and near to the articular surface.
3. Finally, a tendency was observed for visibly normal cartilage from joint surfaces displaying localised areas of degeneration, to be softer and weaker than corresponding areas of cartilage from entirely normal joint surfaces. This observation is however still

tentative and still remains to be confirmed by future work.

It was concluded from the literature survey that these tests represent the first tension tests performed on articular cartilage parallel to the articular surface. They are therefore the first tension tests which confirm quantitatively the collagen fibre orientation in cartilage as observed by other techniques.

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APPENDIX 1. TABLES.

Table 1 DETAILS OF EACH SPECIMEN

(1) Specimen Number	(2) Age (Years)	(3) Sex	(4) Date of Post-Mortem	(5) Date of Testing	(6) Time Between Post-Mortem and Testing (Days)
1	31	F	Amputation Specimen		
2	46	M	12 Dec:66	15 Dec:66	3
3	30	F	17 Jan:67	23 Jan:67	6
4	55	F	24 Jan:67	26 Jan:67	2
5	59	F	21 Jan:67	1 Feb:67	10
6	75	M	1 Feb:67	3 Feb:67	2
7	68	F	31 Jan:67	7 Feb:67	8
8	73	M	14 Feb:67	15 Feb:67	1
9	66	F	13 Feb:67	23 Feb:67	10
10	61	M	27 Feb:67	6 Mar:67	7
11	19	F	3 Mar:67	9 Mar:67	6
12	37	F	9 Jun:67	16 Jun:67	7
13	52	M	27 Jun:67	30 Jun:67	3
14	78	M	4 Jul:67	6 Jul:67	2
15	59	M	4 Jul:67	10 Jul:67	6
16	80	M	11 Jul:67	14 Jul:67	3
17	60	M	21 Jul:67	24 Jul:67	3
18	35	M	24 Jul:67	28 Jul:67	4
19	69	F	20 Jul:67	2 Aug:67	13
20	33	M	29 Aug:67	1 Sep:67	3
21	67	M	26 Sep:67	9 Oct:67	13
22	67	F	14 Oct:67	17 Oct:67	3
23	54	M	16 Oct:67	20 Oct:67	4
24	78	M	24 Oct:67	6 Nov:67	13
25	40	M	8 Nov:67	10 Nov:67	2
26	80	M	16 Nov:67	22 Nov:67	6
27	86	F	18 Nov:67	26 Nov:67	8
28	86	F	22 Nov:67	30 Nov:67	8
29	75	M	3 Dec:67	11 Dec:67	8
30	71	F	16 Dec:67	18 Dec:67	2
31	61	M	6 Jan:68	9 Jan:68	3

TABLE 1 (Cont'd)

32	73	M	10 Jan:68	12 Jan:68	2
33	66	M	13 Jan:68	23 Jan:68	10
34	82	F	15 Jan:68	25 Jan:68	10
35	55	M	19 Jan:68	30 Jan:68	11
36	73	M	29 Jan:68	3 Feb:68	5
37	66	F	10 Feb:68	15 Feb:68	5
38	56	F	Amputation Specimen		
39	77	M	27 Feb:68	27 Feb:68	Nil
40	71	M	28 Feb:68	1 Mar:68	1
41	77	M	27 Feb:68	7 Mar:68	8
42	26	M	28 Feb:68	12 Mar:68	12
43	65	F	27 Mar:68	1 Apr:68	5
44	66	M	11 Apr:68	19 Apr:68	8
45	69	M	24 Apr:68	2 May:68	8
46	34	F	3 May:68	6 May:68	3
47	27	M	9 May:68	11 May:68	2
48	47	M	7 May:68	13 May:68	6
49	43	F	20 May:68	22 May:68	2
50	21	F	29 May:68	29 May:68	Nil
51	36	F	29 May:68	29 May:68	Nil
52	61	M	30 May:68	10 Jun:68	11
53	23	M	5 Jun:68	14 Jun:68	9
54	60	F	1 Jul:68	9 Jul:68	8
55	70	F	30 Aug:68	12 Sep:68	13
56	88	M	4 Oct:68	16 Oct:68	12
57	69	M	23 Oct:68	29 Oct:68	6
58	55	M	29 Oct:68	9 Nov:68	11
59	39	M	12 Dec:68	18 Dec:68	6
60	60	M	23 Jan:69	4 Feb:69	12
61	62	M	5 Feb:69	6 Feb:69	1
62	73	M	4 Feb:69	7 Feb:69	3
63	56	M	6 Feb:70	7 Feb:70	1
64 (RAND L).	72	F	6 Feb:70	7 Feb:70	1

Table 2 SPECIMENS CONTRIBUTING TO DIFFERENT PARTS OF THE INVESTIGATION

	Experiment	Specimens Contributing
1.	Detailed Stiffness Surveys	2,3,4,5,6,7,10,11,20,21,35,22,34,57,8,9.
2.	Storage Effects	1,38,63,64.
3.	Temperature Variation Effect	12,17,19,58.
4.	Repeatability of Indentation Measurements	1,2,3,4,5,6,7,8,9,10,11,20,21,22,23,25,38, 58,13,14,15,17.
5.	Deformation of Sub-Chondral Bone and Apparatus	13,14,15,16,20,22,56,57,60,61,62.
6.	Comparison of Indentations Under Synovial Fluid and Ringer's Solution	Knee (N.60), 36,55,58.
7.	Comparison of Results for Different Indenters and Loads	39.49.54.56.57.60,61,62.
8.	Correlation Between Creep Modulus and Permeability	24,31,34,41,44,46,53,54,42.
9.	Correlation Between Creep Modulus and Chemical Analysis	22,39,43,47,48,49,50,51.
10.	Effect of pH Variation and Composition of Ringer's solution	55,61,62.

Table 3 DEFORMATIONS OF SUB-CHONDRAL BONE PLUS TESTING APPARATUS. PLANE-ENDED INDENTER WITH LOADS OF 5lbf (2.28Kg) AND 2lbf (0.92Kg)

DEFORMATIONS (cm)

2 lbf (0.92Kg)	5 lbf (2.28Kg)
0.00254	0.00532
0.00279	0.00532
0.00279	0.00532
0.00304	0.00610
0.00330	0.00610
0.00228	0.00660
0.00203	0.00430
0.00228	0.00508
0.00254	0.00610
0.00330	0.00660
0.00330	0.00710
0.00254	0.00558
0.00330	0.00685
0.00203	0.00456
0.00279	0.00760
0.00254	0.00584
0.00254	0.00508
0.00254	0.00710
0.00203	0.00760
0.00279	0.00430
0.00355	0.00430
0.00355	0.00456
0.00380	0.00508
0.00304	0.00584
0.00304	0.00508
0.00355	0.00558
0.00304	0.00685
0.00355	
0.00330	
0.00330	
0.00304	

TABLE 6:

AMPUTATION SPECIMEN NUMBER 1 (SPECIMEN 1, TABLE 1)

VALUES OF REPEATED INDENTATIONS:

TIME AT 37°C and -20°C DURING TESTING

TEST NUMBER	RECORDED INDENTATIONS (Cm.)			DIFFERENCE BETWEEN REPEAT INDENTATION AND ORIGINAL AS % OF '0'*			TIME OF TEST AFTER AMPUTATION (HOURS AND MINUTES)		
	AREA 1	A2	A3	AREA 1	A2	A3	AREA 1	A2	A3
*0(ORIGINAL)	0.050	0.037	0.044	ORIGINAL VALUES			0-13	0-45	1-10
1	0.052	0.038	0.039	+ 4.0	+ 2.7	-11.0	1-45	2-20	2-45
2	0.051	0.038	-	+ 2.0	+ 2.7	-	3-15	3-35	-
FEMORAL HEAD STORED FOR 13 HOURS AT -20°C									
3	0.049	0.036	0.041	- 2.0	- 2.7	- 6.8	22-0	22-30	22-45
4	0.052	0.038	0.045	+ 4.0	+ 2.7	+ 2.3	24-0	24-50	25-10
FEMORAL HEAD STORED FOR 66 HOURS AT -20°C									
5	0.052	0.037	0.042	+ 4.0	0.0	- 4.8	93-0	93-20	93-30
DEFORMATION SURVEY PERFORMED. FEMORAL HEAD AT 37°C FOR A FURTHER 14 HOURS, THEN STORED FOR 32 HOURS AT -20°C									
6	0.056	0.043	0.046	+12.0	+16.0	+ 4.8	141-30	141-0	141-15
FEMORAL HEAD STORED FOR 16 HOURS AT -20°C									
7	0.076	0.044	0.050	+52.0	+19.0	+14.0	160-0	161-0	162-0

AFTER REMOVAL FROM DEEP FREEZE THE FEMORAL HEAD WAS ALLOWED APPROXIMATELY 1 HOUR IN RINGERS SOLUTION AT 37°C TO EQUILIBRATE WITH ITS SURROUNDINGS. THESE TIMES ARE NOT INCLUDED IN THE TABLE.

*'0' Denotes original indentation.

TABLE 7

AMPUTATION SPECIMEN NUMBER 2 (SPECIMEN 38, TABLE 1)

VALUES OF REPEATED INDENTATIONS:

TIME AT 37°C AND -20°C DURING TESTING

TEST NUMBER	RECORDED INDENTATIONS (cm.)			DIFFERENCE BETWEEN REPEAT INDENTATION AND ORIGINAL AS A %AGE			TIME OF TEST AFTER AMPUTATION (HOURS AND MINUTES)		
	AREA 1	A2	A3	AREA 1	A2	A3	AREA 1	A2	A3
0(ORIGINAL)	0.038	0.017	0.028	ORIGINAL VALUES.			0-15	0-45	1-05
1	0.036	0.018	0.028	-5·3	+5·9	0	1-30	1-45	2-15
2	0.039	0.018	0.027	+2·6	+5·9	-3·6	2-35	2-50	3-05
FEMORAL HEAD STORED FOR 13 HOURS AT -20°C									
3	0.038	0.017	0.027	0	0	-3·6	19-15	19-40	19-55
4	0.038	0.017	0.028	0	0	0	21-55	22-05	23-00
5	0.038	0.017	0.028	0	0	0	24-00	24-15	25-10
FEMORAL HEAD STORED FOR 17 HOURS AT -20°C									
6	0.039	0.017	0.032	+2·6	0	+14·3	44-0	45-0	45-45
7	0.038	0.017	0.031	0	0	+10·7	47-0	47-10	47-25
8	0.038	0.017	0.031	0	0	+10·7	49-0	49-25	49-45
FEMORAL HEAD STORED FOR 18½ HOURS AT -20°C									
9	0.036	0.017	0.030	-5·3	0	+7·2	69-40	70-05	70-20
10	0.037	0.017	0.031	-2·6	0	+10·7	73-30	73-40	73-55

EQUILIBRATION OF 1 HOUR AT 37°C ALLOWED AFTER REMOVAL OF FEMORAL HEAD FROM DEEP FREEZE, THESE TIMES ARE NOT INCLUDED IN THE TABLE.

TABLE 9

RESULTS OF REPEATED INDENTATIONS ON AMPUTATION, AND STIFFNESS SURVEY SPECIMENS

A	B	C	D		E	
SPECIMEN NUMBER	NUMBER OF AREAS TESTED	ORIGINAL INDENTATION AT 2 Sec. Cm.	SUBSEQUENT INDENTATIONS		DIFFERENCE AS A PERCENTAGE OF ORIGINAL DEFORMATION	
			FIRST REPEAT	SECOND REPEAT	FIRST REPEAT	SECOND REPEAT
1.	AMPUTATION SPECIMEN NUMBER ONE. THREE AREAS WERE TESTED REPEATEDLY UNTIL A SYSTEMATIC AND PROGRESSIVE INCREASE IN THE INDENTATION OF ALL THREE AREAS WAS RECORDED. (SEE TABLE 6 IN APPENDIX).					
	NUMBER OF REPEATED INDENTATIONS 14					
	" " " " SHOWING LESS THAN 5% DIFFERENCE FROM ORIGINAL 12					
	NUMBER OF INDENTATIONS BETWEEN 5% AND 10% 1					
	" " " GREATER THAN 10% 1					
2	3	0.024	0.023		-4.2	
		0.033	0.034		+3.0	
		0.064	0.067		+4.7	
3	2	0.027	0.030		+10	
		0.031	0.032		+3.2	
4	2	0.032	0.031		-3.1	
		0.045	0.046		+2.2	
5	2	0.044	0.043		-2.3	
		0.052	0.054		+3.8	
6	2	0.037	0.037		0.0	
		0.046	0.049		+6.5	
7	2	0.051	0.050		-2.0	
		0.038	0.041		+7.9	
8	2	0.031	0.032		+3.2	
		0.037	0.038		+2.7	

TABLE 9 (CONTINUED)

A	B	C	D		E	
SPECIMEN NUMBER	NUMBER OF AREAS TESTED	ORIGINAL INDENTATION AT 2 Sec. Cm.	SUBSEQUENT INDENTATIONS		DIFFERENCE AS A PERCENTAGE OF ORIGINAL DEFORMATION	
			FIRST REPEAT	SECOND REPEAT	FIRST REPEAT	SECOND REPEAT
9	2	0.026 0.025	0.026 0.026		0.0 +4.0	
10	2	0.093 0.063	0.093 0.061	0.096 0.066	0.0	+3.2 +4.8
11	2	0.053 0.027	0.053 0.028	0.056 0.026	0.0 +3.7	+5.6 -3.7
20	5	0.038 0.039 0.063 0.047 0.038	0.039 0.040 0.065 0.049 0.035	0.038 - 0.065 0.049 -	+2.6 +2.6 +3.2 +4.3 -7.9	0.0 - +3.2 +4.3 -
21	2	0.066 0.038	0.067 0.038	0.068 0.039	+1.5 0.0	+3.0 +2.6
22	1	0.028	0.028	0.029	0.0	+3.6
23	3	0.049 0.056 0.056	0.050 0.061 0.058	0.042 - -	+2.0 +8.9 +3.6	-14.0 - -
25	3	0.048 0.059 0.041	0.046 0.057 0.044	- 0.057 -	-4.2 -3.4 +7.3	- -3.4 -
58	1	0.022	0.022	0.022	0.0	0.0

38 AMPUTATION SPECIMEN NUMBER TWO. THREE AREAS REPEATED FOR DETAILED RESULTS SEE TABLE 7 (APPENDIX)

NUMBER OF REPEATED INDENTATIONS 25

" " " " LESS THAN 5% DIFFERENT 25

TABLE 10

RESULTS OF REPEATED INDENTATIONS ON FOUR FEMORAL HEADS

(1) SPECIMEN NUMBER	(2) NUMBER OF AREAS TESTED	(3) ORIGINAL INDENTATION AT 2 Sec. Cm.	(4) SUBSEQUENT INDENTATIONS FIRST REPEAT	(5) SUBSEQUENT INDENTATIONS SECOND REPEAT	(6) DIFFERENCE AS A PERCENTAGE OF ORIGINAL DEFORMATION FIRST REPEAT	(6) DIFFERENCE AS A PERCENTAGE OF ORIGINAL DEFORMATION SECOND REPEAT
13	2	0.117	0.117	0.123	0.0	+5.1
		0.041	0.041	0.039	0.0	-4.9
A THIRD REPEAT ON THE SECOND AREA GAVE DIFFERENCE OF +1.2						
14	5	0.049	0.049	-	0.0	-
		0.021	0.021	-	0.0	-
		0.032	0.031	-	-3.1	-
		0.032	0.031	-	-3.1	-
		0.047	0.048	-	+2.1	-
15	8	0.109	0.114	-	+4.6	-
		0.070	0.074	-	+5.7	-
		0.027	0.027	-	0.0	-
		0.026	0.027	-	+3.8	-
		0.086	0.089	-	+3.5	-
		0.044	0.040	-	-9.1	-
		0.046	0.046	-	0.0	-
		0.025	0.026	-	+4.0	-
17	4	0.023	0.024	0.023	+4.4	0.0
		0.025	0.027	0.026	+8.0	+4.0
		0.021	0.023	0.021	+9.6	0.0
		0.087	0.088	-	+1.2	-

Table 13 THE EFFECT OF TEMPERATURE ON INDENTATION VALUES Indentation (cm) At:

Specimen Number	20°C	24°C	25°C	30°C	31°C	34°C	37°C	40°C	44°C	54°C
12			0.0 426		0.0424	0.0424	0.0424	0.0434		
			0.0426		0.0434	0.0434	0.0434	0.0437		
			0.0218		0.0226	0.0234	0.0234	0.0234		
17		0.0158								0.0163
		0.0127								0.0140
19		0.0127				0.0129			0.0134	0.0129
		0.0363							0.0373	0.0368
58	0.0554	0.0551		0.0564				0.0558		
	0.0512			0.0520				0.0513		
	0.0361			0.0361				0.0376		
	0.0282			0.0289				0.0287		

EFFECT OF pH VARIATION AND COMPOSITION OF RINGER'S SOLUTION.

Table 15

pH VARIED BY USING BOTH TAPWATER AND DISTILLED WATER TO MAKE RINGER'S SOLUTION

Specimen Number	Group	Indentation (cm) (A) Distilled Ringer's	(B) Tap Ringer's	(C) Distilled Ringer's	(A-B) %(A)	(A-C) %(A)
55	(1)	0.0226	0.0231	0.0225	2.2	0.4
		0.0391	0.0396	0.0387	1.3	1.0
61	(1)	0.0464	0.0472	0.0470	1.7	1.3
		0.0426	0.0421	0.0430	1.2	0.9
		0.0396	0.0381	0.0400	3.8	1.0
		0.0453	0.0440	0.0448	2.9	1.1
		0.0378	0.0396	0.0375	4.8	0.8
		Tap Ringer's	Distilled Ringer's	Tap Ringer's		
62	(2)	0.0320	0.0315	0.0325	1.6	1.6
		0.0406	0.0419	0.0416	3.1	2.5
		0.0493	0.0477	C.0487	3.4	1.2
		0.0539	0.0524	0.0530	2.9	1.7
		0.0398	0.0406	0.0412	2.0	3.5

	p H (1)	(2)
Fresh Distilled Ringer's	6.9	6.9
Used Distilled Ringer's	7.1	6.9
Fresh Tap Ringer's	7.9	7.7
Used Tap Ringer's	7.8	8.1

TABLE 20

SERIES 1. INDENTATION SURVEYS USING A PLANE - ENDED CYLINDRICAL INDENTER 0.125 in. IN DIAMETER (3.175mm) WITH A LOAD OF 5lbf (2.28Kg).

TABULATED
1. Position (Reference to Fig.20facing p.51.)
2. Thickness
3. Indentation } at 2 seconds loading time
4. Creep Modulus }

SPECIMEN 2. 046

Position	A Thickness (cm)	B Indentation (cm)	C Creep Modulus (Kg/cm^2)	Position	A Thickness (cm)	B Indentation (cm)	C Creep Modulus (Kg/cm^2)
0	0.246	0.0456	31.6	3 - 1	0.135	.0174	65.2
1 - 1	.102	.0274	49.5	2	.186	.0091	172.5
2	.168	.0102	139.0	3	.239	.0137	147.0
3	.191	.0201	80.0	4	.246	.0146	142.0
4	.244	.0297	69.0	5	.254	.0219	98.0
5	.297	.0520	48.0	6			
6	.234	.0260	76.0	7	.229	.0201	95.8
7	"	.0173	114.2	8	.186	.0137	114.0
8	.191	.0109	148.0	9	.150	.0073	173.5
9	.178	.0137	110.0	10	.117	.0055	180.0
10	.191	.0155	103.5	4 - 1	.196	.0320	52.0
2 - 1	.118	.0183	54.0	2			
2	.160	.0283	47.7	3	.234	.0219	89.5
3	.196	.0274	60.5	4	"	.0247	80.0
4	.203	.0283	60.4	5	.244	.0219	94.0
5	.272	.0614	37.2	6			
6	.208	.0512	34.3	7			
7	.218	.0328	55.8	8	.191	.0457	35.1
8	.178	.0228	65.5	9	.170	.0256	56.0
9	.147	.0183	67.8	10	.102	.0274	31.2
10	.137	.0228	50.5				

SPECIMEN 3 Q30

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0				3 - 1	0.107	0.0238	37.8
1 - 1	0.097	0.0183	44.7	2	.114	.0192	50.2
2	.129	.0183	59.3	3	.162	"	71.2
3	.150	.0220	57.6	4	.158	.0206	64.5
4	.259	.0389	56.0	5	.208	.0320	55.0
5				6			
6	.361	.0121	25.1	7			
7	.193	.0411	40.6	8			
8	.165	.0196	71.0	9	.135	.0352	32.3
9	.152	.0156	83.0	10	.112	.0183	51.2
10	.112	.0128	74.0	4 - 1	.104	.0146	60.0
2 - 1	.109	.0165	56.0	2	.112	"	64.8
2	.135	"	69.3	3	.142	.0169	70.7
3	.168	.0200	70.6	4	.180	.0237	63.9
4	.191	.0260	61.7	5	.228	.0425	45.0
5	.198	.0384	43.5	6			
6				7			
7	.178	.0425	35.3	8	.201	.0388	43.8
8	.170	.0302	47.5	9	.147	.0206	60.0
9	.142	.0200	59.7	10	.107	.0183	49.2
10							

SPECIMEN 4 ♀55

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.272	0.0803	28.6	3 - 1	0.066	0.0547	10.4
1 - 1	.102	.0256	33.5	2	.155	.0214	61.4
2	.165	.0246	56.5	3	.193	"	75.7
3	.204	.0334	51.2	4	.213	.0288	62.1
4	.259	.0383	57.2	5	.269	.0384	59.2
5	.267	.0585	39.0	6			
6	.274	.114	20.3	7			
7	.234	.0458	43.0	8			
8	.203	.0342	50.0	9	.160	.0343	39.3
9	.173	.0288	50.6	10	.064	.0228	23.7
10				4 - 1	.064	.0384	14.0
2 - 1	.099	.0260	32.0	2	.147	.0174	71.0
2	.137	.0210	55.3	3	.204	.0306	56.0
3	.221	.0315	59.0	4	.236	.0480	41.5
4	.254	.0411	52.0	5	.228	.0795	24.2
5	.272	.0526	43.8	6			
6				7			
7				8	.183	.0503	30.6
8	.160	.0329	41.0	9	.155	.0251	52.6
9	.130	.0160	68.0	10			
10							

SPECIMEN 5 ♀59

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.277	0.0895	26.2	3 - 1	0.127	0.0450	23.8
1 - 1	.114	.0256	37.9	2	.191	.0452	35.5
2	.162	.0302	45.2	3	.238	.0494	40.5
3	.221	.0466	40.0	4	.241	.0384	53.0
4				5	.228	.0640	30.0
5	.300	.0990	25.4	6			
6	.236	.0785	"	7			
7	"	.0516	38.6	8	.117	.0503	20.0
8	.213	.0424	42.2	9	.122	.0348	29.5
9	.155	.0334	39.1	10			
10	.127	.0302	35.4	4 - 1	.142	.0366	32.7
2 - 1				2	.175	.0292	50.4
2	.180	.0457	33.0	3	.223	.0392	48.2
3	.251	.0410	51.5	4	.239	.0502	40.2
4	.254	"	52.2	5	.218	.0695	26.4
5	.249	.0566	37.1	6			
6				7			
7	.183	.0720	21.4	8	.170	.0870	16.5
8				9	.160	.0535	25.2
9	.165	.0137	10.2	10	.097	.0302	27.0
10							

SPECIMEN 6 075

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.249	0.0665	31.6	3 - 1	0.180	0.0520	29.0
1 - 1	.147	.0265	46.6	2	.185	.0398	39.1
2	.216	.0356	51.0	3	.193	.0274	59.3
3	.269	.0366	62.0	4	.201	.0242	70.0
4	.244	.0384	53.4	5	.244	.0398	51.8
5	.264	.0457	48.8	6			
6	.262	.0685	32.1	7			
7	.246	.0430	48.1	8			
8	.254	.0352	60.5	9	.195	.0356	46.2
9	.216	.0228	80.0	10	.137	.0343	33.7
10	.147	.0146	85.0	4 - 1	.160	.0329	41.0
2 - 1	.191	.0411	39.1	2	.163	.0238	57.6
2	.208	.0329	53.4	3	.195	"	69.0
3	.228	.0320	60.0	4	.239	.0357	56.3
4	.249	.0366	57.4	5	.254	.0443	48.4
5	.241	.0366	55.5	6	.287	.0704	34.4
6				7	.254	.0457	47.0
7	.254	.0356	60.2	8	"	.0440	48.7
8	.223	.0456	41.0	9	.221	.0393	47.4
9	.196	.0256	64.6	10	.168	.0229	62.0
10							

SPECIMEN 7 068

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.284	0.0750	32.0	3 - 1	0.145	0.0210	58.0
1 - 1	.107	.0260	34.6	2	.178	.0200	75.5
2	.140	.0320	36.8	3	.198	.0246	67.7
3	.191	.0352	45.5	4	"	.0265	63.0
4	.196	.0402	41.0	5	.224	.0311	60.6
5	.224	.0440	43.0	6			
6	.228	.0540	35.5	7	.234	.0795	24.7
7	.241	.0510	40.0	8			
8	.224	.0539	35.0	9			
9	.208	.0315	55.8	10			
10	.170	.0274	52.2	4 - 1	.199	.0242	69.5
2 - 1	.117	.0247	40.0	2	.180	.0274	58.5
2	.165	.0274	51.0	3	.196	.0283	58.5
3	.170	.0256	56.0	4	.203	.0310	55.0
4	.216	.0274	66.5	5	.224	.0374	50.4
5	.233	.0292	67.5	6			
6				7			
7				8	.175	.0320	46.0
8	.196	.0400	41.3	9	.142	.0251	47.7
9	.155	.0329	40.0	10	.125	.0265	39.6
10							

SPECIMEN 8 073

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.200	0.0430	39.4	3 - 1	0.160	0.0183	73.6
1 - 1	.140	.0297	40.0	2	.163	"	75.3
2	.173	.0183	80.0	3	.203	"	93.5
3	.191	.0219	73.4	4	.216	.0200	91.0
4	.213	"	82.1	5	.216	.0256	71.0
5	.211	.0265	67.2	6			
6	.180	.0374	40.6	7			
7	.203	.0320	53.5	8			
8	.191	"	50.2	9	.140	.0435	27.1
9	.180	.0247	61.6	10	.203	.0288	59.4
10	.158	.0187	71.6	4 - 1	.142	.0238	50.3
2 - 1	.140	.0215	54.7	2	.163	.0196	70.3
2	.163	.0173	79.7	3	.175	.0200	74.0
3	.211	.0200	89.0	4	.203	.0292	58.6
4	.229	.0238	81.0	5	.127	.0283	38.0
5	.223	.0235	80.1	6	.218	.0456	40.0
6	.211	.0475	37.5	7			
7	.155	.0388	33.7	8	.165	.0228	61.0
8	.173	.0251	58.3	9	.147	.0228	54.2
9	.175	.0238	62.2	10			
10	.147	.0260	47.7				

SPECIMEN 9 ♀66

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.185	0.0594	26.4	3 - 1	0.145	0.0256	47.9
1 - 1	.119	.0141	71.4	2	.162	.0196	70.0
2	.127	.0137	78.5	3	.188	.0187	85.0
3	.168	.0146	97.5	4	.191	.0228	70.8
4	.165	"	95.5	5	.188	.0352	45.2
5	.193	.0215	76.0	6			
6	.180	.0251	61.8	7			
7	"	.0192	79.2	8			
8	.170	.0173	83.0	9	.145	.0210	58.4
9	.140	"	68.4	10	.122	.0224	46.0
10	.119	.0201	50.0	4 - 1	.127	.0210	51.1
2 - 1	.			2	"	.0165	65.0
2	.125	.0133	79.5	3	.140	.0114	104.0
3	.183	.0137	113.0	4	.180	.0183	83.0
4	.191	.0283	57.0	5	.178	.0283	53.2
5				6	.188	.0274	58.0
6				7	.185	.0165	94.7
7				8	.160	.0155	87.2
8	.162	.0297	46.1	9	.130	.0132	83.2
9	.130	.0183	60.0	10	.117	.0142	69.5
10	.130	.0160	68.7				

SPECIMEN 10 061

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.196	0.0519	32.0	3 - 1	0.130	0.0152	72.1
1 - 1	.124	174	60.1	2	.140	122	97.0
2	."	152	69.0	3	.132	152	73.4
3	.132	203	55.0	4	.150	117	108.4
4	.158	356	37.5	5	.173	117	125.0
5				6			
6	.226	300	80.0	7			
7	.196	183	90.5	8			
8	.208	158	111.0	9	.168	254	55.9
9	.152	122	105.0	10	.122	158	65.3
10	.124	152	69.0	4 - 1	.109	259	35.5
2 - 1	.196	168	98.5	2	.127	193	55.6
2	.130	112	98.1	3	.145	119	103.0
3	.132	112	99.6	4	.173	102	143.0
4	.150	152	83.5	5	.193	142	99.0
5	.170	195	73.7	6			
6	.196	284	58.3	7			
7	.193	236	69.1	8			
8	.147	185	67.0	9	.147	163	76.1
9	.130	157	70.0	10	.114	203	47.5
10							
2a - 8	0.160	0.0211	64.0				
- 9	0.140	.188	63.0				

SPECIMEN 11 ♀19

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.127	0.0320	33.5	3 - 1	0.107	0.0183	50.0
1 - 1	.297	.0444	56.3	2	.140	.0174	68.0
2	.155	.0238	54.9	3	.175	.0133	111.0
3	.165	.0183	76.0	4	.145	.0114	107.0
4	.228	.0247	77.8	5	.175	.0178	82.6
5	.272	.0292	78.5	6			
6	.221	.0320	58.1	7			
7	.191	.0251	64.0	8	.191	.0238	67.5
8	.173	.0192	76.0	9	.122	.0174	59.0
9	.160	.0169	80.0	10	.107	.0137	66.0
10	.127	"	63.4	4 - 1	.117	.0228	43. 0
2 - 1	.117	.0178	55.2	2	.152	.0174	73.6
2	.130	.0165	66.5	3	.158	.0206	64.8
3	.137	.0160	72.1	4	.173	.0201	72.5
4	.147	."	77.4	5	.183	.0251	61.6
5	.165	.0174	80.0	6			
6	.236	.0520	38.2	7			
7	.249	.0507	41.5	8	.162	.0155	88.0
8	.223	.0525	35.7	9	.127	.0165	65.0
9	.228	.0379	50.6	10	.114	.0174	55.5
10	.114	.0178	54.0				

TABLE 21

SERIES 2. INDENTATION SURVEYS USING A HEMISPHERICALLY - ENDED CYLINDRICAL INDENTER 0.125in. IN DIAMETER WITH A LOAD OF 21bf (0.92Kg)

SPECIMEN 20. ♂33

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.377	0.077	39.6	3 - 1			
1 - 1	.172	.021	253.0	2	0.137	0.029	112.0
2	.175	.030	128.0	3	.188	.033	115.0
3	.239	.034	164.0	4	.216	.042	82.0
4				5	.249	.057	51.2
5				6			
6	.213	.049	59.5	7			
7	.334	.033	168.0	8	.160	.027	148.0
8	.203	.039	89.5	9	.109	.026	111.0
9	.155	.034	90.0	10	.076	.024	88.0
10	.117	.035	65.0	4 - 1	.114	.035	64.0
2 - 1	.127	.043	41.0	2			
2	.168	.032	112.0	3	.196	.044	68.0
3	.216	.033	128.0	4	.216	.042	82.0
4	.239	.034	129.0	5	.221	"	83.5
5	.292	.056	60.0	6			
6	.219	.042	82.0	7			
7	.183	.039	"	8	.208	.033	124.0
8	"	.043	67.8	9	.163	.028	140.0
9	.145	.026	146.0	10	.127	.025	"
10	.112	"	114.0				

SPECIMEN 21. 067

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.256	0.064	41.0	3 - 1	0.155	0.034	91.5
1 - 1	.150	.027	141.0	2			
2	.196	.032	127.0	3	.194	.027	173.0
3	.221	.036	115.0	4	.206	.031	139.5
4	.267	.038	114.0	5	.211	.060	40.0
5	.282	.058	56.5	6			
6	.224	.043	80.3	7			
7	.226	.041	86.8	8	.183	.055	41.0
8	.214	.032	136.0	9	.165	.039	74.2
9	.178	.028	114.3	10			
10	.137	.023	176.5	4 - 1	.122	.025	134.5
2 - 1	.173	.041	70.5	2	.158	.033	98.2
2	.203	.031	138.0	3			
3	.224	"	148.5	4	.210	.044	73.0
4	.234	.028	146.0	5	.200	.048	59.5
5	.257	.058	50.5	6			
6	.229	.051	59.5	7			
7	.214	.043	77.0	8	.249	.046	61.4
8				9	.193	.038	91.5
9	.173	.028	147.0	10	.153	.027	138.0
10							

SPECIMEN 22 ♀67

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.193	0.056	42.9	3 - 1	0.148	0.036	78.9
1 - 1				2	.173	33	107.5
2	.165	.037	82.0	3	.211	43	76.6
3	.200	.055	45.1	4	.226	40	92.9
4	.229	.067	34.5	5	.198	46	63.3
5	"	.068	33.9	6			
6				7			
7				8	.163	49	46.7
8	.137	.033	87.0	9	.117	40	50.0
9	.130	.031	93.5	10	.127	39	57.3
10	.140	"	99.6	4 - 1			
2 - 1	.144	.037	72.6	2	.155	30	118.0
2	.175	.042	67.8	3	.180	48	53.4
3	.244	.046	75.5	4	.190	57	40.3
4	.241	.052	59.3	5			
5	.208	.055	46.9	6			
6				7	.183	40	78.0
7				8	.142	31	102.0
8	.120	.042	46.5	9	.120	38	57.0
9	.114	.032	76.6	10			
10							

SPECIMEN 34 ♀82

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0				3 - 1			
1 - 1	0.155	0.0528	38.4	2	0.185	0.0390	82.0
2	.211	.0548	48.2	3	.254	.0482	71.5
3	.259	.0580	50.6	4	.254	.0665	38.4
4	.317	.0660	46.5	5	.254	.0810	26.2
5	.335	.0675	46.5	6			
6				7			
7	.183	.0590	36.0	8			
8	.211	.0560	46.0	9	.150	.0370	75.0
9	.193	.0405	79.0	10	.147	.0380	70.5
10	.153	.0365	78.0	4 - 1	.127	.0279	111.0
2 - 1	.135	.0269	128.0	2	.160	.0304	115.0
2	.249	.0580	49.0	3	.208	.0451	69.0
3	.279	.0525	64.0	4	.201	.0594	39.2
4	.307	.0595	56.0	5			
5	.300	.0655	45.5	6			
6				7			
7	.173	.0635	29.4	8	.244	.0584	48.2
8	.160	.0492	45.5	9	.170	.0304	123.0
9	.145	.0386	66.5	10	.140	.0410	57.5
10							

SPECIMEN 35 055

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)	Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0	0.330	0.070	43.1	3 - 1			
1 - 1	.165	.036	87.0	2	0.241	0.041	93.5
2	.221	.039	96.0	3	.229	"	101.0
3	.247	.043	86.6	4	.249	..047	73.5
4	.259	.045	82.5	5	.297	.058	57.3
5	.274	.060	50.0	6			
6				7			
7	.302	.051	73.0	8	.191	.087	17.4
8	.267	.040	72.0	9	.147	.038	70.4
9	.208	.043	75.5	10			
10	.137	.030	87.0	4 - 1	.197	.041	79.0
2 - 1	.216	.056	59.0	2	.252	.040	90.1
2	.145	.036	77.4	3	.267	.041	100 .0
3	.170	.034	100.0	4	.287	.050	73.0
4	.234	.044	79.5	5	.297	.053	65.2
5	.310	.069	41.8	6			
6				7			
7				8	.224	.045	73.0
8	.191	.041	76.6	9	.191	.043	69.6
9				10	.170	.047	53.1
10							

SPECIMEN 57 069

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
0			
1 - 1			
2	0.198	0.056	43.0
3	.254	.057	51.8
4	"	.069	35.8
5	"	.064	42.0
6			
7			
8			
9	.206	.056	44.7
10			
2 - 1	0.203	.057	42.0
2	.254	.064	42.1
3	.259	.069	42.3
4	.241	.059	46.7
5			
6			
7			
8			
9			
10			
2a - 2	0.274	.065	43.0
- 3	.229	.066	35.8
- 4	.211	.071	28.8
- 9	.175	.040	76.6
3a - 2	.163	.074	20.2

Position	Thickness (cm)	Indentation (cm)	Creep Modulus (Kg/cm^2)
3 - 1			
2	0.229	0.058	46.0
3			
4			
5			
6			
7			
8			
9			
10			
4 - 1			
2	0.170	0.078	19.1
3			
4			
5			
6			
7			
8	0.198	0.062	35.5
9			
10			

TABLE 22

THE CORRELATION BETWEEN INDENTATION AND THICKNESS FOR EACH FEMORAL HEAD.

(THE ORDER IS THE SAME AS THAT OF TABLES 20 AND 21)

GRADIENT TO INDENTATION Y AXIS	INTERCEPT WITH THICKNESS X AXIS	CORRELATION COEFFICIENT r	NUMBER OF POINTS	SIGNIFICANCE OF r EXPRESSED AS A PROBABILITY p
1.62841	0.15560	0.43569	37	$0.001<p<0.01$
2.30527	0.10475	0.40286	32	$0.01<p<0.02$
1.48968	0.13242	0.38506	31	$p\approx 0.02$
1.49086	0.12114	0.54001	31	$p\approx 0.001$
1.93654	0.14445	0.61028	36	$p<0.001$
1.87658	0.12633	0.59260	32	$p<0.001$
0.44249	0.16902	0.12958	36	$p>0.10$
1.35025	0.12881	0.44551	34	$p<0.01$
0.72980	0.13671	0.36176	33	$p\approx 0.02$
3.23773	0.09194	0.73683	37	$p<0.001$
4.05307	0.04087	0.70767	33	$p<0.001$
1.76059	0.13332	0.51709	32	$p\approx 0.001$
2.70199	0.05611	0.69939	29	$p<0.001$
3.91167	0.03022	0.71840	29	$p<0.001$
2.29575	0.12160	0.51284	30	$0.01<p<0.001$
0.43332	0.19484	0.10583	17	$p>0.10$

TABLE 23

THE CORRELATION BETWEEN CREEP MODULUS AND THICKNESS FOR EACH FEMORAL HEAD

(ORDER SAME AS TABLE 20)

GRADIENT TO CREEP MODULUS Y AXIS	INTERCEPT WITH THICKNESS X AXIS	CORRELATION COEFFICIENT r	NUMBER OF POINTS	SIGNIFICANCE OF r EXPRESSED AS A PROBABILITY p
-0.00003	0.19756	-0.02373	37	$p > 0.10$
-0.00140	0.23813	-0.34787	32	$p \approx 0.05$
0.00141	0.11712	0.35539	31	$p \approx 0.05$
0.00158	0.13982	0.32367	31	$p > 0.05$
-0.00029	0.23251	-0.09442	36	$p > 0.10$
0.00009	0.18560	0.02929	32	$p > 0.10$
0.00067	0.13915	0.42000	36	$p \approx 0.01$
0.00014	0.14697	0.10243	34	$p > 0.10$
0.00009	0.14713	0.11870	33	$p > 0.10$
-0.00020	0.18100	-0.07301	37	$p > 0.10$
-0.00023	0.21269	-0.13486	33	$p > 0.10$
-0.00044	0.23901	-0.36124	32	$p < 0.05$
-0.00048	0.20528	-0.27373	29	$p > 0.10$
-0.00105	0.28451	-0.37234	29	$p < 0.05$
-0.00066	0.27885	-0.24271	30	$p > 0.10$
0.00048	0.20278	0.17719	17	$p > 0.10$

TABLE 24

COMPARISON OF CREEP MODULUS VALUES

HEMISPHERICALLY - ENDED INDENTER, LOAD 2lbf (0.92 Kg) vs

PLANE - ENDED INDENTER, LOAD 5lbf (2.28 Kg)

CREEP MODULUS (Kg/cm^2)

SPECIMEN NUMBER	HEMISPHERICALLY-ENDED INDENTER	PLANE-ENDED INDENTER
57	48	50
	68	74
	49	52
	52	55
	53	54
	80	82
	49	47
	30	40
	40	49
	48	57
	52	52
	23	32
	21	27
60	76	69
	102	96
	166	137
	84	91
	79	73
61	85	75
	76	70
	37	35
	94	99
62	79	72
	120	110
	129	112
	79	75
56	84	76
	148	134
	180	166
	154	139

TABLE 26

COMPARISON OF CREEP MODULUS VALUES HEMISPHERICALLY - ENDED INDENTER, LOADS OF 21bf (0.92 Kg) and 11bf (o.46 Kg)

SPECIMEN NUMBER	CREEP MODULUS Kg/cm^2	
	2 lbf	1 lbf
60	76	73
	102	101
	166	147
	84	86
	79	69
56	143	130
	180	155
	154	136
54	69	68
	34	36
	73	79
	57	64
39	135	126
	123	130
	71	60
49	127	124
	129	110
	126	111
	138	145
	124	127

TABLE 27

COMPARISON BETWEEN RESULTS OF INDENTATION TESTS AND CHEMICAL ANALYSIS

TABULATED:
(1) Creep Modulus
(2) Chondroitin Sulphate Content
(3) Keratan Sulphate Content
(4) Total Mucopolysaccharide Content [(2)+(3)]
(5) Collagan Content

SPECIMEN NUMBER	CREEP MODULUS (Kg/cm^2)	CHONDROITIN SULPHATE 'A' CONTENT ($\mu g/mg$) dry weight	KERATAN SULPHATE CONTENT ($\mu g/mg$) dry weight	TOTAL MUCOPOLYSACCHARIDE CONTENT ($\mu g/mg$) dry weight	COLLAGEN CONTENT percentage of dry weight
22	93.50	60.90	30.80	91.70	46.90
"	76.70	67.00	30.90	97.90	47.60
"	107.0	55.20	50.70	105.9	58.70
"	33.30	61.50	15.20	76.70	57.50
"	50.00	69.00	14.40	83.40	56.70
"	40.50	48.70	19.00	67.70	55.40
"	46.00	54.60	14.40	69.00	55.90
"	57.00	62.40	17.10	79.60	53.40
"	76.00	57.60	33.10	90.70	41.00
"	59.00	60.20	2.80	63.00	69.90
"	116.0	81.00	29.00	110.0	51.60
"	82.00	67.00	19.90	86.90	50.70
39	54.00	69.00	17.40	86.40	59.90
"	30.10	53.50	12.40	66.00	57.20
"	42.00	56.40	14.60	71.00	56.10
"	46.00	68.50	21.50	90.00	48.30
"	55.60	73.30	19.60	93.00	57.30

TABLE 27 continued

	MODULUS	CSA	KS	TMPS	COLLAGEN
43	55.60	78.20	13.90	93.00	43.10
"	100.0	80.00	29.10	109.0	46.40
"	80.50	78.00	"	107.0	41.40
"	105.0	83.50	"	112.6	39.50
"	50.20	"	11.85	95.30	51.80
"	77.50	77.60	28.30	105.9	38.60
"	101.0	86.20	22.60	108.8	47.20
"	111.0	79.00	31.70	110.7	48.60
47	98.00	86.50	33.00	119.5	60.80
"	138.0	93.50	37.00	130.5	61.90
"	123.5	85.20	30.00	115.2	63.90
48	116.0	69.60	44.40	114.0	53.20
"	133.0	78.70	36.50	115.2	49.90
"	102.0	67.50	27.80	95.30	57.50
"	93.00	66.20	28.30	94.50	67.40
49	127.0	64.50	45.20	109.7	67.10
"	126.0	63.00	56.50	119.5	61.90
"	138.0	73.80	57.50	131.5	65.90
"	124.0	54.10	38.70	92.80	88.50
50	98.00	95.90	32.60	128.5	71.00
"	87.60	79.80	37.80	117.6	67.90
"	82.00	82.00	29.10	111.0	68.00
"	113.0	95.50	30.90	126.4	67.90
"	29.00	34.10	46.60	80.70	85.50
51	115.0	98.30	28.20	126.5	60.90
"	132.0	117.5	27.20	144.7	55.10
"	91.00	88.70	27.40	116.1	61.70
"	102.0	97.00	26.70	123.7	52.20

TABLE 28

COMPARISON BETWEEN RESULTS OF INDENTATION TESTS AND PERMEABILITY MEASUREMENTS

SPECIMEN NUMBER	CREEP MODULUS (E) (Kg/cm^2)	PERMEABILITY COEFFICIENT (K) ($cm^3/sec/g$) x 10^{-13}
24	54.0 128.0	4.45 1.71
31	130.0 90.5 170.0	1.92 2.36 1.40
34	70.0 177.0	1.58 1.20
41	135.0 123.5 71.0	1.08 1.21 1.71
42	59.0 117.0 76.7 93.4 103.0	2.87 1.87 2.45 " 2.22
44	75.5 89.5	2.32 "
46	66.0 70.4 32.6 82.5 53.1	3.00 3.30 4.16 2.35 3.04
53	96.5 51.2 89.5 70.5 86.0	2.46 2.92 2.68 3.20 1.75
54	97.5 42.0 58.2 94.6 88.4	1.75 3.00 2.49 1.82 2.10

APPENDIX 2.

COMPUTER PROGRAMME FOR FITTING THE "BEST" LINE TO THE EXPERIMENTAL RESULTS OF TWO VARIABLES, AND ESTIMATING THE CORRELATION COEFFICIENT.

```
      DIMENSION X(1000),Y(1000)
      READ(5,1) N,M
      READ(5,2)(X(I),Y(I),I=1,N)
      SUMX=0
      SUMY=0
      SUMXY=0
      SUMSQX=0
      SUMSQY=0
      DO 3 I=1,N
      SUMX=SUMX+X(I)
      SUMY=SUMY+Y(I)
      SUMXY=SUMXY+X(I)*Y(I)
      SUMSQX=SUMSQX+X(I)*X(I)
      SUMSQY=SUMSQY+Y(I)*Y(I)
      EN=N
      DENOM=SUMX*SUMX-EN*SUMSQX
      TOPE=SUMX*SUMY-EN*SUMXY
      TOPF=SUMXY*SUMX-SUMY*SUMSQX
      E=TOPE/DENOM
      F=TOPF/DENOM
      TOPG=EN*SUMXY-SUMX*SUMY
      XDEV=EN*SUMSQX-SUMX**2
      YDEV=EN*SUMSQY-SUMY**2
      R=TOPG/SQRT(XDEV*YDEV)
      WRITE(6,4) E,F,R
      IF(M.EQ.1) GO TO 5
    1 FORMAT(2I5)
    2 FORMAT(10F7.5)
    4 FORMAT(1H0,3E20.5)
    5 STOP
      END
```

APPENDIX 3

DERIVATION OF ELASTIC MODULUS FOR THIN RUBBER SHEETS [WATERS (1965)]

Timoshenko and Goodier (1951) showed, that the indentation and radius of the contact area arising from the compression of two elastic spheres, are given by the equations:

$$d = \left[\frac{9\,\pi^2\,P^2\,(k_1 + k_2)^2\,(R_1 + R_2)}{16\,R_1 R_2} \right]^{1/3} \qquad (1)$$

and

$$a = \left[\frac{3\pi P\,(k_1 + k_2)\,R_1\,R_2}{4\,(R_1 + R_2)} \right]^{1/3} \qquad (2)$$

where $K_{1,2} = (1 - \nu_{1,2}^2)/\pi E_{1,2}$

ν = Poissons Ratio

E = Young's modulus.

(1) Theory applied to the case of a cylindrical hemispherically-ended indenter

Considering the case of a rigid, hemispherically-ended, cylindrical indenter, pressed into a semi-infinite elastic medium of which $\nu = 0.5$, then making the following assumptions about the following quantities which appear in equations (1) and (2) that:

$$E_1 = \infty \quad \text{and} \quad E_2 = E$$

and $$\frac{1}{R_1} \gg \frac{1}{R_2} \quad \therefore \quad \frac{1}{R_1} + \frac{1}{R_2} \simeq \frac{1}{R_1} = \frac{1}{R}$$

then equations (1) and (2) may be re-written in the form:

$$d = \frac{K_1 P^{2/3}}{E^{2/3} R^{1/3}} \qquad (3)$$

and

$$a = \frac{K_2 R^{\frac{1}{2}} d^{\frac{1}{2}}}{K_1^{\frac{1}{2}}} \qquad (4)$$

where $K = (81/256)^{1/3} = (K_2)^2$

Timoshenko and Goodier (1951) stated, that the stresses in a semi-infinite medium may be defined in terms of magnitude and position by 'q_o' , the maximum pressure at the centre of the surface of contact, and by 'a', the radius of the area of contact, respectively. Thus, for a sheet of finite thickness, although the stress distribution is distorted by the boundaries, it would be expected that the distribution would be geometrically similar for any given value of the ratio of the thickness 't', to the radius of the area of contact, 'a'.

Waters proposed that equation (3) may be written in the form:

$$d_t = \frac{K_1 P^{2/3}}{E^{2/3} R^{1/3}} f(t/a_t) \qquad (5)$$

where the 't' refers to a sheet of material of thickness 't', and where $f(t/a_t)$ is a dimensionless function.

Hence $$\frac{d_t}{d_\infty} = f(t/a_t) \qquad (6)$$

and $$a = \frac{K_2 R^{1/3} P^{1/3}}{E^{1/3}} \qquad (7)$$

where the suffix '∞' refers to a semi-infinite sheet of the material. Assuming that a_t can be derived from equation (4) by substitution for d_t, then

$$a_t = \frac{K_2 R^{\frac{1}{2}} d_t^{\frac{1}{2}}}{K_1^{\frac{1}{2}}} = R^{\frac{1}{2}} d_t^{\frac{1}{2}} \qquad (8)$$

If therefore $f(t/a_t) = \frac{d_t E^{2/3} R^{1/3}}{K_1 P^{2/3}}$ is plotted against

$$(\frac{t}{a_t}) = (\frac{t}{R d_t})^{\frac{1}{2}}$$

then the resulting curve should represent a universal function, valid for a wide range of indenter radius, load and Young's modulus. From this function, E can be deduced from a knowledge of d_t, t and the known experimental factors R and P.

Waters plotted experimental values of the two dimensionless groups shown above, for a wide range of indenter diameter, sheet thickness and Young's modulus. He obtained a single curve (see Fig. 4la, facing p. 60), which was fitted by the equation:

$$f\left({}^{t}/_{a_t}\right) = 1 - \exp\left(-A\ {}^{t}/_{a_t}\right) \qquad (9)$$

The constant A had two values; 0.67 and 0.42 dependent on whether the lower boundary was lubricated, or not. In the case when it was not lubricated, Waters stated[1] that adhesion effectively occurred between the rubber and this lower boundary. Thus the situation is almost analagous to the continuous cartilage–bone interface, which is relevant to the present investigation.

Substituting $f\left({}^{t}/_{a_t}\right)$ from equation (9) into equation (5), and rearranging the latter gives:

$$E = \frac{(K_1)^{3/2} P}{(R)^{\frac{1}{2}}} \left[\frac{1 - \exp\left(-0.42\ {}^{t}/_{a_t}\right)}{d_t}\right]^{3/2} \qquad (10)$$

Equation (10) was used with the results from the second series of indentation tests, which employed the hemispherically-ended indenter, to calculate values of E for all of the indented areas of cartilage.

Data $(K_1)^{3/2} = 9/16$

$P = 2$ lbf (0.92 Kg)

$R = 1/16$ in. (0.159 cm).

which give:

$$E = 1.28 \left[\frac{1 - \exp\left(-0.42\ {}^{t}/_{a_t}\right)}{d_t}\right]^{3/2} \qquad (11)$$

---

1. Personal communication with Mr. Waters.

The value of E calculated for cartilage in this manner is not strictly the Young's modulus, because the effective elastic behaviour of cartilage is restricted to a limited period. It seemed more appropriate to call E, for cartilage, the creep modulus at two seconds after load application.

(2) Theory applied to the case of a cylindrical, plane-ended indenter

Timoshenko and Goodier (1951) showed that the depth of indentation produced by a rigid, cylindrical plane-ended indenter is given by the equation:

$$d = \frac{P\ (1 - \nu)^2)}{2\ RE} \qquad (12)$$

Employing the same assumptions as those used in Para. (1) of this Chapter, equation (12) above may be re-written in the form:

$$d = \frac{3P}{8RE} \qquad (13)$$

Waters $(1965)_2$ proposed that equation (13) could be written, for sheets of finite thickness as:

$$d_t = \frac{3P}{8RE}\,\emptyset \qquad (14)$$

where $\emptyset$ is a dimensionless function which takes into account the finite and variable thickness of the material. It was then pointed out, that Gent (1957) had shown, that the compression characteristics of flat rubber pads between flat anvils, could be expressed by the rates between the area of the stress-free surfaces and the area of one loaded surface. This suggested that $\emptyset = \emptyset\ (^t/R)$, and Waters consequently plotted values of:

$$\emptyset(^t/R) = \frac{8\ d_t\ R\ E}{3P}$$

against $^t/R$ for a wide range of values of E, R and P as before. The results were plotted in the form of two curves, relating to a lubricated and non-lubricated lower boundary respectively. Fig. 41b (facing p. 60), shows the curve obtained by Waters for a non-lubricated boundary. It is apparent that the

graph is essentially linear between the values $0 \leqslant {}^{t}/R \leqslant 1.8$, with a gradient of 0.25.

Equation (14) may be re-arranged into the following form:

$$E = \frac{3P}{8R} \left(\frac{\emptyset}{d_t}\right) \qquad (15)$$

and this equation was used with the results of the first series of indentation surveys, to calculate values of E, the creep modulus, for all of the indented areas of cartilage.

Data P = 5 lbf (2.28 Kg)

R = 1/16 in. (0.159 cm).

which give:

$$E = 5.37 \left(\frac{\emptyset}{d_t}\right) \qquad (16)$$

The values of $\emptyset$ were determined from the graph shown in Fig. 41b.

APPENDIX 4

PARTIAL CORRELATION COEFFICIENTS

The computer programme given in Appendix 2 enabled the total correlation coefficients between two variables to be calculated. However when a situation is encountered in which there are three or more variables, the correlation between any two of them is best expressed by a partial correlation coefficient, which is calculated whilst the other variablees are effectively held constant.

The following expressions were used in calculating the partial correlation coefficients given in Figs. 37 to 40 inclusive.

(1) Three variables (1st Order)

$$r_{12.3} = \frac{r_{12} - r_{13} \times r_{23}}{(1-r_{13}^2)^{\frac{1}{2}}(1-r_{23}^2)^{\frac{1}{2}}} \qquad (A)$$

where r_{ij} is the total correlation coefficient between variables i and j and where $r_{ij.K}$ is the partial correlation coefficient between the variables i and j with K held constant.

(2) Four variables (2nd Order)

$$r_{12.34} = \frac{r_{12.3} - r_{14.3} \times r_{24.3}}{(1-r_{14.3}^2)^{\frac{1}{2}}(1-r_{24.3}^2)} \qquad (b)$$

where the 1st order partial correlation coefficients on the R.H.S. of equation (B) are calculated from equation (A) above, and where $r_{ij.Km}$ is the partial correlation coefficient between the variables i and j with K and m held constant.

(3) Application to results

Figs. 37 to 40 inclusive show graphs of the creep modulus of cartilage at two seconds, plotted against the following quantities:

1. Total mucopolysaccharide content.
2. Chondroitin sulphate content.

3. Keratan sulphate content.

4. Collagen content.

The partial correlation coefficients between creep modulus and (2) and (3) (see above), were calculated holding (3) and (4) constant, and (2) and (4) constant, respectively.

The particl correlation coefficients between creep modulus and (1) and (4) were calculated holding each of these two variables constant, in turn.

The total correlation coefficients between each two variables were determined from the computer programme shown in Appendix 2; these are tabulated below.

Variables	Total Correlation Coefficient
MOD : CSA	0.618
MOD : KS	0.607
MOD : COL	0.071
CSA : KS	-0.035
CSA : COL	-0.182
KS : COL	-0.097
COL : KS	0.299
KS : CSA	0.012
COL : CSA	0.183
TM : COL	0.024
COL : TM	0.018

Key to abbreviations used in the table:

MOD - Creep Modulus

CSA - Chondroitin Sulphate A

KS - Keratan sulphate

COL - Collagen content

TM - Total muclpolysaccharide.

(*Reprinted from Nature, Vol. 220, No. 5172, pp. 1127–1128, December 14, 1968*)

Tensile Properties of Articular Cartilage

ARTICULAR cartilage consists principally of collagen fibres embedded in a mucopolysaccharide ground substance. Electron microscopy studies[1] show that the collagen fibres near the articular surface are predominantly parallel to the surface; viewed perpendicularly to the surface, they also show a dominant orientation which varies systematically over the whole joint surface. In contrast, the fibres in the deeper zones have a more random distribution with a tendency to be perpendicular to the surface. The fibre diameter and distance between adjacent fibres appear to increase with depth from the articular surface. Chemical and physico-chemical studies (unpublished results of H. Muir and A. Maroudas) confirm that the collagen density decreases with depth from the articular surface.

The mechanical function of collagen in connective tissues seems to be to resist tensile loads. Any change in the orientation or fibre density should therefore be reflected in the tensile characteristics of the tissue. This investigation was carried out to measure the tensile properties of articular cartilage in planes parallel to the articular surface. Tests were performed on specimens taken from four different levels through the cartilage thickness and at two angles, namely 0° and 90°, to the dominant direction of the surface collagen.

Having exposed the femoral condyles of a number of knees, the articular surface was pricked with a pin and the pricks stained. A reproducible pattern of fissures was observed which was thought to illustrate the collagen orientation at, and close to, the articular surface[2]. This pattern was used as a reference for cutting specimens from five other knees which were not pricked. These five knees were taken at autopsy from human cadavers within the age range 50 to 67 and stored at −20° C. The presumed orientation was confirmed by pricking and staining each surface layer after testing. The articular cartilage was dissected from the subchondral bone and full thickness rectangular specimens which were visually normal, with a presumed collagen orientation, were cut from the sheet. These were then sliced into four layers of equal thickness, including the articular surface, using a Leitz Thermal Module freezing microtome. Each layer was then cut into a dumbbell shaped tension specimen using a specially prepared die. Each specimen was equilibrated for at least 15 min in buffered Ringer

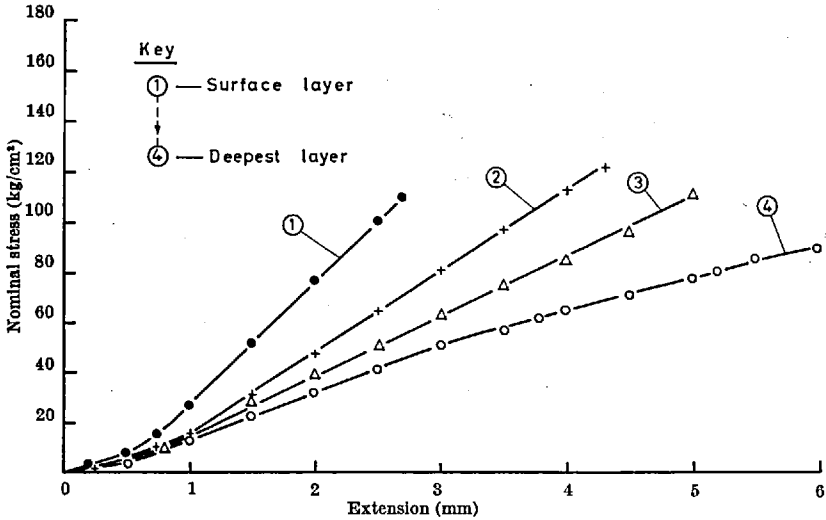


Fig. 1. Curves of nominal stress (load÷original cross-sectional area) versus extension for all four layers of a specimen taken from a 60 year old male. The prick lines were parallel to the load axis.

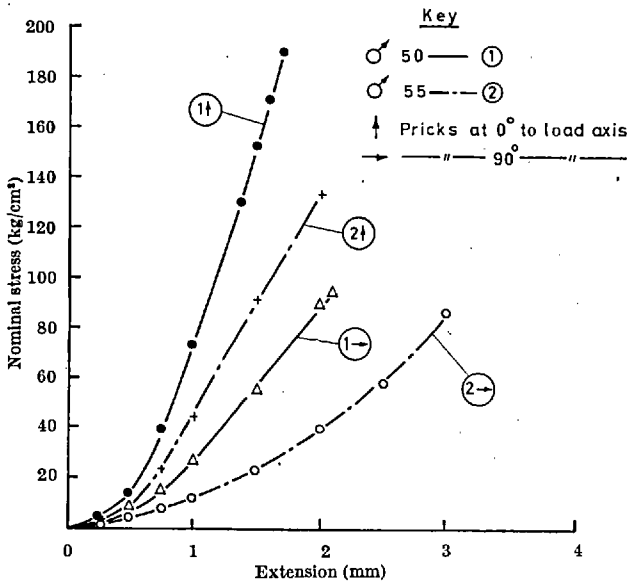


Fig. 2. Curves for the surface layers of two specimens taken from each of two different femoral condyles. They are cut at 0° and 90° to the prick lines.

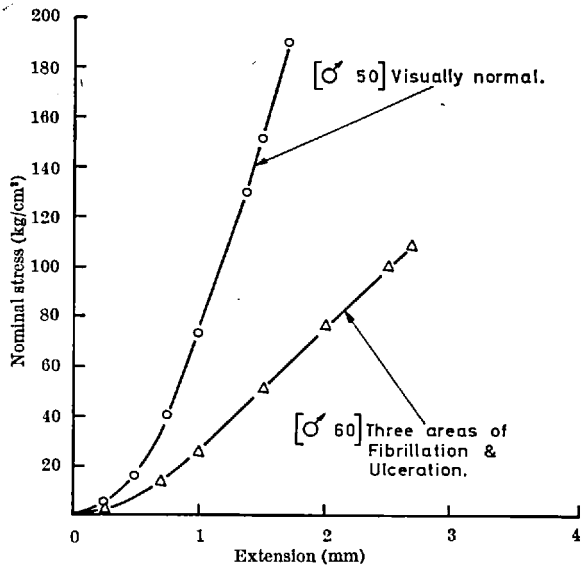


Fig. 3. Stress versus extension for the surface layers of two specimens.

solution at 37° C. Its width and thickness were then measured and finally the specimen was subjected to a tensile test in a Tensometer type E materials testing machine using a constant cross head speed of 4·3 mm/min. A continuous graph of the load versus extension was recorded, which was replotted as nominal stress versus extension to compensate for variations in specimen cross-sectional area. The results obtained are shown in Figs. 1–3.

The slope of each curve in Fig. 1 represents the stiffness of the relevant specimen, and it is clear that the surface layer was the stiffest and the deepest layer the least stiff. Similar behaviour was exhibited by specimens in which the load axis was across the prick lines, the only difference being that the stiffness of all layers was reduced, the surface layer somewhat more than the others.

Fig. 2 shows that the stiffness of a specimen loaded parallel to the prick lines was two or three times greater than that of a specimen from the same condyle loaded at 90° to the prick lines.

The stress at fracture (Table 1) for specimens loaded parallel to the prick lines was appreciably greater than for specimens loaded across the prick lines.

Referring to Fig. 3, one specimen was taken from a visually normal joint (male 55) whereas the other was taken from a joint displaying areas of fibrillation and ulceration (male 60) although the tensile specimens

Table 1. ULTIMATE TENSILE STRESS FOR TWO ORIENTATIONS

Specimen	Parallel	Across
(1) Female 67	160	47
(2) Female 51	160	68
(3) Male 60	111	74
(4) Male 55	133	81
(5) Male 50	190	94

themselves were taken from areas showing no fibrillation. Both specimens were loaded parallel to the prick lines, and the curves show how much stiffer was the specimen from the normal joint than that from the visually degenerate joint.

These results show how the stiffness and strength of normal articular cartilage depend on three factors: (1) the surface collagen orientation; (2) the depth from the articular surface; (3) the amount of cartilage degeneration in areas of the joint surface other than those tested. The results also confirm the structure of articular cartilage as observed by other workers.

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[1] Maroudas, A., and Bullough, P., *Nature*, **219**, 1260 (1968); Weiss, C., Rosenberg, L., and Helfet, A. J., *J. Bone Joint Surg.*, **50A** (4), 663 (1968).
[2] Benninghof, A., *Z. Zellforsch.*, 2, 783 (1925).

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RESULTS OF CURRENT TENSION TESTS TO SUPPORT THE ASSUMPTION OF ISOTROPY IN THE DEEPER ZONES OF ARTICULAR CARTILAGE

Tension tests were performed, parallel to the articular surface, on waisted specimens 200μ thick, $\frac{1}{16}$ inch wide with a parallel-sided gauge length of $\frac{1}{4}$ inch. The specimens were taken from both condyles of human cadaveric knees, and at 200μ intervals through the full thickness of cartilage. From each condyle two sets of specimens were taken:- one set was cut parallel to the cleavage pattern displayed on the articular surface, and the other set was cut perpendicular to the cleavage lines. The rate of extension, on the Tensometer E-Type materials testing machine, was set at 5.0 m.m. per minute. Nominal stress versus extension ratio curves were plotted.

The tensile stiffness modulus for each slice was measured from the slope of the stress vs. extension ratio curve, over the approximately linear range. The ratio of these values, between specimens cut parallel to the cleavage pattern and those cut perpendicularly to the pattern, was evaluated for corresponding slices on each condyle separately.

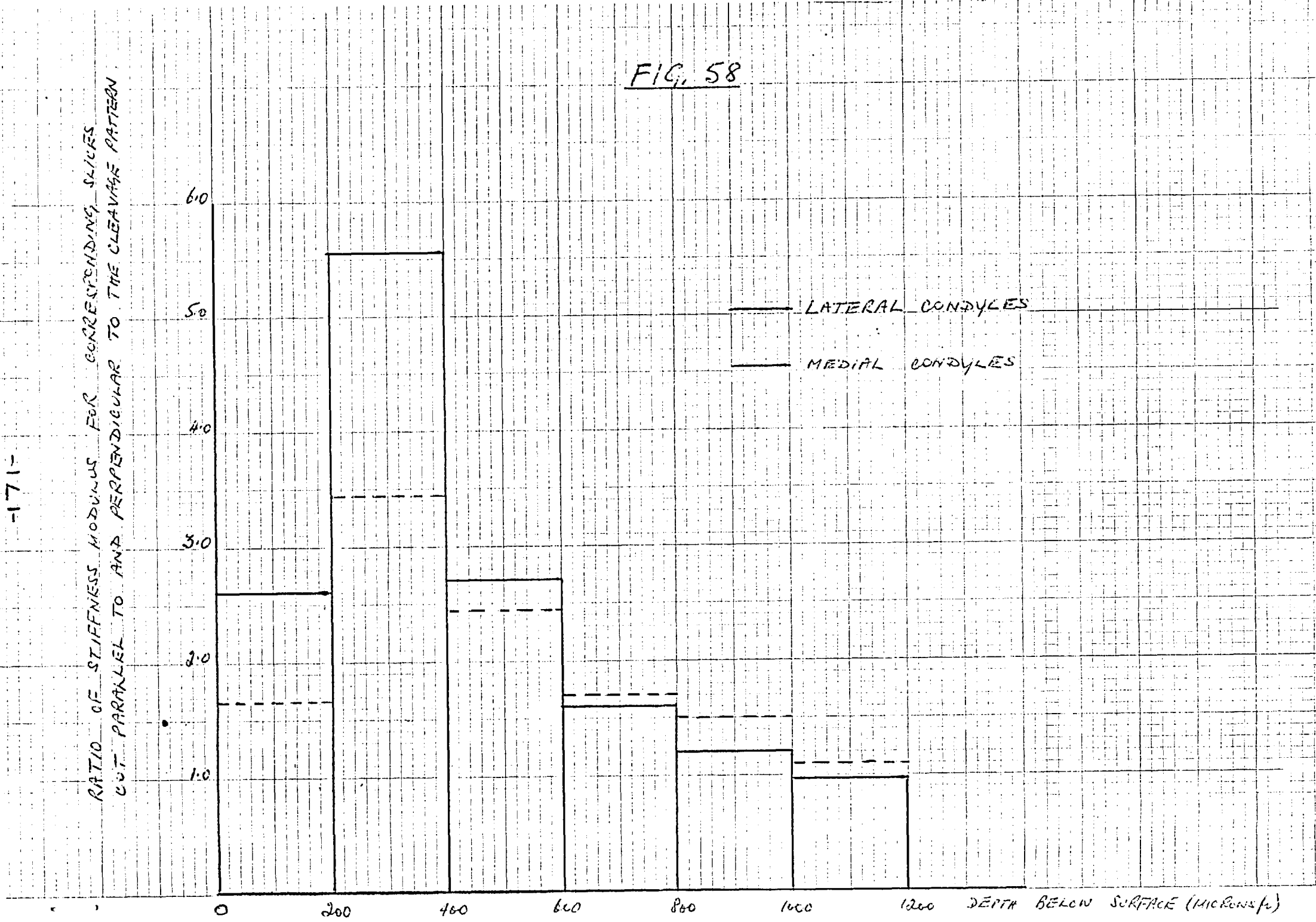
Table 30 shows the arithmetic mean of these ratio values for 20 sets of specimens, (approximately 6 slices per set), taken from 5 knees between the ages of 18 and 77 years.

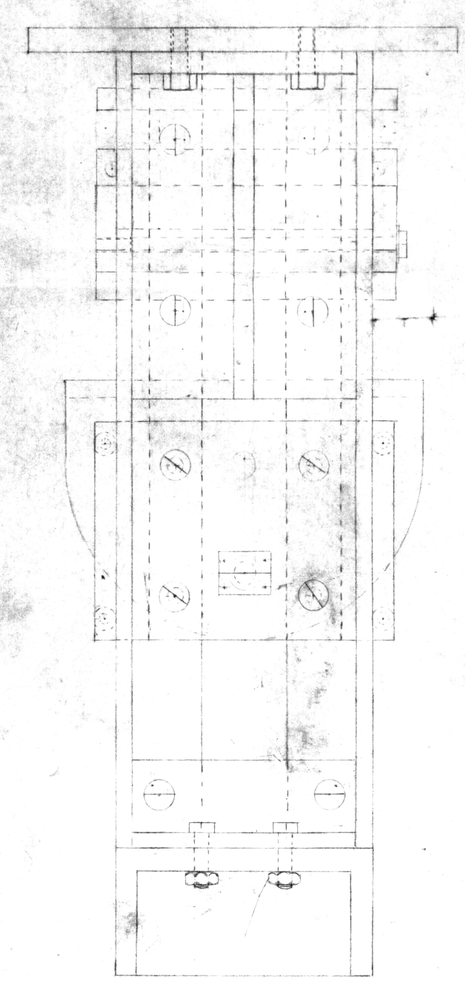
TABLE 30

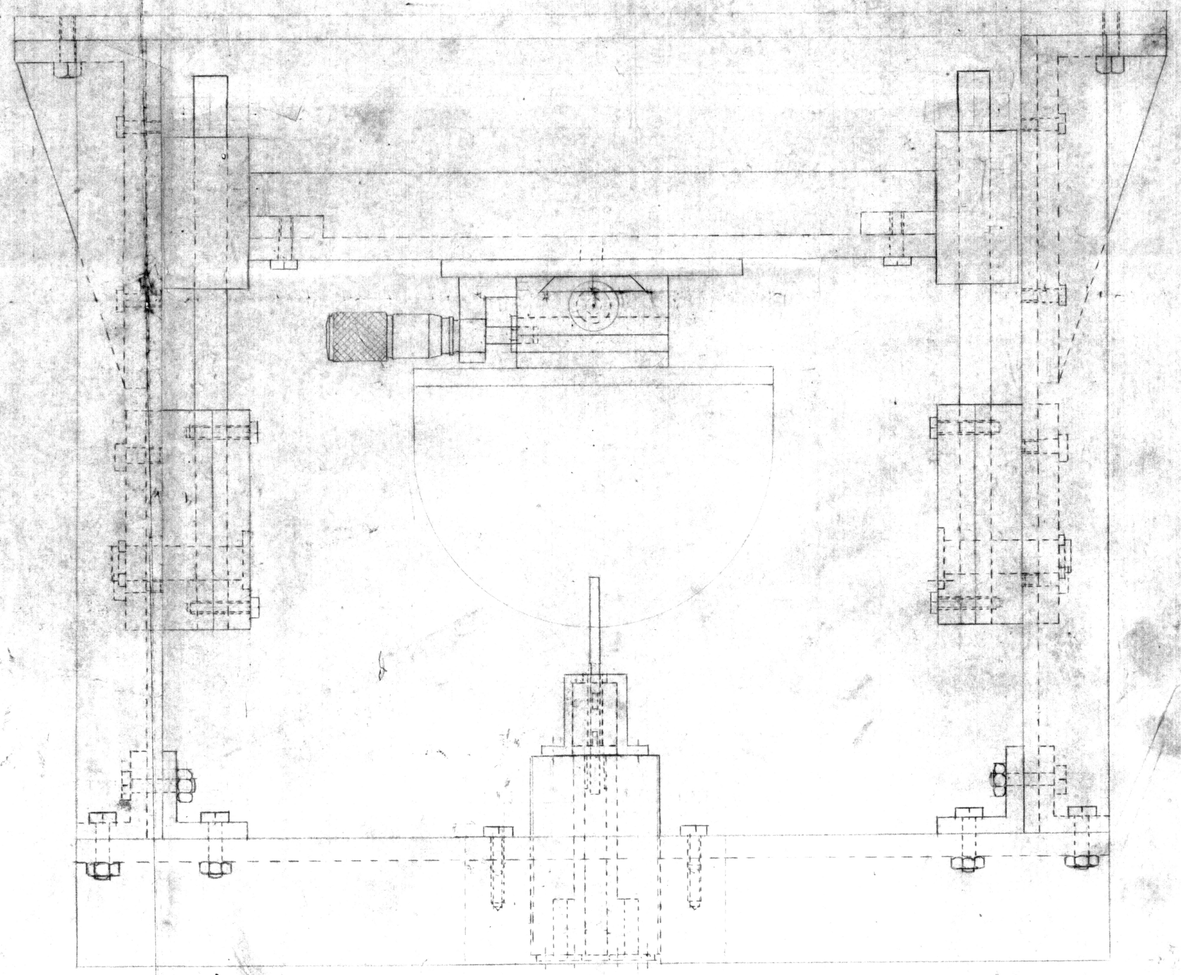
ARITHMETIC MEAN VALUES OF RATIO BETWEEN STIFFNESS MODULUS FOR SPECIMENS PARALLEL TO CLEAVAGE PATTERN AND THOSE PERPENDICULAR TO THE PATTERN.

	RATIO OF MODULI, PARALLEL : PERPENDICULAR	
SLICE NUMBER	LATERAL CONDYLE	MEDIAL CONDYLE
1	2.60	1.67
2	5.55	3.46
3	2.70	2.48
4	1.61	1.70
5	1.22	1.50
6	0.97	1.11

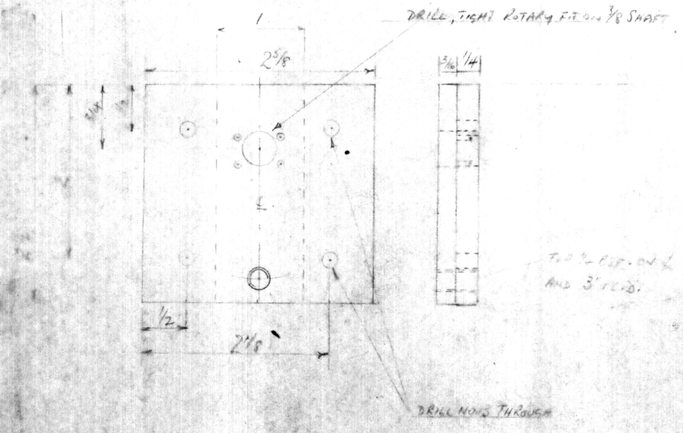
FIG. 58


RATIO OF STIFFNESS MODULUS FOR CORRESPONDING SLICES CUT PARALLEL TO AND PERPENDICULAR TO THE CLEAVAGE PATTERN
6.0
5.0
4.0
3.0
2.0
1.0
LATERAL CONDYLES
MEDIAL CONDYLES
0
200
400
600
800
1000
1200
DEPTH BELOW SURFACE (MICRONS/μ)

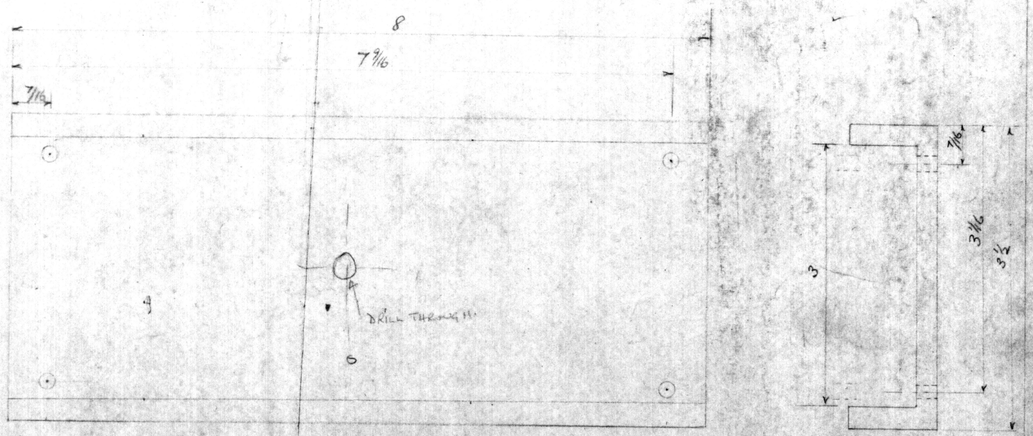




7. UPPER SIDE PLATE. 2OFF.

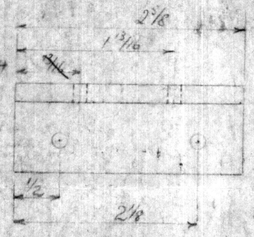


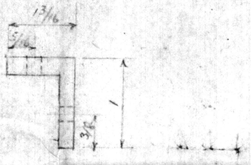
9. SWINGING BASE 1 OFF



ALL HOLES NO 14 DRILL THROUGH

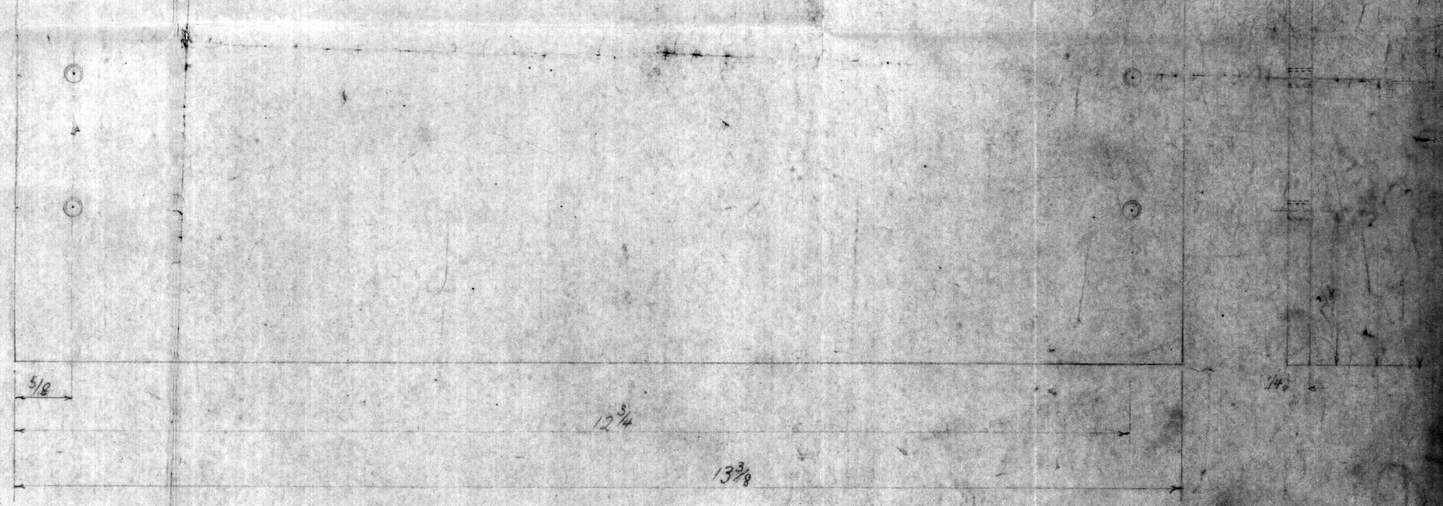
11. ANGLE BRACKET 2OFF (UNDER HAS TOP FACE FULL 1", 2BA CLEAR HOLE THROUGH CENTRE LINE 3/8 FROM END. AFTER 2 OF 5/16 BELOW.



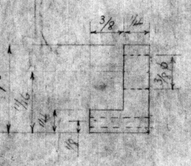


ALL HOLES NO 12 DRILL THROUGH

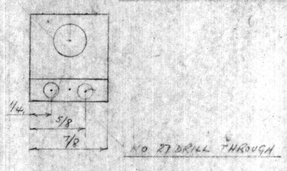
10. BASE PLATE 1 OFF.



13. MICROMETER BRACKET (1) 1 OFF.

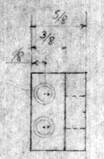


DRILL & REAM FOR INTERFERENCE PRESS FIT WITH MICROMETER COLLAR 3/8 DIA (NOM)



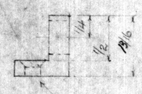
NO 27 DRILL THROUGH

12. MICROMETER BRACKET (2) 1 OFF.



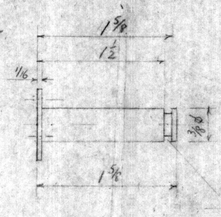


DRILL & REAM FOR INTERFERENCE PRESS FIT ON MIC. COLLAR 7/16 DIA (NOM)

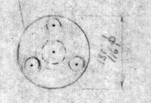


DRILL & C'BORE NO 27 DRILL

18 TRUNNION SHAFT 2OFF.

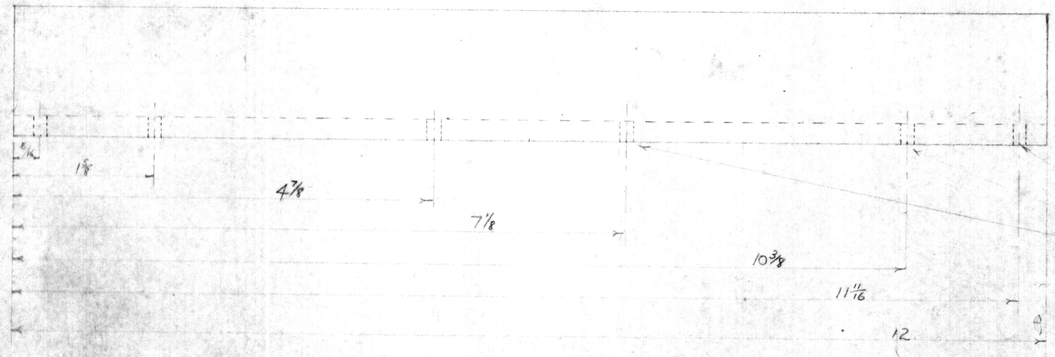


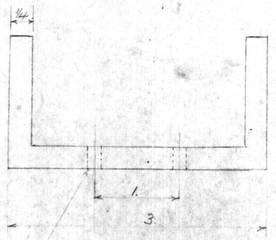
NO 27 DRILL THROUGH



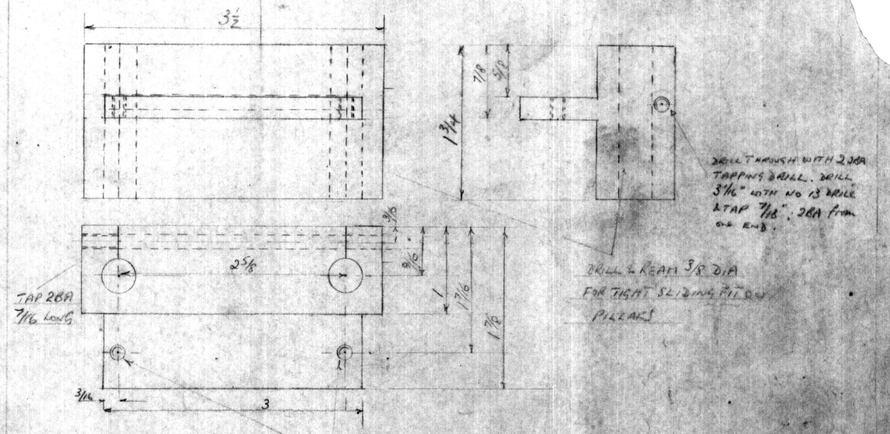
EQUI-SPACED ON 3/8 PCD

1 TOP CROSS BEAM. 1 OFF

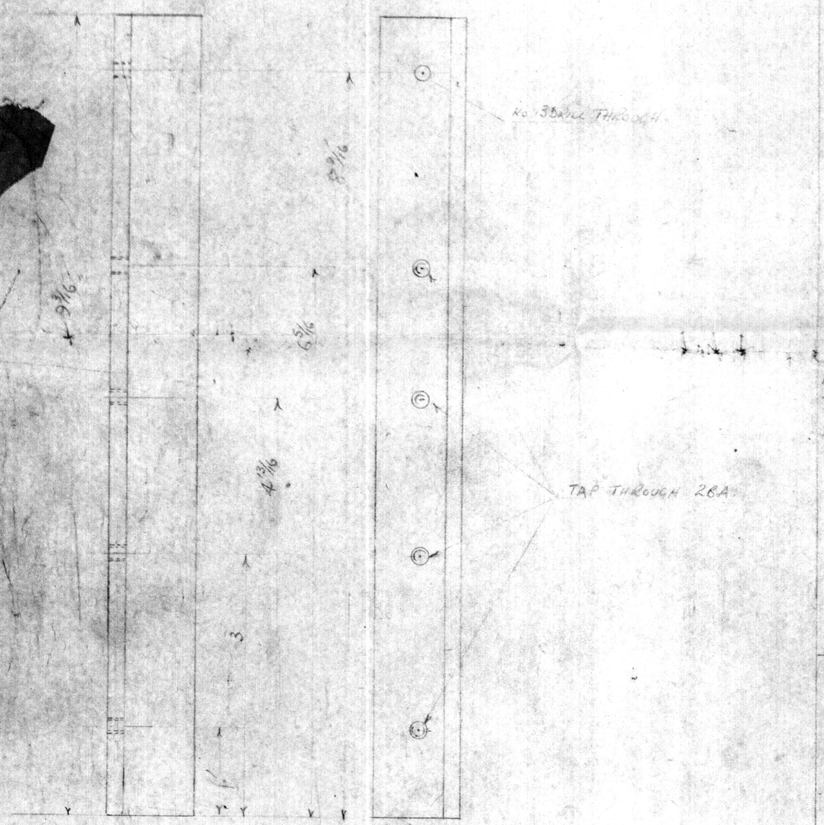




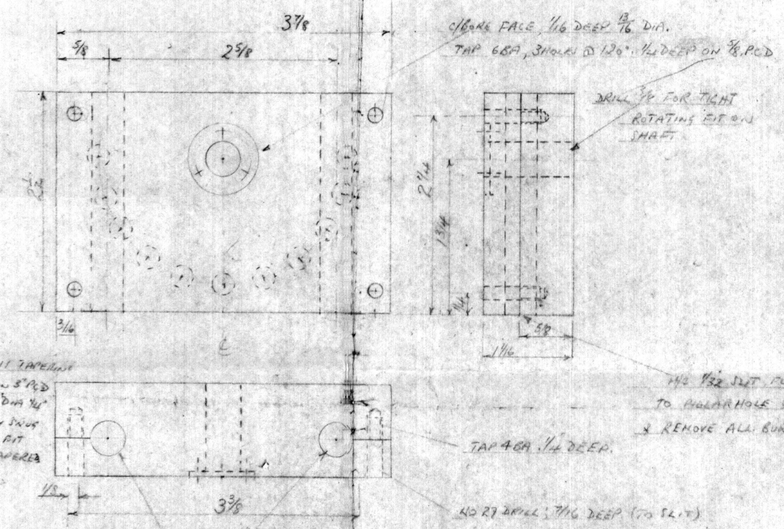
4. SWINGING GATE SUPPORT 2 OFF

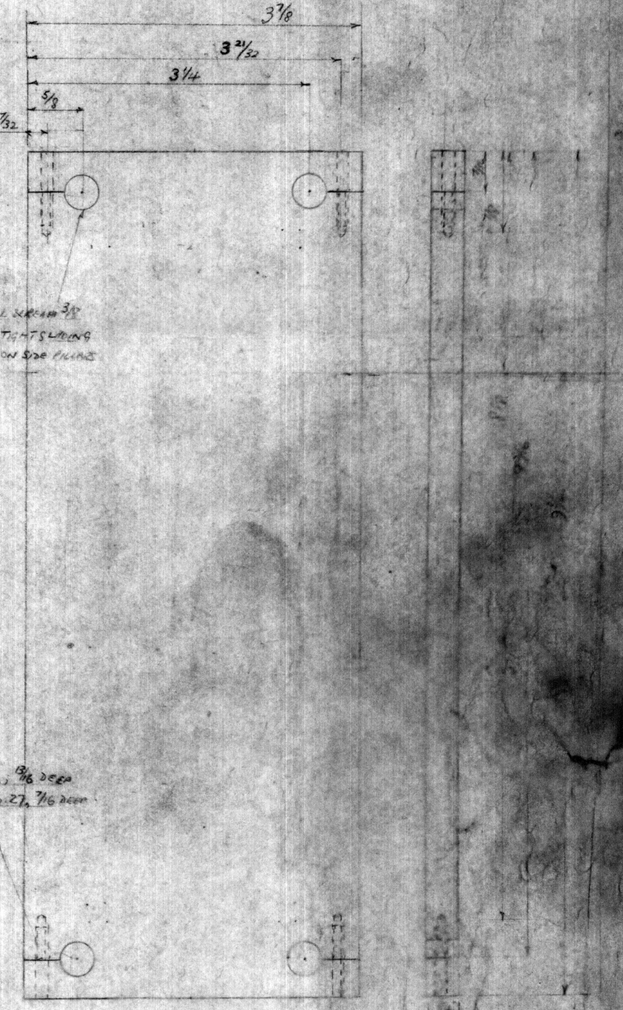


2. SIDE PILLARS 4 OFF

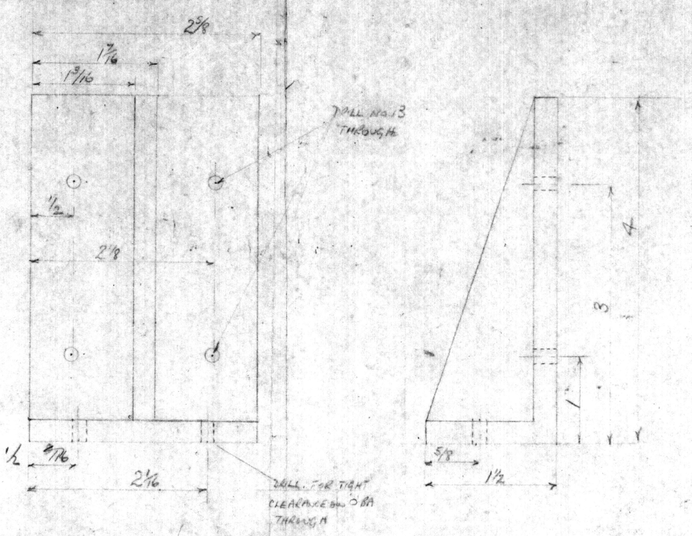


3. TRUNNION PLATE. 2 OFF.





6. LOWER SIDE BRACE 2 OFF



5 BOTTOM BRACE PLATE 1 OFF.