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THE EFFECTS OF REHYDRATION TIME AND ANATOMICAL SOURCE ON THE MECHANICAL RESPONSE OF CANCELLOUS BONE ALLOGRAFTS USED IN CERVICAL ARTHRODESIS

By

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ABSTRACT

THE EFFECTS OF REHYDRATION TIME AND ANATOMICAL SOURCE ON THE MECHANICAL RESPONSE OF CANCELLOUS BONE ALLOGRAFTS USED IN CERVICAL ARTHRODESIS

BY

Kathleen Marie Cowling

The purpose of this research was to investigate the mechanical response of cancellous bone allografts. These bone dowels, used in the Cloward technique of cervical arthrodesis, were tested fresh and rehydrated for various time intervals after lyophilization to determine which time of rehydration restored the grafts mechanical properties to those of the fresh bone. Comparisons of anatomical source sites were done to discover if there are differences between the sites where the allogenic bone was taken from the donor.

Results from these tests indicate that differences were found between the donor's right/left leg, medial/lateral condyle, and flexion contact points. These differences were in the mechanical response after rehydration for longer than two hours. Within each donor, transverse isotropy was seen at dominant sites and was sensitive to the rehydration effects.

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I. INTRODUCTION

Arthrodesis (artificial fusion) of the cervical spine is used to treat both traumatic and nontraumatic conditions of the spine, including idiopathic cervical spondylosis, vertebral fracture, and flexion-extension cervical spine injuries. The objective of the procedure is to remove all degrees of freedom from the joint so bone ingrowth can permanently fuse the vertebrae together. The most common reasons stabilization via fusion is desired are: osteoarthritis with nerve root irritation, disc degeneration, and unstable fractures. [54] Various anterior approach techniques have been developed by three separate groups in the late 1950's; Smith and Robinson 1958 [55], Cloward 1958 [22], Dereymaker 1965 [23]. The anterior approach made it possible to not only provide stabilization and decompression by fusion of the vertebral bodies but also to obtain access for removal of symptomatic pathological lesions previously inoperable. [22] The difference between each of these techniques is basically whether the operation is limited to simple discectomy and interbody fusion or if entrance into the spinal cord to remove osteophytes is desired, [46] Figure 1.

The Cloward technique, which uses a cylindrical bone dowel, permits direct access to the spinal cord before the graft is put in place. Unfortunately, this creates the possibility for iatrogenic spinal cord injury due to improper use of the instruments or posterior displacement of the graft either during surgery or postoperatively. However, this technique also permits the removal of lesions and complete decompression

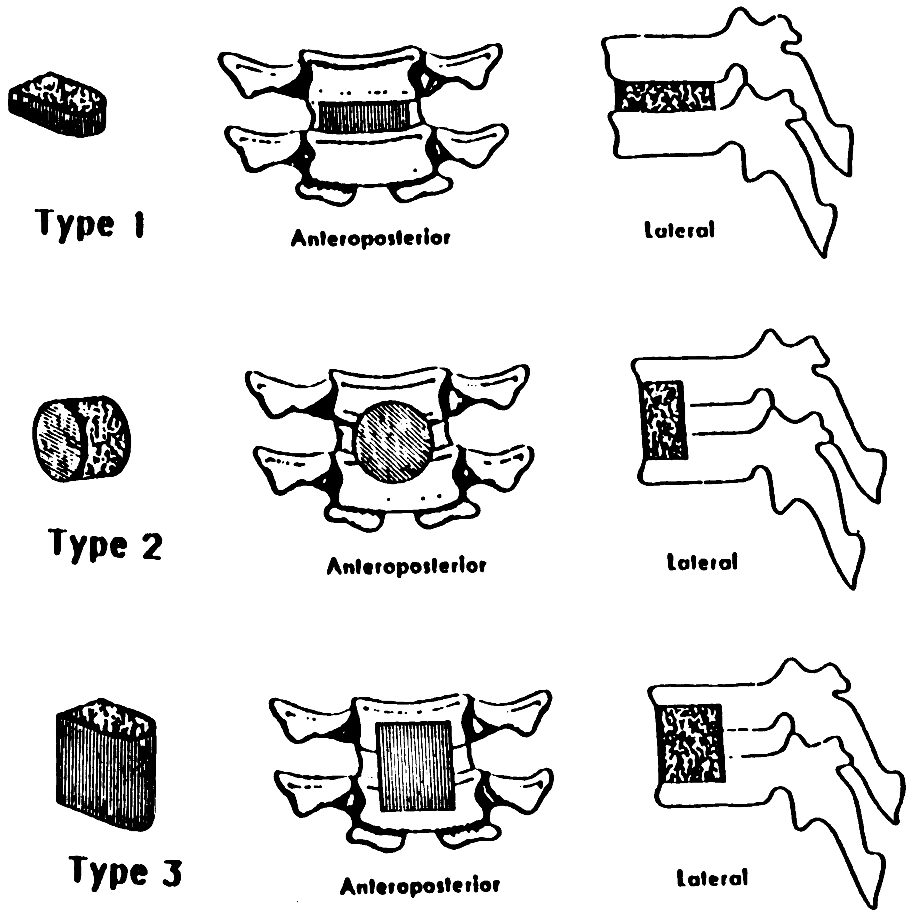

FIGURE 1. Different techniques developed for cervical arthrodesis. Type 1, Robinson and Smith, Type 2, Cloward, Type 3, Bailey and Badgely. White [62]

of the nerves, occasionally a necessity. Rectangular grafts have a firmer fixation than dowel grafts, but do not always immobilize the joint from rotation.

The operation is performed with the patient lying supine with the cervical spine extended. The surgeon enters anteriorly, Figure 2 A, and obtains direct access to the fusion site using retractors, Figure 2 B. The vertebrae involved are separated using spreaders, and a hole is made between the superior and inferior vertebra using a drill, Figure 2 C. All fragments are removed and the disc is cleared away. The graft is seated in place and the spreaders removed, Figure 2 D. The soft tissue retracters are removed with subsequent closing. Following implantation, the graft must bear the weight of the head and superior vertebrae as well as muscular loads.

The graft must sustain its structural function for the duration of the bone induction . Schneider recorded 85% of patients having complete fusion within four months. [54] During that time the graft's primary purpose is to maintain the disc space so nerve impingement does not occur, and until union is complete, to serve as an inorganic supportive matrix for new bone growth. White and Hirsch found that autogenic grafts maintain integrity under loads up to 2 1/2 times body weight. Mertz [44] found that injury could occur under compressive neck loads of 4000 N for 0 msec and only 1110 N applied for 30 msec, Figure 3. However, serious neck injuries can occur from several loading conditions including bending or torsional moments and tension forces. 1110 N was considered to be the minimum load the transplanted grafts should withstand so as not to be a weak link while the joint is still unfused.

Autogenic bone is considered to be ideal for grafts because no

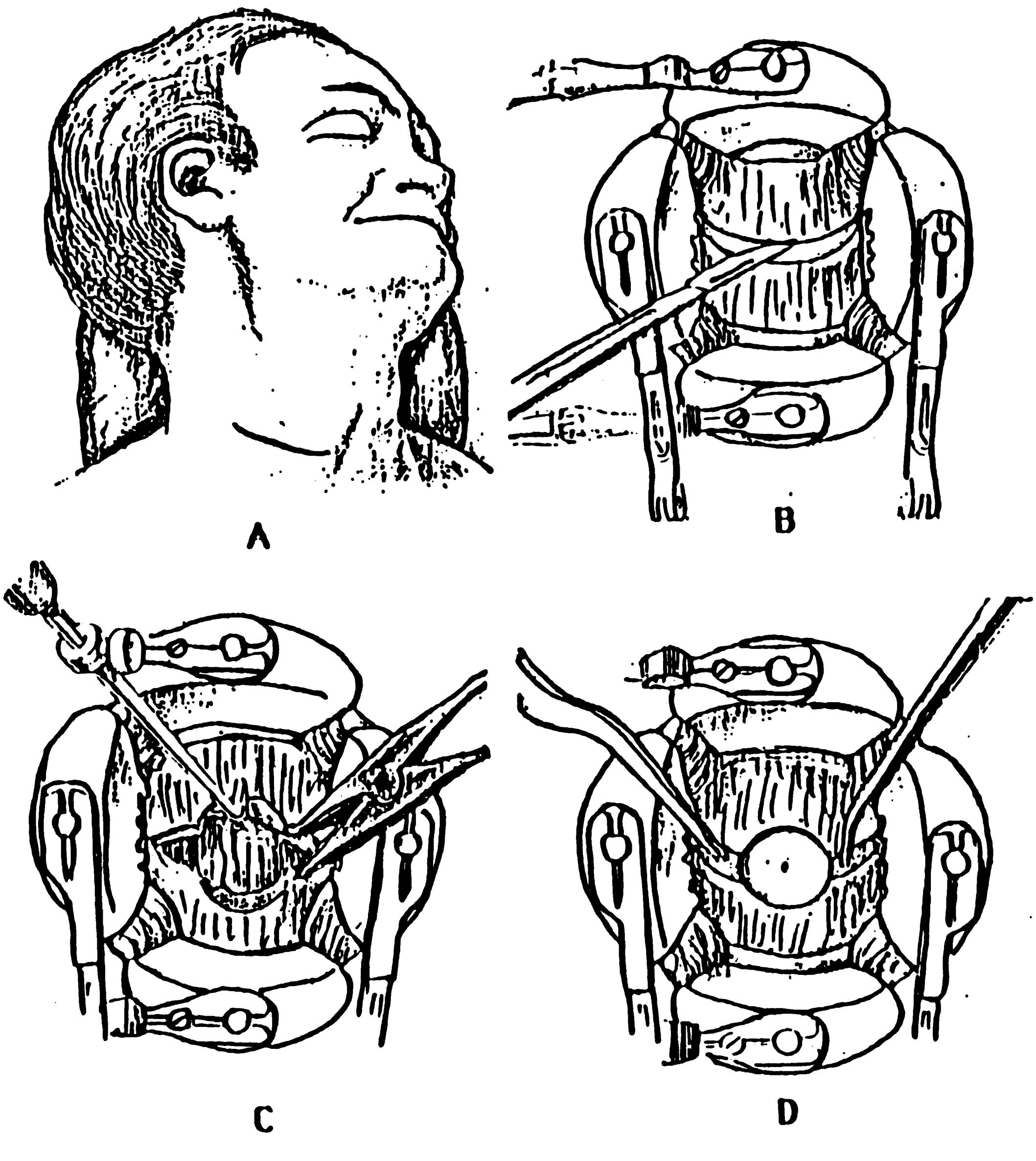

FIGURE 2. The Cloward technique, A; the anterior approach, B; exposure of the vertebral bodies, C; hole drilled to size, to accept corresponding graft, D; graft seated in place, and spreaders removed.

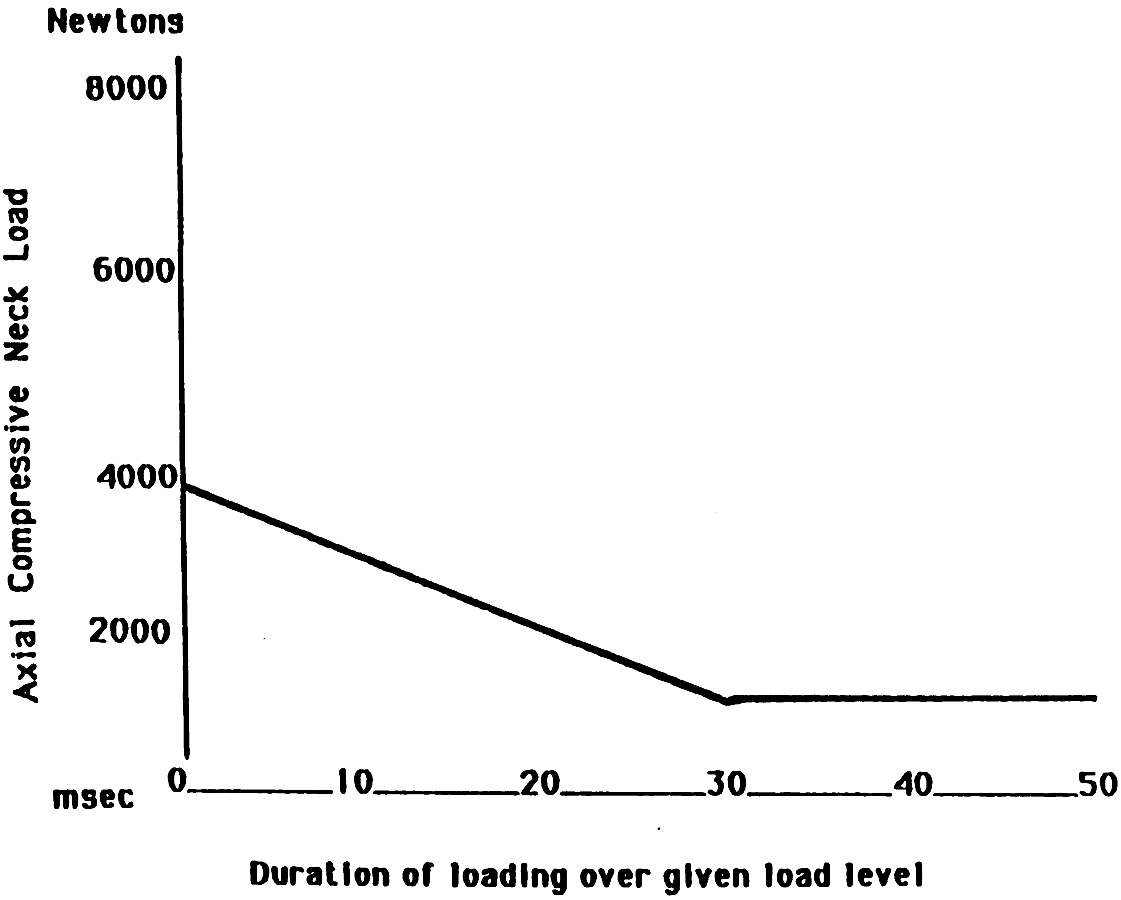

FIGURE 3. Proposed neck injury references for axial compressive neck force, for adult population. Mertz [44]

antigenicity is involved and viable cells are thought to survive and speed healing. In actuality, only osteocytes within 300 μ of the surface survive, and the rest die from lack of vascularization. [7] The major objections to the use of autogenic bone are that a second invasion is required to retrieve the bone, and often the volume or quality of the bone desired, is not available from the patient. Consequently, if performed, the procedure will increase morbidity and make recuperation longer for the patient.

Since the beginning of the United States Navy Tissue Bank in 1950, many tissue grafts have been processed for use in reconstructive surgery. From the start, research interest has focused on the field of bone grafting because it is the easiest tissue to transplant, and the need for deriving an equivalent replacement for autologous bone is high. [7]

Cancellous bone for preparation of allografts used by tissue banks can be harvested from several sites, including: the distal femur, femoral head, proximal tibia and vertebrae. The demand for intact femoral heads, and the difficulty of retrieval from vertebrae often preclude those sites from being utilized. The distal femur and proximal tibia are left as good consistent sources for obtaining several grafts from the same donor. Due to the geometry of its planar contact surface, the proximal tibia is also a source for cancellous blocks, commonly used in reconstruction; therefore, the distal femur is the most likely source of dowel grafts.

There are several different ways allografts can be processed, varying both storage and sterilization: [2] lyophilization (freezing at -70° C while dehydrating in vacuum so final water content is less than 5%) or storage at -70° C and kept fresh. Both methods greatly decrease the antigenicity of the bone by killing the viable cells which might be viewed as immunogens by the recipient's body. Both can be sterilized using $Co^{60}\gamma$

radiation, but only lyophilized bone can be sterilized using ethylene oxide gas. Due to ease of processing, shelf life, and shipping considerations, the preferred method is to lyophilize and irradiate the bone.

This study concentrated on the lyophilized grafts and attempted to determine the rehydration time required to return their material properties to those of fresh bone. Also, it was important to determine which site(s) of the distal femur supplied the maximum strength in resistence to compression, that is, whether from a dominant leg, condyle or position as specified with degree of flexion.

A parallel study investigated the effects of the irradiation sterilization process on the bone and constructed a theoretical model using finite element method to compare the experimental results to the analytical ones.[47]

II. SURVEY OF LITERATURE

One of medicine's oldest needs has been to be able to replace diseased or damaged parts of the body. For awhile, this was limited to using artificial limbs or dentures, but in 1878, MacEwan [40] performed the first successful bone transplant from one person to another. In 1895, an introduction to bone grafting techniques was published by Barth. [3] Then in 1937, Orell [48] began using cadaver bone for graft material. The demand for grafts increased dramatically during the Korean conflict and prompted the Navy to develop the first large scale collection and preservation facility. Since 1950, the U.S. Navy Tissue Bank has taken over 37,000 tissue deposits, and dispensed two-thirds of the grafts made for clinical use. [7]

Since Barth's first introduction of bone grafting, many applications have developed including: arthrodesis of joints, filling bone cavities after infection, replacing joint surfaces or bone lost to trauma or tumor removal. The two primary forms of material used in these procedures are autogenic (person's own) bone and allogenic (taken from a donor) bone.

The anterior approach to the cervical spine was first described by Chipault [20] in a textbook of Neurosurgery published in 1895. This specific approach was not used again until the 1950's when three groups independently developed it for the use in spinal fusion. Smith and Robinson [55] designed a horizontally seated graft using rectangular block.

Bailey and Badgely [2] used rectangular block also but positioned vertically, providing the option of fusing more than one joint with one graft. Cloward [22] developed a cylindrical dowel graft, which was limited to single joint fusion as was Smith and Robinson. Recently, in 1984, Gore [31] created a new technique using a dovetail design with an odd shaped rectangular graft which is seated vertically. Like that of Bailey and Badgely, it can be used for multi-level fusions, adding to the options available to orthopaedic surgeons.

Increased use of bone grafts turned attention to the preparation of the tissue. The first attempt at preserving bone using refrigeration for later transplant was done in 1912 by Carrel. [17] Shortly afterwards, Inclan [34] tested the viability of frozen human bone for use in orthopaedic surgery.

Flosdorf [27] developed the processing method of lyophilization, and bone was the first tissue lyophilized and used clinically. Schneider [54] reported a success rate of 92% in 57 patients of cervical arthrodesis using lyophilized allografts. 85% of the unions demonstrated bridging within 4 months, as examined radiographically. Kline [37] found that freeze-dried allografts induce the recipient's mesenchymal cells to differentiate into mature osteoblasts aiding in the incorporation of the graft. Kreuz [38] reported no difference in the remodelling and repair of lyophilized bone when compared to fresh autogeneous bone, except that the rate was slower. Carr and Hyatt [16] using histology to confirm these results. Brown [9] showed no radiologically demonstrable significant difference in the process of incorporation between autografts and allografts. Pelker [49] determined lyophilization did not have a deleterious effect on the compressive strength of bone allografts. However, Triantafyllou [59] found a considerable reduction in the mechanical properties of the bone allografts, due to lyophilization and sterilization by irradiation.

Burwell [12] in 1963 listed several criteria for using allografts: the bone must be sterile, nonantigenic, capable of induction and resorption, permit vascularization, and finally be implanted close to cancellous bone.

According to Elves [25], lyophilized grafts did not stimulate humoral immunity and produced a decreased cell-mediated response compared to that of fresh bone. A year later, in 1977, Bright [7] stated that the freeze-drying process kills the viable cells in the graft, subsequently decreasing its antigenicity. Bright [6] found that rehydration of 24 hours restored the lyophilized cortical bone so that its mechanical properties were closest to those of fresh bone.

Studies done by several different groups and reported by Galante [29] indicated that cancellous bone acts as an anisotropic material resulting in different strengths depending on the direction in which the load is applied. Gibson [30] determined a dependence of the mechanical properties of cancellous bone on density, cell wall properties and cell geometry. She also stated that the symmetry of the cancellous structure is dependent on the direction of the applied loads , and the density was dependent upon magnitude of those loads. Galante [29], Weaver and Chalmers, [60] and Behrens, [4] found a strong linear relationship between compressive strength and density. Martens, [42] and Lindahl, [39] found the correlation to be 0.7 between strength and density.

Behrens [4] also showed that bone strength varied with position in the joint . They chose to differentiate the sites using the contact points at 0°, 45°, and 90° of flexion as their specified sample sources. They found the bone from 0° and 45° of the lateral condyle and 45° of the medial condyle to be the strongest sites. They ranked the sites with 45° being the strongest, 0° slightly lower, and 90° the weakest. They determined the medial condyle to be stronger than the lateral one (all sites from each condyle considered collectively). These results suggest not only magnitude but duration and frequency of the applied loads on the bone site are the determining factors of bone strength.

Medawar [43], in 1944, investigated the immunologic basis for tissue rejection and, found autogenic bone generally was a superior graft material. Chalmers, [19] in 1959, showed bone to have the same histocompatibility antigens as skin, but, in 1979, Richards [53] discovered that bone cells have a smaller number of antigens than skin.

Duthie [24] in 1958, tested the rates of vascularization of cortical and cancellous bone, and found the cancellous had a faster rate, confirming the earlier work of Abbott [1] in 1947.

Lindahl [39] discovered a quantitative deterioration in most of the strength parameters of bone with age. The change in strength was greater than the decrease in density (increased porosity) could explain, leaving the possibility that there is a qualitative change as well. Specifically, that trabecular reorganization may be responsible for the change. Weaver and Chalmers [60] found that after the age of forty there is an faster, rate of decrease in strength as aging continues. Bright [6] hypothesized that the changes in aging bone might be due to increased cross-linking of the bone collagen. Weaver and Chalmers also noticed that trabecular reorganization might explain the variations in bone strength which occur independently of changes in density. Behrens [4] suggested this possibility too, and considered it to be a significant factor. Therefore, strength is dependent on several factors which must all be considered together.

This study examined the effects that anatomical site and processing have on the mechanical properties of cancellous bone allografts. Specifically, whether lyophilized grafts could respond similar to fresh bone, and if so, what rehydration time would be required. The site of the bone is an important question from the viewpoint of the tissue bank, since the findings could change the choice of sites utilized for load bearing grafts.

III. MATERIALS AND METHODS

Tissue samples of cancellous bone used for testing were supplied by the Michigan Regional Tissue Bank, a division of Edward W. Sparrow Hospital in Lansing Michigan. Samples were obtained using the Tissue Bank's standard procedure of procurement and then stored at -70°C until processed. When thawed, they were cleaned of any remaining soft tissue and cut according to predetermined guidelines using a Black and Decker hole saw with an attached 1.4 cm bit. The cortical bone plates on the end were removed with a Stryker autopsy saw, so the samples were composed of cancellous bone only.

Since donor variability was a concern, consideration was given to maximize the number of allografts obtained from one donor. This study was restricted to one sex, male, and an age range of 34 to 55. This removed the sources of scatter due to decreasing bone density caused either by aging, disease, or the onset of menopause in women. A repeatable procedure was desirable so that all donors could be processed the same, and differences due to anatomical site could be analyzed. For consistency in protocol, both right and left distal femurs from the same donor were prepared as follows; six allografts were drilled from each side with three coming from each condyle. The grafts were numbered according to the condyle and postion at approximately equal spacing, from anterior to posterior of the articulating surface, Figure 4. The dowels, taken from the medial condyle were numbered 1, 2, and 3, with

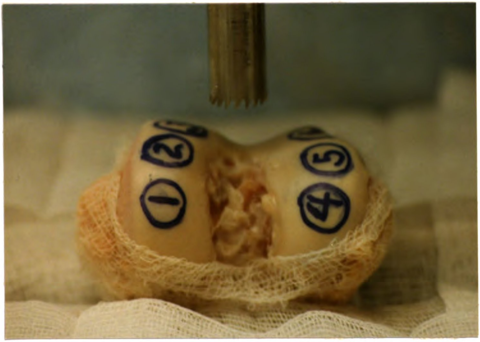

FIGURE 4. Distal femur showing site locations, and set up for hole saw with 1.4 cm bit.

site 1 being the most posterior, and sites 2 and 3 then taken from increasingly anterior positions, ultimately dividing the articulating surface into thirds. The same was done to the lateral condyle using the numbers 4, 5, and 6, with 4 taken as the most posterior. Each prepared graft was identified by donor, leg side (right/left), condyle (medial/lateral), and corresponding site. All dowels were taken from these designated sites and varied only in length, a result of the differing condylar sizes. The lengths ranged from 9.5 mm to 25 mm with the typical graft being around 20 mm, Figure 5.

The grafts were sorted according to experimental design, Figure 6. This specimen matrix was used to insure that all variables were considered equitably. Donor, side, condyle, site, and treatment were all treated as possible influences on the responses observed. The dowels were then stored fresh in refrigeration or lyophilized to reduce the water content to less than 5% of total composition.[27] The fresh grafts were tested within 48 hours of drilling and the lyophilized ones were rehydrated in sterile saline the specified time. Rehydration times ranged from 2 to 24 hours with 6, 12, and 18 hours covering the intermediate range.

Since the major load on the vertebrae, *in vivo*, is that of axial compression, the grafts were tested under compression using diametrically opposed loading as applied by grips geometrically analagous to the inferior and superior vertebrae simulating the *in vivo* conditions. Deformation tests were run to a maximum value of 30 % diametral strain corresponding to the loss of disc space. Also of concern was the mode of failure, since brittle fracture would produce loose fragments which could cause damage in the spinal canal.

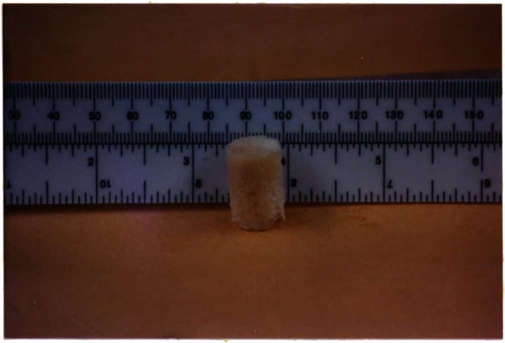

FIGURE 5. Typical allograft approximately 1.4 cm X 2.0 cm shown here scaled next to a standard metric ruler.

TREATMENT

DONOR AND LEG	1	2	3	4	5	6
	SITE (anterior/ posterior position and condyle)					
1 RIGHT	1	2	3	4	5	6
1 LEFT	2	3	4	5	6	1
2 RIGHT	3	4	5	6	1	2
2 LEFT	4	5	6	1	2	3
3 RIGHT	5	6	1	2	3	4
3 LEFT	6	1	2	3	4	5

TREATMENT CODE 1=FRESH, 2=2 HOURS, 3=6 HOURS, 4=12 HOURS, 5=18 HOURS, 6=24 HOURS

FIGURE 6. Experimental specimen matrix. Treatment number corresponds to different lengths of rehydration time or the fresh control.

Test fixtures were designed to simulate the loading as would occur postoperatively. The grip compression surfaces were made to geometric specifications to approximate the superior and inferior vertebrae as prepared surgically, Figure 7. The grips had compression surfaces with concave rounds of radius 7 mm running the length of each. When the test sample was positioned before testing, a gap between grips of 4 mm would approximate the same distance as the desired disc spacing found *in vivo*.

Before testing, the grafts' lengths were measured with a micrometer so that density values could be determined using the volumetric measurements. Since Galante [29] had reported the effects of trabecular orientation during testing, all grafts were aligned before testing with the apparent trabecular grain running vertically, Figure 8. Even though the primary axis of loading would be parallel with the long axis of the cylinder, the effects of the radial trabecular structure were considered potentially significant.

Tests were performed using an Instron Servohydraulic Materials Testing Machine with stroke control at 3%/sec strain rate to a maximum 30 % strain, Figure 9. Both load and stroke data were recorded using a Nicolet oscilloscope and stored using an attached mini-floppy disk drive. A DEC PDP 11/23 minicomputer was used to transfer the data from the floppy disks and plot load / deformation curves on a PRINTRONIX p-series printer, Figure 10. This was done using four programs which changed the initial data recorded as volts/seconds into values of Newtons/millimeters while removing the time base. The first program moved the data from the floppy disks recorded by the Nicolet and transferred it to the hard disk on the PDP. The second program then

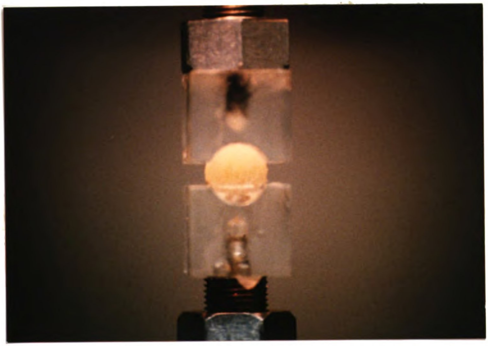

FIGURE 7. Allograft as seen end on before compression between lucite grips, shown here 8/7 actual size.

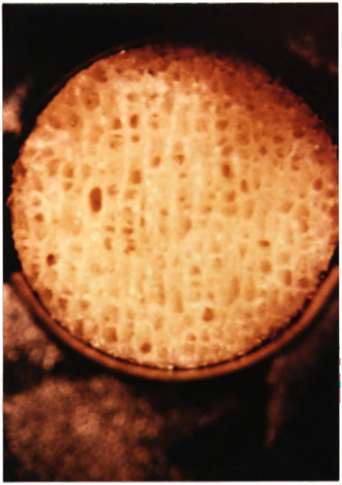

FIGURE 8. End of allograft before testing as seen through a 10X microscope.

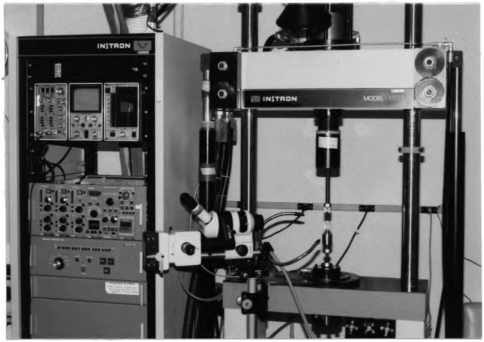

FIGURE 9. Testing apparatus, showing Instron materials testing machine, lucite grips, Nicolet oscilloscope and Wild microscope with camera attachment.

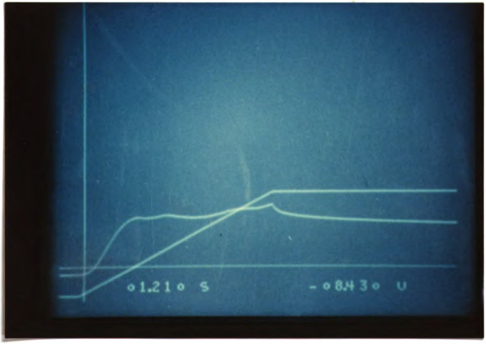

FIGURE 10. Screen of Nicolet oscilloscope showing typical stroke and load curves recorded in volts/seconds.

converted the values which are recorded in pounds per inch into Newtons per millimeter. The third program removed the time base and paired the load and stoke data point to point. Finally, the fourth program plotted the values calculated by the previous program onto a pair of axes.

The load vs. deformation curves were analyzed using a 2% offset method to determine the peak load and stiffness values. The stiffness was defined as the slope of the linear portion, line 1, Figure 11. A second line was then drawn, parallel to the first but offset to the right on the deformation axis, line 2. Then peak load, line 3, and deformation, line 4 were the values determined from where lines 3 and 4 intersect with the curve. Stiffness was calculated from the peak load and deformation values. Those values were then normalized by the mineral densities obtained from ashing. Linear regression analysis was used to calculate the correlation between age, density and compressive strength. Line graphs were constructed from the peak load and stiffness values obtained for the different rehydration times and anatomical sites. A t-test was done to compare the statistical means of the different treatments to that of the fresh control. After testing, the grafts were ashed in a muffle furnace at 850°C for 90 minutes to remove all organic material and water. The grafts were then weighed on a Mettler analytical balance to calculate percent mineral density. The ash densities were used as the normalization parameter for peak load and stiffness, in an attempt to remove some of the variance due to length and donor differences.

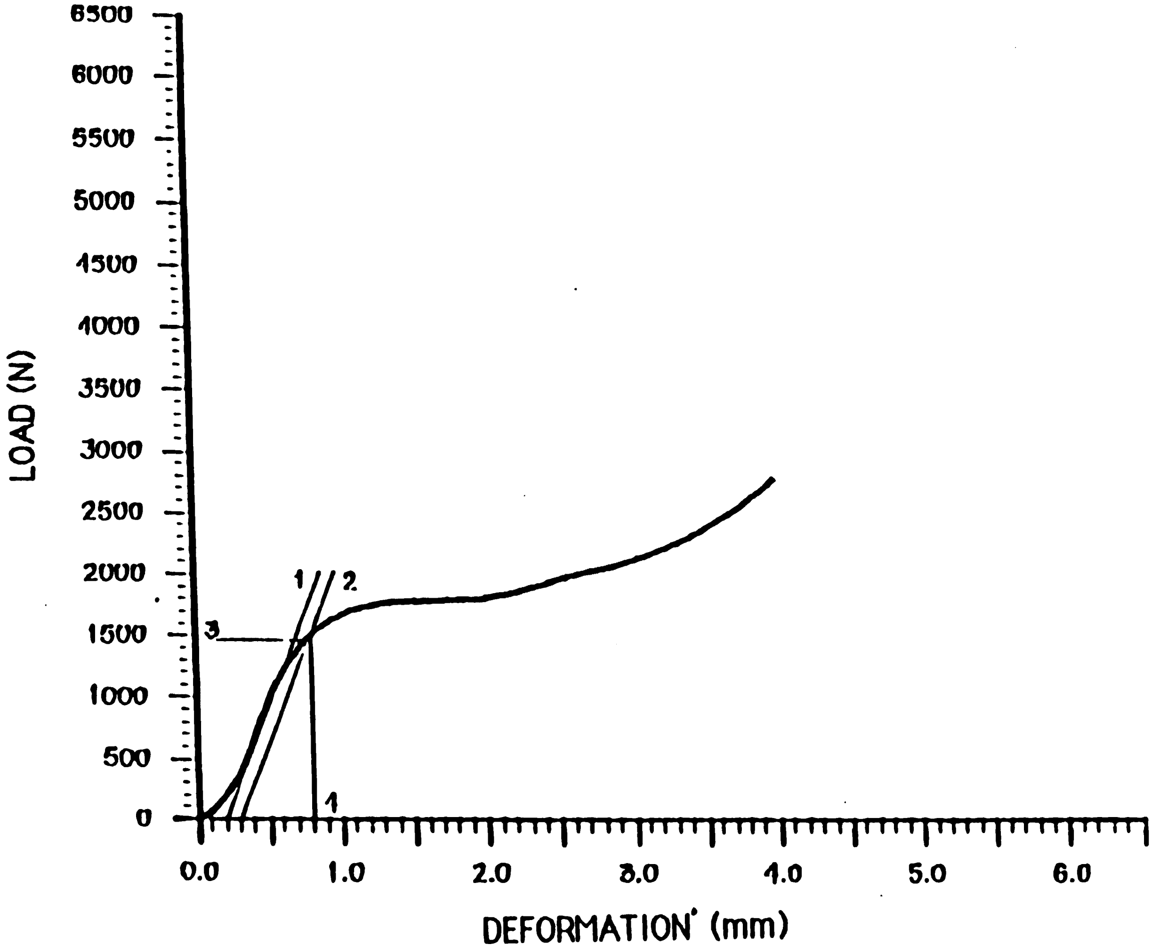

FIGURE 11. Typical Load vs. Deformation curve showing 2% offset method used for obtaining peak load and stiffness.

IV RESULTS

Upon first examination the raw data showed considerable scatter, possibly attributable to the fact that the prior health of each individual donor varied greatly and could not be controlled for in this study. Previous researchers have used ash weight density as a normalization parameter for compressive strength of cancellous bone. The first analysis made was a comparison of compressive strength and ash weight density using the method of linear regression, Figure 12. The results were very similar to the values reported by Weaver [60] for vertebral and calcaneal cancellous bone. The correlation coefficient determined for N=52 of all sites tested fresh was 0.79, and all data were then normalized by ash weight density.

The analysis involved examination of the potential changes in response of grafts from each donor, left and right legs considered independently, to different rehydration times. All sites within the knee were assumed equitable, with each donor's grafts separated according to experimental design. Two groups, each comprised of three donors were tested after four different treatments. Grafts from both groups were tested fresh and at two hours rehydration. The first group was then tested at 6 and 18 hours, while the second group was then tested at 12, and 24 hours, Tables 1 and 2. The normalized loads for each graft of a particular treatment were averaged, and a t-test was done to compare their means to that value determined for the control of each group, which was the fresh bone, Table 3. The results of this test revealed that for the first group there was no significant difference between treatments. However,

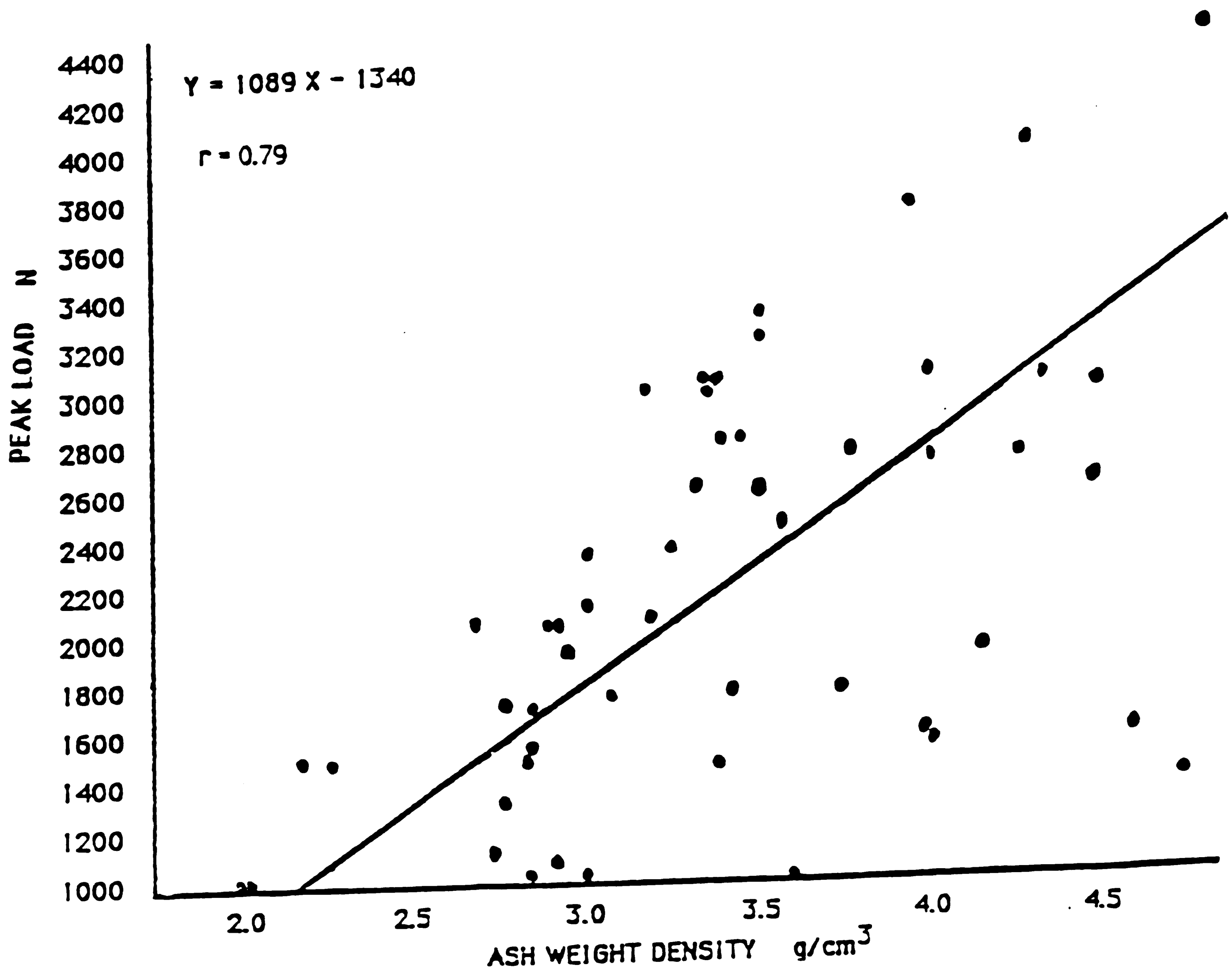

FIGURE 12. Peak load vs. ash weight density.

TABLE 1. Group 1 data. D=donor, SI=side, ST=site, TX=treatment, ASH=ash weight, LOAD=peak load, LENGTH=length, ΔX=deformation, DENSITY=density, NLOAD=normalized load, and NSLOPE=normalized stiffness.

D	SI	ST	TX	ASH g	LOAD N	LENGTH mm	ΔX mm	DENSITY $g/\pi r^2 l$	NLOAD N/∂ x 10^1	NSLOPE N/∂mm x 10^1
1	1	3	3	1.14	2600	18.37	0.80	4.03	645.2	806.5
3	2	6	3	0.96	3050	14.48	0.70	4.31	707.7	1011.0
2	2	4	3	1.06	2650	16.18	0.70	4.26	622.1	888.7
2	1	5	3	0.60	1180	14.47	0.60	2.69	438.7	731.2
1	2	2	3	0.93	2600	18.35	0.72	3.29	790.3	1097.6
3	1	1	3	1.30	3900	19.46	0.77	4.34	898.6	1167.0
3	1	6	2	0.90	3000	17.27	0.55	3.39	885.0	1609.1
3	2	5	2	1.06	1600	15.06	0.52	4.57	350.1	673.7
1	2	1	2	1.03	3700	17.26	0.65	3.88	953.6	1467.1
2	1	4	2	0.99	1300	13.33	0.50	4.83	269.2	538.4
1	1	2	2	0.53	600	9.48	0.50	3.63	165.3	330.6
2	2	3	2	1.09	4400	15.04	0.82	4.70	936.2	1139.3
3	1	3	5	0.75	1750	14.45	0.60	3.37	519.3	865.5
2	1	1	5	0.83	1700	14.47	0.57	3.73	455.8	799.7
1	2	4	5	0.67	1750	16.10	0.52	2.70	648.1	1246.4
1	1	5	5	1.13	1550	18.31	0.40	4.01	386.5	966.3
2	2	6	5	0.88	1900	19.47	0.60	2.94	646.3	1077.2
3	2	2	5	1.18	1900	18.34	0.50	4.18	454.5	909.0
2	1	2	0	1.04	2700	18.32	0.54	3.69	731.7	1355.0
3	2	3	0	1.00	3050	14.40	0.60	4.51	676.3	1127.2
3	1	4	0	1.07	2600	15.51	0.57	4.48	580.4	1018.3
1	2	5	0	1.06	2600	19.40	0.65	3.55	732.4	1126.8
1	1	6	0	0.50	1050	11.10	0.47	2.93	358.4	762.5

TABLE 2. Group 2 data. D=donor, SI=side, ST=site, TX=treatment, ASH=ash weight, LOAD=peak load, LENGTH=length, ΔX=deformation, DENSITY=density, NLOAD=normalized load, and NSLOPE=normalized stiffness.

D	SI	ST	TX	ASH g	LOAD N	LENGTH mm	ΔX mm	DENSITY $g/\pi r^2 l$	NLOAD N/∂ x 10[1]	NSLOPE N/∂mm x 10[1]
4	2	1	6	0.82	2075	18.31	0.65	2.91	713.1	1097.1
5	1	2	6	0.96	2725	18.33	0.53	3.41	799.1	1507.7
5	2	3	6	0.80	2325	16.12	0.65	3.23	719.8	1107.4
6	1	4	6	0.85	1700	17.25	0.60	3.19	532.9	888.2
6	2	5	6	0.97	2975	18.35	0.60	3.43	867.3	1445.5
4	1	6	6	1.00	3200	18.35	0.70	3.53	906.5	1295.0
4	1	1	0	0.97	3050	19.50	0.65	3.23	944.3	1452.8
4	2	2	0	0.97	3000	18.40	0.70	3.43	874.6	1249.4
5	1	3	0	0.98	3050	16.10	0.65	3.95	772.2	1188.0
5	2	4	0	0.86	2050	17.20	0.52	3.25	630.8	1213.1
6	1	5	0	0.67	1550	18.30	0.50	2.38	651.3	1302.6
6	2	6	0	0.77	2040	18.30	0.50	2.73	747.3	1494.6
5	1	1	4	1.01	2800	19.48	0.58	3.37	830.9	1432.6
5	2	2	4	1.09	3275	20.00	0.63	3.54	925.1	1468.4
6	1	3	4	0.59	875	18.32	0.60	2.09	418.7	697.8
6	2	4	4	0.90	2150	19.48	0.60	3.00	716.7	1194.5
4	1	5	4	0.84	2300	17.27	0.65	3.15	730.2	1123.4
4	2	6	4	0.82	1675	18.36	0.50	2.90	577.6	1155.2
4	2	5	2	0.90	2000	20.06	0.58	2.91	687.3	1185.0
6	2	3	2	0.54	875	17.34	0.48	2.03	431.0	897.9
6	1	2	2	0.86	1500	19.42	0.55	2.88	520.8	946.9
4	1	4	2	0.86	1450	20.02	0.48	2.79	519.7	1082.7
5	2	1	2	0.97	2475	17.24	1.00	3.66	676.2	676.2
5	1	6	2	0.50	1500	14.43	0.68	2.26	663.7	976.0
6	1	1	1	1.12	1450	17.06	0.60	4.28	338.8	564.7
4	1	3	1	0.89	1300	21.45	0.55	2.69	483.3	878.7
4	2	4	1	0.94	800	19.41	0.50	3.15	254.0	508.0
5	1	5	1	0.92	975	20.99	0.67	2.85	342.1	510.6
5	2	6	1	0.74	1450	14.00	0.60	3.43	422.7	704.5

TABLE 3. T-test comparing means for normalized load at different rehydration times to fresh control, both sides, all sites, all donors of each matrix included.

TREATMENT	NLOAD MEAN ± sd $N/g/cm^3$	N sample size	p value
	MATRIX 1 (3 DONORS)		
FRESH	615.8±156.7	5	-----
2 HOURS	593.2±368.7	6	>0.40
6 HOURS	683.8±157.1	6	>0.25
18 HOURS	518.4±108.2	6	>0.10
	MATRIX 2 (3 DONORS)		
FRESH	770.1±122.7	6	-----
30 MINS	368.2± 87.8	5	<0.0005
2 HOURS	583.0±106.8	6	<0.01
12 HOURS	699.9±180.6	6	>0.20
24 HOURS	765.5±134.0	6	>0.40

the second group had significance at both the 30 minute and 2 hour treatments. The difference for the 30 minute interval can be disregarded because, grafts tested at this time failed in a brittle mode. The other finding possibly indicates that although 2 hours is adequate to remove the brittle failure, it may not be restoring the bone completely back to the level of fresh bone.

An analysis was made to compare age and compressive strength using the method of linear regression. Eleven donors ranging from 34 to 55 had six grafts, one from each site, tested fresh for each. The average of those six sites was then used to determine the linear regression line and correlation coefficient, Figure 13. When all 11 donors were included the correlation coefficient was -0.78. This line agrees with the literature from Lindahl and Weaver, in that a decrease in strength is seen after age 40, and continues to decline. Lindahl also found that the decrease in strength with age, was greater than the decrease in density could explain, implying that there must also be a qualitative change in the structure as well. Without further research, including ages covering the range more evenly, it is difficult to conclude what the actual trend would be, specifically where the line begins to decrease most rapidly.

Of the total 193 grafts tested, 8.8% failed to meet the minimum criterion of sustaining load up to 1110 N. These grafts had no traits in common which could explain this weakness. They came from all treatments, several donors of different ages, lengths, and densities. This finding would indicate the need to examine the trabecular structure closely to determine if any specific pattern would correlate with the failing grafts.

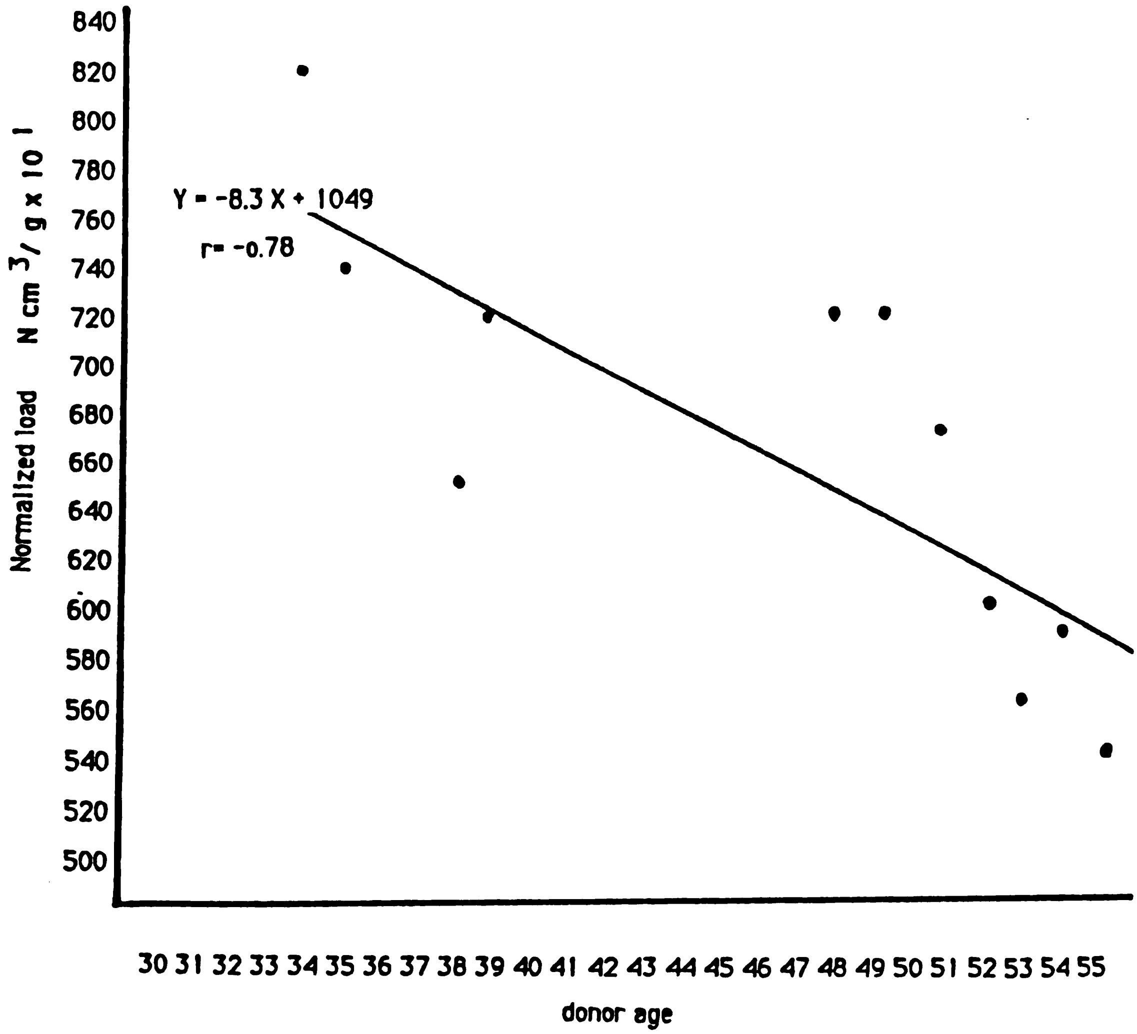

FIGURE 13. Linear regression analysis of normalized peak load vs. donor age.

V DISCUSSION

As well substantiated in the literature, cancellous bone is an anisotropic material, having preferred axes aligned in the directions of *in vivo* principal stresses. Bone grafts from the femoral condyles, Figure 14, are taken such that the cylindrical axis corresponds with the direction of maximum compressive stress. The radial axes would then be in the transverse plane to this principal stress. Milne-Thomson, [45] described this particular type of anisotropy as transverse isotropy. With the primary axis being the axis of monotropy, and all directions perpendicular to the axis of monotropy being equivalent. Williams and Lewis, [64] also commented on this particular case of orthotropy, after testing lyophilized cancellous blocks from the tibial epiphysis, rehydrated overnight.

In a parallel study done by Mukherjee [47], 2 hours was determined to be the minimum time of rehydration required for non- irradiated lyophilized grafts to avoid brittle fracture failure. Based on this finding, all strength and stiffness data were normalized additionally by the 2 hour rehydration value for each donor. The results from the first group are shown in, Figure 15. The most obvious difference is between legs within any given donor. The strength of grafts taken from one leg of each donor remains fairly constant with increased rehydration while the other shows an increase of compressive stength after 2 hours up to the 6 hour interval. The second group shows similar results with the exception of one donor, number 5, who consistently had very high strength values for grafts from both legs at all the treatments, Figure 16. The values for

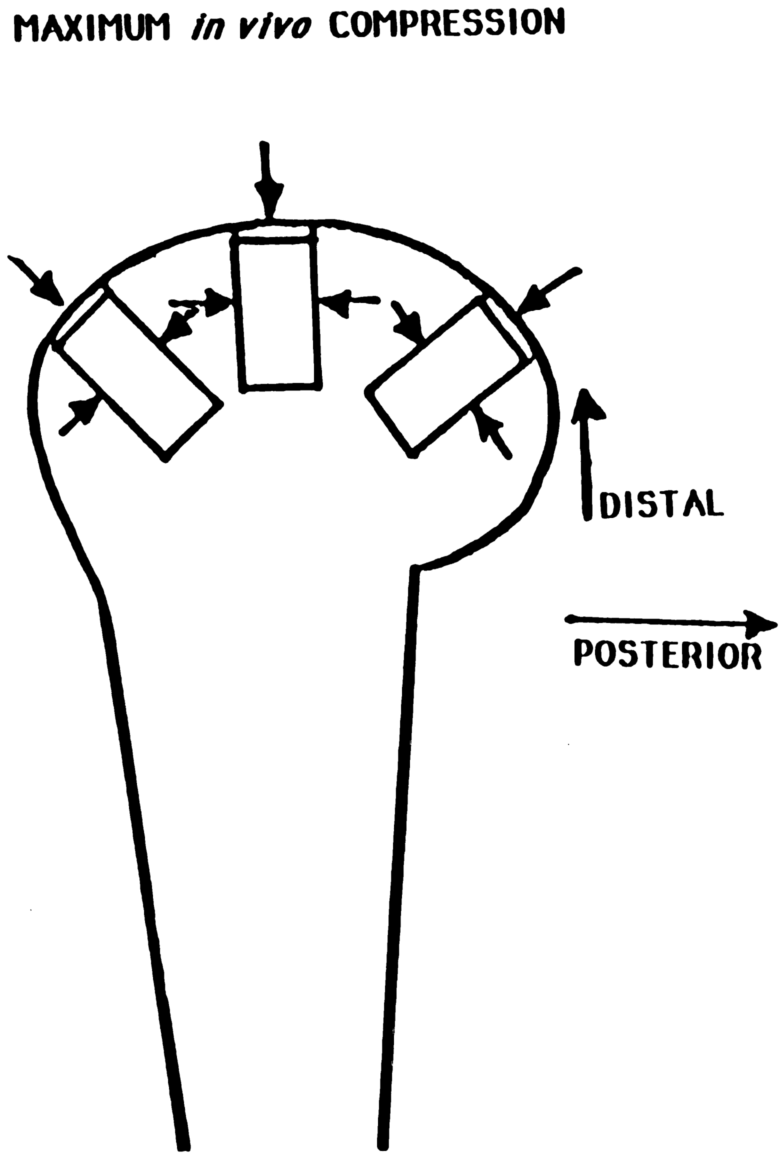

FIGURE 14. Illustration of distal femur showing directions of applied physiological and testing loads.

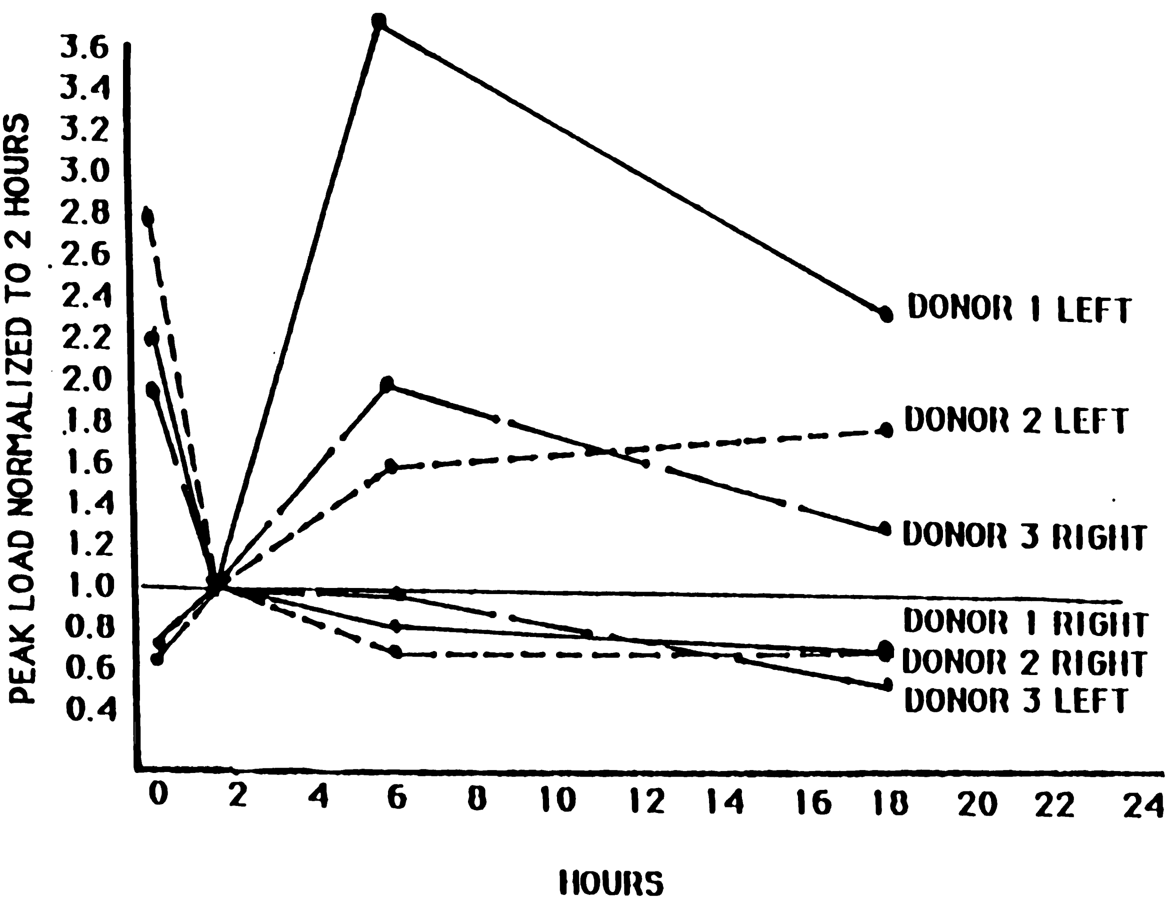

FIGURE 15. Line graph of normalized peak load values for donors 1, 2, and 3.

stiffness for group one were comparable to the trends of compressive strength, Figure 17. However, the values for stiffness of donor 5 of group two were also higher compared to that of the other two donors, Figure 18. Donor 4 showed little changed in stiffness while donor 6 had similar results to that of the first group.

Williams described the different types of trabecular structure as forming cylindrical arrangements in the primary vertical direction. Some consist of interconnected rods forming circular or elliptical holes in the transverse plane, while the other has curved plates running parallel to the primary axis. Gibson,[30] used a hexagonal model to represent the different types both based on the cylindrical shape, but one having an open framework, while the other more closed, Figure 19.

If the 2 hour minimium rehydration time sufficiently saturated the structure enough to remove the chance of brittle fracture failure, but did not completely fill the inner trabecular microstructure with fluid, then when tested in compression along the minor axis, the plate-like structure would respond different than the rod-like structure which would be less effected by the enhanced hydraulic effect. However, with increased reconstitution, the plate-like structure would demonstrate considerable increase in compressive strength as the trabecular microstructure was saturated giving the properties of fluid filled cylinders. Ultimately responding with greater increase in strength than the rod-like structure, which may have a dominant axis but which does not demonstrate the anisotropy of the plate-like framework. This is supported by several groups which have commented on the anisotropic design of cancellous bone, but have not dealt with the problem of rehydration. As mentioned, Williams rehydrated overnight, missing the period of time where the

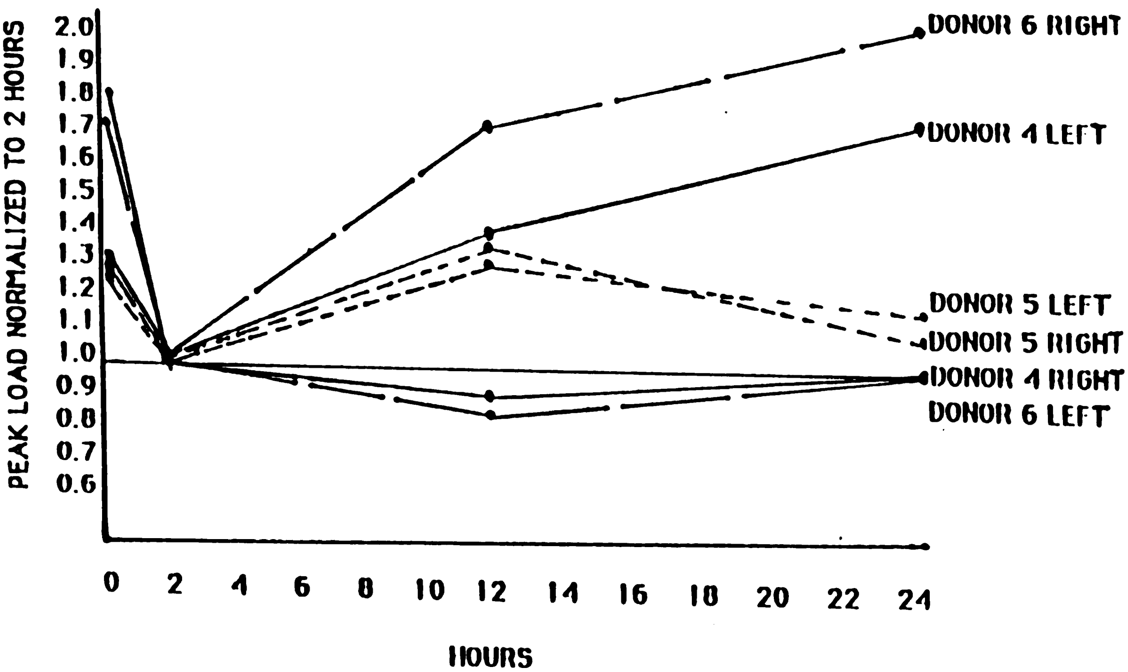

FIGURE 16. Line graph of normalized peak load values for donors 4, 5, and 6.

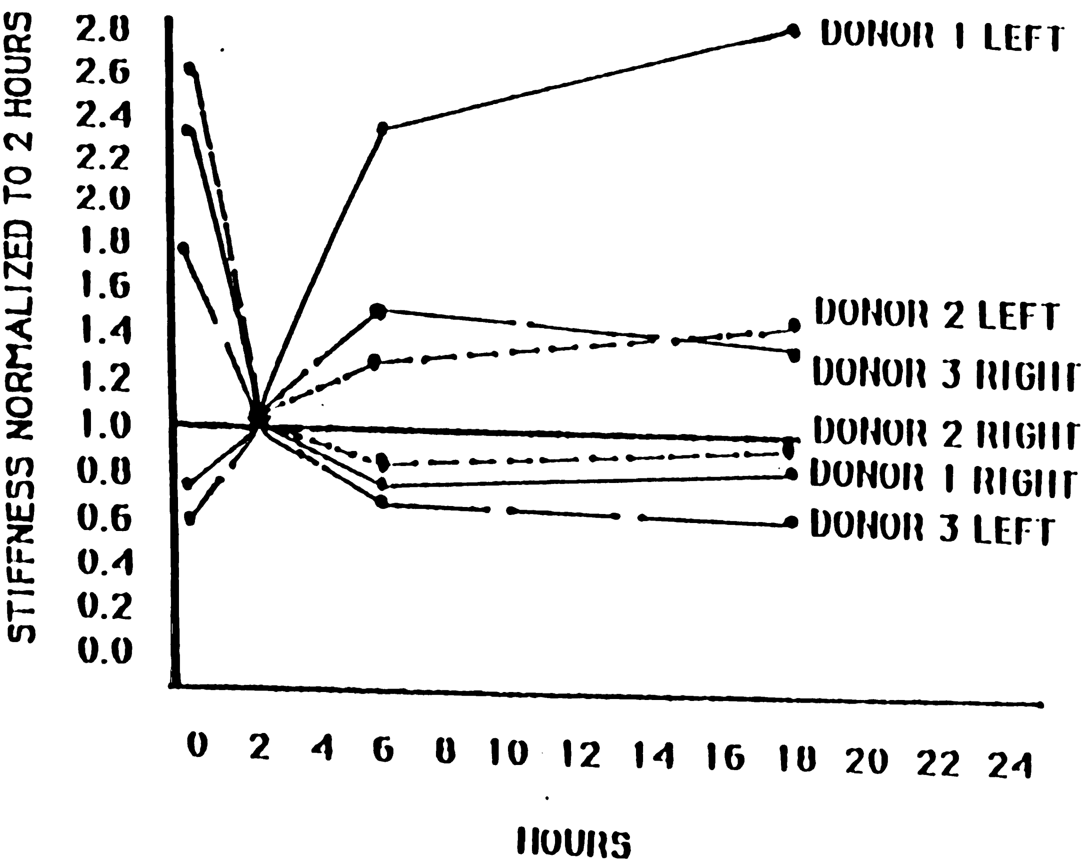

FIGURE 17. Line graph of normalized stiffness values for donors 1, 2, and 3.

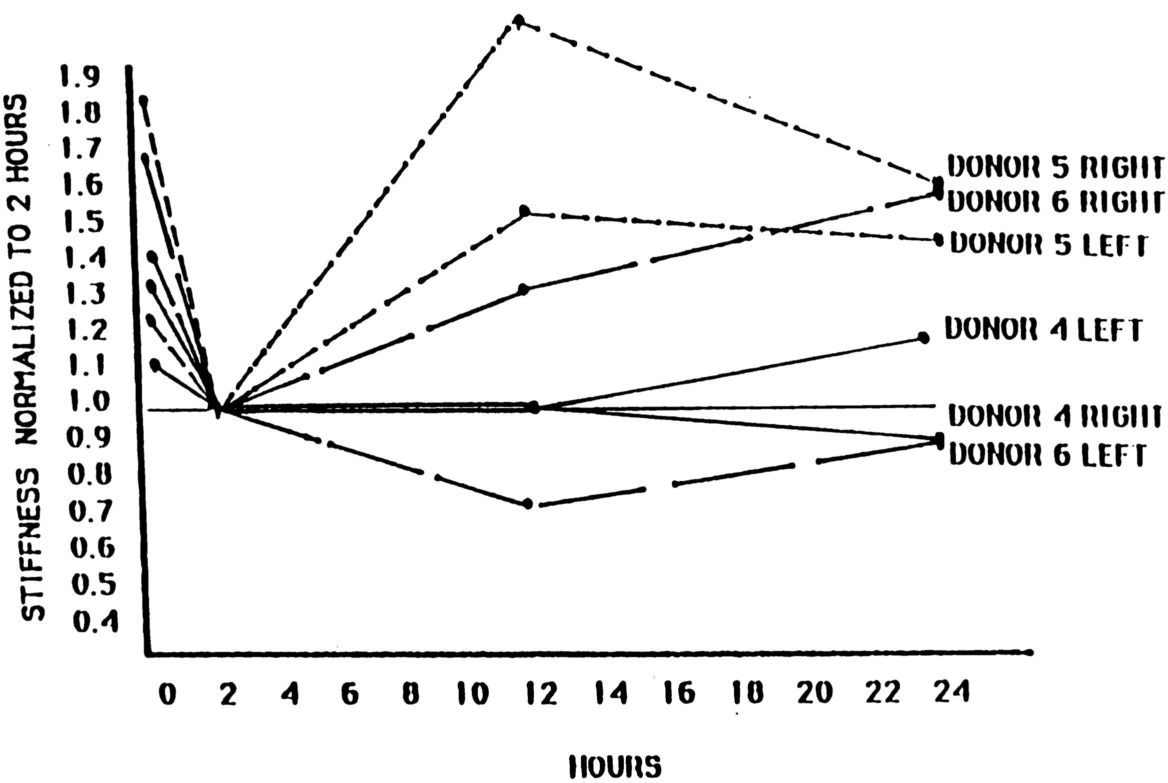

FIGURE 18. Line graph of normalized stiffness values for donors 4, 5, and 6.

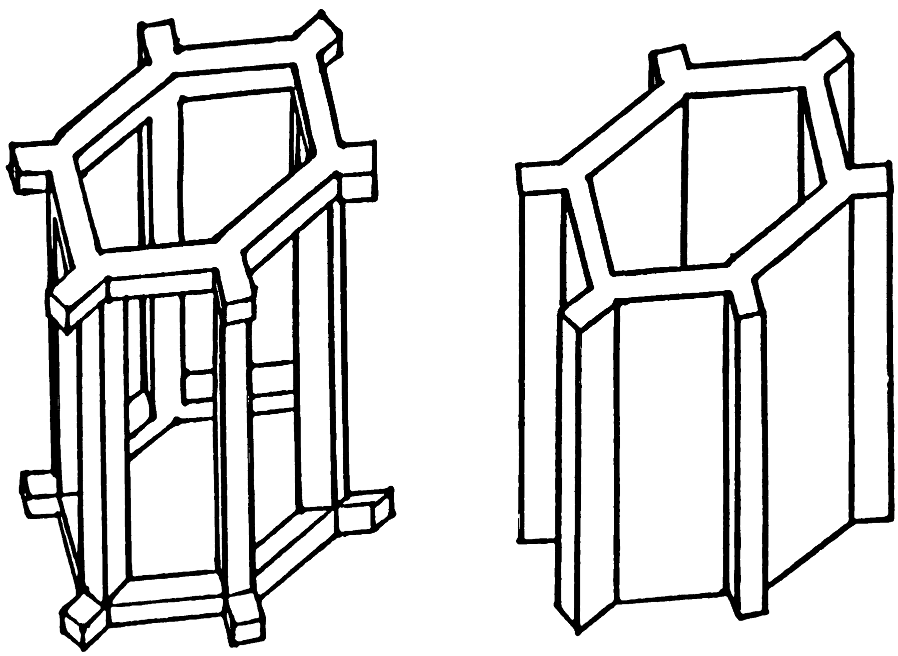

FIGURE 19. Hexogonal models of rod-like and plate -like columnar structure of cancellous bone. Gibson, L. J.. J. Biomechanics .vol. 18, no. 5, 1985, p. 317.

transverse isotropic effects are the greatest.

It is consistent with previous studies that the dominant leg would have a more plate-like trabecular microstructure due to higher load while the less dominant would have a rod-like trabecular structure. Since none of the cancellous bone grafts were loaded along their axis of monotropy, the increase in strength and stiffness in the radial direction with rehydration would be a measure of greater transverse isotropy and therefore leg dominance. From this view, it is probable that the response demonstrated by the different legs of a donor, is due primarily to the involvement of rehydration and transverse isotropy. With the most dramatic results seen in the dominant leg, where this particular structure is most apparent. The exceptional donor from group two, as mentioned, showed increased strength and stiffness, consistently throughout the treatments, possibly due to an increased fitness level in that individual. A very active donor would probably not show the difference between legs, as would someone whose dominant leg carries the load for a greater proportion of the time.

The analysis of strength dependency on site location yielded results somewhat consistent with the literature . Wismans, [65] found that the contact points with flexure angle followed irregular patterns on the condyle surfaces. A surface map showing these contact points is shown in Figure 20. Comparison with Figure 4 shows that the samples sites did not correspond with contact points of 0°, 45°, and 90°, the locations used by Behrens. On the lateral condyle most contact occurs between sites 4 and 5, while on the medial condyle sample sites correspond closer to contact points. Using strength sensitivity to rehydration as a measure of transverse isotropy and therefore strength, Figure 20, shows that site 2 or approximately 45° would be the heaviest loaded *in vivo*. This is consistent with the results reported by Behrens and in Figure 21, but on the lateral condyle, the transverse isotropy increases around the most posterior site, site 4, Figure 22. Behrens reported higher strengths between 0° and 45° which would correspond to site 5. Three factors might explain this discrepancy; the contact area for all flexure angles is concentrated in the area of both sites 4 and 5 and second, the results presented here do not differentiate between right or left leg or age, and finally the raw data shows similar transverse strength values for all areas on the lateral condyle.

The question of proper rehydration time depends upon the application. From the point of view of basic tissue biomechanics, strength will depend upon rehydration time and type of trabecular structure requiring care in comparison of results from different laboratories. For the tissue banks, removing the possibility of brittle fracture failure is the most important aspect and therefore 2 hours rehydration appears adequate.

This thesis has presented a possible explanation for the change in strength and stiffness with continued rehydration. By use of this hypothesis, increase in strength or stiffness would indicate there would be greater transverse isotropy and therefore higher *in vivo* loading along the axis of monotropy. To fully examine if this theory holds true, cubes of the material should be taken and adjoining specimens tested along different orthogonal axes. This will be left as a future research project.

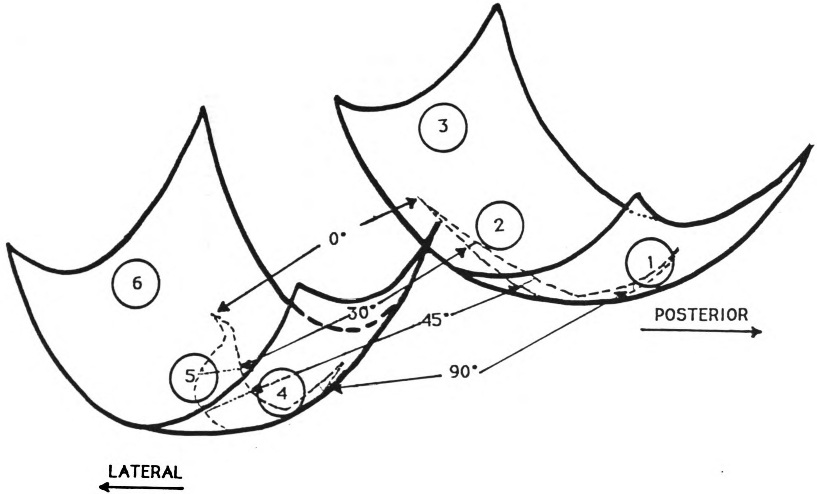

FIGURE 20. Schematic representation of the sample sites as they relate to the condylar surface. Typical pattern of the contact area frequently loaded is shaded. Wisman, J. Biomech. 13:677, 1980.

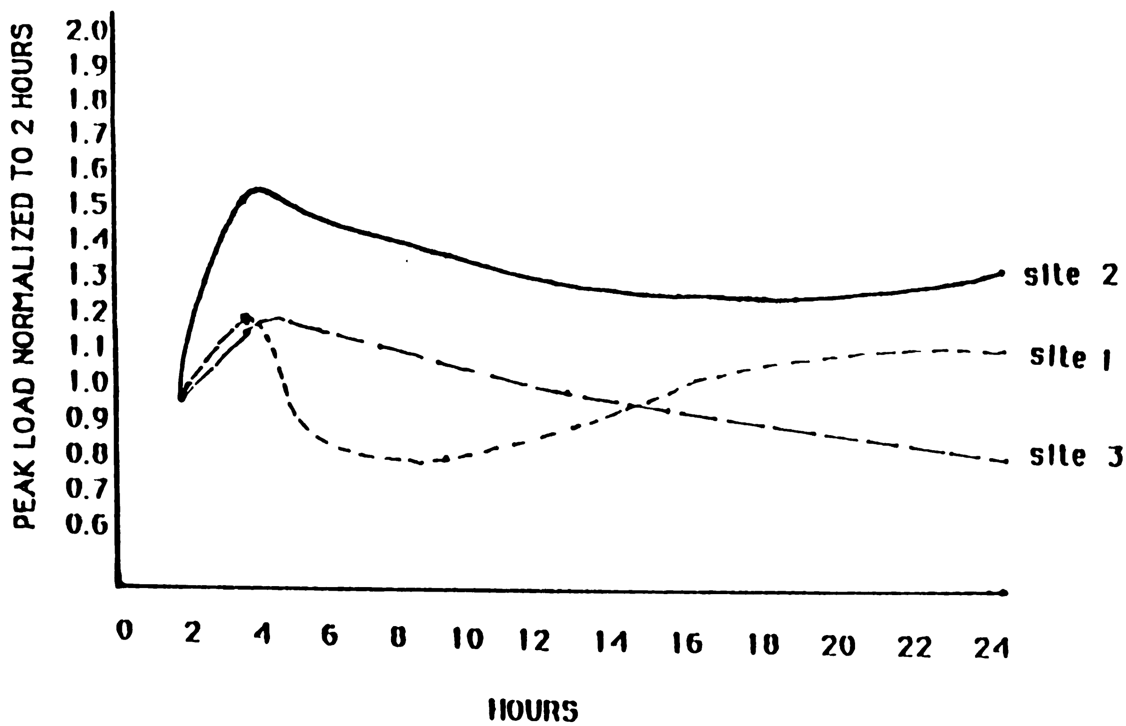

FIGURE 21. Comparison of sites from the medial condyle.

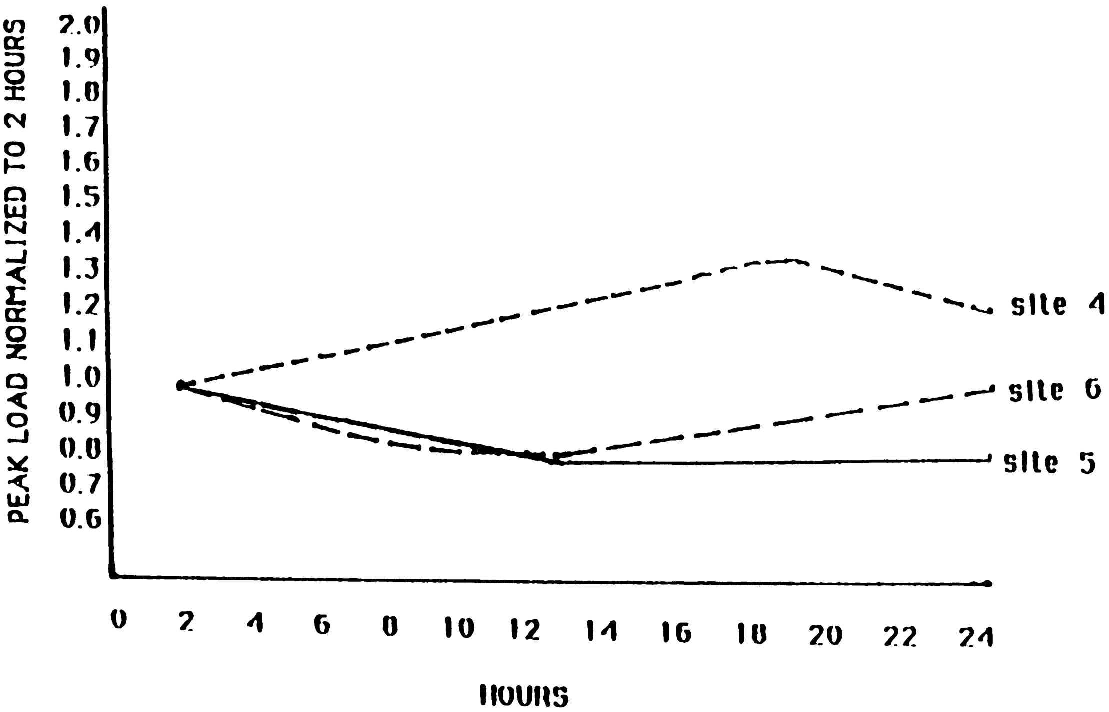

FIGURE 22. Comparison of sites from the lateral condyle.

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