

ABSTRACT

G. M. Seligman, M. D.

The Transplantation of Whole Knee Joints in the Dog

Department of Experimental Surgery

M. Sc.

An attempt has been made to clarify the role played by immunity in the late degenerative changes in the transplantation of whole joints.

Thus mature female dogs were divided into 4 groups as follows:

- i A control group, in which the joint was removed and reimplanted immediately.
- ii A group in which the joint was stored at -20°C for one to four weeks and then homotransplanted.
- iii Similar to group 2, but in addition the graft was irradiated at some point during storage.
- iv Similar to group 2, but in addition the recipient was placed on an immunosuppressant agent post-operatively.

Immune studies with respect to circulating and cell-bound antibodies to cartilage were also carried out.

When each group was examined radiologically, clinically, and histologically, group 1 did the best, followed in turn by group 3, group 4 and finally group 2. We therefore concluded that immunological factors do play a role in the late degenerative changes seen and this was specifically related to the degree and rate of bone replacement and subsequent preservation of joint architecture. The cartilage did not appear to be highly antigenic.

THE TRANSPLANTATION OF WHOLE KNEE JOINTS IN THE DOG

BY

G. M. SELIGMAN, M. D.

A Thesis submitted to the Faculty of Graduate Studies
and Research, McGill University, in partial fulfilment
of the requirements for the Degree of Master of Science
(Experimental Surgery)

Department of Experimental Surgery,
McGill University, Royal Victoria Hospital,
Montreal, Quebec.

PREFACE

This thesis has attempted to show that the late degenerative changes seen in the transplantation of whole weight bearing joints are due to the resorption of homotransplanted graft bone at a faster rate than its replacement by the recipient's new bone. Consequently, the subchondral bone-plate collapses and the joint architecture is thereby disrupted. By decreasing the antigenicity of the bone (by freezing, freezing and irradiation, freezing plus immunopressant drugs to the recipient) we could lessen but not prevent the joint degeneration. The cartilage degeneration was most likely due to mechanical factors, since studies to detect antibodies to cartilage were completely negative.

ACKNOWLEDGEMENTS

I wish to thank especially Dr. R. L. Cruess for his timely advice and guidance and for the use of all the laboratory facilities. I also am most grateful for the aid given by Dr. Isadore Yablon and Dr. E. George. Mr. Gordon Nutik, Miss Linda Spence, Miss Louise Dolly and Miss Mary Lee Coffin offered much help technically. Their surgical assistance is gratefully appreciated. A special word of thanks goes to those individuals who looked after the dogs post-operatively. Mr. N. Yiannou, Mr. G. Michalopoulos, Mr. E. Caney and Mr. A. Nagy, Mr. B. Bedoui and Dr. L. Lord. Mrs. Joyce Gilpin prepared all the microscopic sections and without her skills, there would have been no experiment. Many thanks go to the secretarial staff. Miss Gaby (our girl Friday) Micaloff for her assistance and help in general and to Miss Ann O'Connor who typed the thesis. Mrs. Judy Smith and the Department of Visual Aids were most helpful and their cooperation and guidance is most appreciated. Last but not least, to my wife, I thank her for her extreme patience and understanding and help in preparing this work.

INDEX

	Page
Preface	i
Index	ii
List of Illustrations	v
List of Tables	viii
1 <u>Review of Literature</u>	1
1) Historical Review	1
2) The Experiment of Herndon & Chase	6
3) The Immunology of Skeletal Tissue	9
a) General	9
b) Bone Immunology	10
c) Cartilage Immunology	14
4) <u>The Principles of Immunofluorescence</u>	17
a) Technique	18
b) Controls for Specificity	21
c) Non Specific Staining	21
d) The Preparation of Tissues	21
e) Applications	22
5) <u>Cartilage</u>	23
a) Histology	23
b) Metabolism	27
c) Nutrition	31
d) The Enzymes of Cartilage	32
e) Histochemistry	34

	Page
II <u>The Experiment</u>	36
1) Purpose	36
2) Material and Methods	36
a) Selection and Grouping of Animals	36
b) Operative Technique	38
c) Immunological Investigations	48
III <u>Results</u>	50
a) Radiological Observations	50
b) Clinical Observations	65
c) Gross Examination	71
d) Histological Examination	87
e) Immune Studies	105
IV <u>Discussion</u>	110
V <u>Summary</u>	118
VI <u>Conclusions</u>	120
VII <u>Bibliography</u>	121

LIST OF ILLUSTRATIONS

	Page
<u>The Indirect Fluorescent Antibody Technique</u>	
Figure 1 Indirect Localization of Antigen	19
Figure 2 Antigen - Antibody - Antiantigen Complex	20
<u>The Normal Histology of Articular Cartilage & Bone</u>	
Figure 3 Hematoxylin and Eosin Stain	24
Figure 4 Toluidine Blue Stain	26
<u>Radiosulphate in Normal Cartilage</u>	
Figure 5 Low Power	28
Figure 6 High Power - Cartilage Surface	29
Figure 7 High Power - Cartilage Bone Junction	30
<u>Operative Technique</u>	
Figure 8 Incision	39
Figure 9 Exposure of Knee Joint	42
Figure 10 Osteotomy Cut	43
Figure 11 Transplantation Complete	45
Figure 12 Application of Hip Spica	46
<u>Radiological Observations</u>	
Figure 13 Control 6 wks lateral view	51
Figure 14 Control 6 wks A P View	52
Figure 15 Control 18 mos. A P View	53
Figure 16 Control 18 mos. lateral view	54
Figure 17 Frozen Homotransplant 6 wks A P View	56
Figure 18 " 6 wks lateral view	57
Figure 19 " 6 wks.	58

Page

Figure 20	Frozen Homotransplant	3 mos.	59
Figure 21	"	9 mos. A P view	60
Figure 22	"	9 mos. lateral view	61
Figure 23	"	12 mos.	62
Figure 24	"	16 mos.	63
Figure 25	"	16 mos	64
Figure 26	Frozen Irradiated Homotransplant	12 mos.	66
Figure 27	"	13 mos.	67
Figure 28	"	14 mos.	68
Figure 29	Frozen Homotransplant Imuran	12 mos.	69
Figure 30	"	12 mos.	70

Gross Observations

Figure 31	Normal articular cartilage - femur		73
Figure 32	" " " tibia		74
Figure 33	Control	18 mos.	75
Figure 34	Control	18 mos.	77
Figure 35	Control	6 wks	78
Figure 36	Frozen Homotransplant	6 wks	79
Figure 37	"	14 mos.	81
Figure 38	"	16 mos.	82
Figure 39	"	16 mos.	83
Figure 40	Frozen Irradiated Homotransplant	14 mos.	84
Figure 41	"	14 mos.	85
Figure 42	Frozen Homotransplant Imuran	12 mos.	86

Histological Observations

Figure 43	Microscopic changes - diagramatic	88
Figure 44	Frozen Irradiated 6 wks	90
Figure 45	Control H & E 3 wks	91
Figure 46	Control Toluidine Blue 6 wks	92
Figure 47	Frozen Irradiated Toluidine Blue 6 wks	93
Figure 48	Control H & E 3 wks	94
Figure 49	Control H & E 6 wks	95
Figure 50	Control H & E 5 mos	96
Figure 51	Control H & E 16 mos	98
Figure 52	Autoradiograph 16 mos	99
Figure 53	Control Toluidine Blue 9 mos	100
Figure 54	Normal Cartilage synovial junction	101
Figure 55	Control Toluidine Blue 16 mos	102
Figure 56	Control Autoradiograph 18 mos	103
Figure 57	Control H & E 18 mos	104
Figure 58	Frozen Homotransplant H & E 14 mos	106

Immune Studies

Figure 59	Fluorescein labelled articular cartilage	107
Figure 60	Blocking Test	108

LIST OF TABLES

	Page
Table (1)	72

REVIEW OF THE LITERATURE

1 - Historical Review

The restoration of motion to a joint by the autogenous or homogenous transplantation of a comparable joint has been the basis of considerable experimental and clinical investigation for many generations. Tietze in 1902 was the first to perform a genuine joint transplantation; he replaced the resected lower end of the radius with a phalanx of the great toe. This was therefore a hemi-transplant. The first experimental studies on this subject were performed by Judet in 1906 and Axhausen in 1912 described the histological changes that occurred following whole joint transfer in animals. Lexer in 1907 carried out the first joint transplant in the human; this involved the homologous transplantation of a whole knee joint without its capsule. He performed such transplantations in 23 cases of which 12 had good function and mobility for a number of years. Later, however, mobility decreased and became painful due to extensive arthritic changes. Two joints were examined pathologically and it was found that the cartilage had been replaced by fibrous tissue and that there was extensive subarticular breakdown of the subchondral cancellous bone. May (1942) also performed homogenous whole joint and half joint transplantation clinically with some degree of success. The first real comprehensive study was not published until 1952 when Herndon and Chase reported their studies of transplantation of whole knee joints, both homologous and autologous, in dogs. This study deserves special mention

and will be discussed in greater detail later. More recently transplantation has been done on the metatarsophalangeal joints of the dog. Entin (1968) Erdelyi (1963). Entin and associates noted that the articular cartilage of whole joints and half joints appeared to survive autologous transplantation but ultimately underwent progressive destruction. The reason why such degeneration occurred could not be answered with certainty. They felt that the articular changes were due mainly to "altered joint mechanisms", ie: mechanical stresses, disturbance of vascular and nerve supply, and alteration of synovial fluid. Bruncke (1961) studied autotransplants in joints whose vasculature was maintained and showed that joints survived completely if the vascular hook-up was successful. No evidence of degeneration was seen after two years. They also concluded that lack of nerve supply was likely not a factor in degeneration, since the nerve supply of these joints had not been repaired. Slone and Reeves (1966) were successful in transplanting knee joints in dogs while preserving the blood supply to the joint. Long term studies are to be done by them but as yet nothing has been reported. Fiala (quoted by Vavrda, 1965) transplanted dog knee joints and in order to hasten autologous replacement of new bone, drilled holes to remove the cancellous bone from the graft bone ends. This was done in 12 dogs and 24 months following transplantation, 4 of the animals showed "very satisfactory results" at the time of sacrifice.

However, immunological problems may become more apparent since it has been shown by many workers (Khvorostukkin, 1958, Depalma, 1963, Burwell, 1964) that fresh homologous bone and cartilage transplants do not survive as well as homografts which have been previously treated by either freezing and/or irradiation and other physical means. This will be discussed in more detail later.

The treatment of bone tumors by excision and replacement with massive autologous and homologous osteochondral grafts has been reported recently by Ottolenghi (1966) and Parrish (1966). Ottolenghi stored his grafts at -20°C for periods of up to 6 months. Transplants in both the upper and lower limb were carried out in 6 patients and he noted that the grafts would eventually undergo resorption, collapse and disruption of articular surfaces after several years. The functional results remain good. Parrish stored his grafts at -20°C for three weeks at the most and reported very good long term results both in mortality and functioning graft.

Because of the lack of metal prostheses as a form of arthroplasty in the Iron Curtain countries, whole and half joint transplantations have been done more extensively than in the Western countries. In 1965, Kosinka et al. reported their recommendations and results of homologous joint transplants in humans. In a series of 30 patients whose ages ranged from 20 to 40 years, thirty two joints (30 Hips, 1 shoulder and 1 knee) were operated upon for degenerative arthritis.

They used homologous osteochondral grafts as described by Campbell, Papp and Depalma, which had previously been removed from young adult donors 16 to 24 hours following death. The grafts were then stored in paraffin oil at 4°C for 14 to 28 days. At operation as close a fit between graft and host was obtained so that no fixation was required. As well a capsulectomy and synovectomy were carried out. The patients were then placed in a hip spica for three weeks and then joint mobilization was begun. No weight bearing was allowed for 2 to 3 months. Their follow-up was from 18 months to 5 years, the average being 3 years and they reported that "the patients' progress had been favourable" with most having "good motion free of pain".

Based on their clinical experience, they recommended the following:

- 1 the contact between graft and host be as large as possible, preferably cancellous rather than cortical bone.
- 2 No weight bearing on the graft, the length of time depending on the size of graft used.
- 3 The deforming forces should be perpendicular to the graft-host junction.
- 4 The graft must be from adults and must not contain the epiphyseal plate since grafts from children tend to dissolve.
- 5 The subchondral bone of the graft must remain intact in order to support the overlying articular cartilage and must be transferred to a functional site. The thickness of

the subchondral bone must be no thicker than 3 to 4 mm. or else, with new bone replacement, structural integrity is lost and collapse of the articular cartilage with secondary degenerative changes occurs.

- 6 The synovium has an unfavourable effect on the transferred graft and therefore a synovectomy is performed with the grafting procedure.

These workers do not seemed concerned as to whether immunological phenomena play a role since there is no mention of it in their report.

That homologous as well as autologous osteochondral grafts can survive and continue to function has been shown by Depalma (1963) as well as by Campbell (1963) and Papp and Krompecher (1961). Depalma used radioactive S^{35} to show that osteochondral homografts in dogs, whose subchondral supporting bone was no thicker than 5 mm. and which had been stored in pooled dog plasma at $5^{\circ}C$ for no longer than 30 days, survived transplantation into a functional site (the hip joint) for the duration of their studies which was one year. Fresh homografts however, underwent delayed loss of bulk and degenerated earlier. Campbell also noted this. The bone in all the grafts, whether autologous or fresh or stored homologous, was completely replaced by new bone with no loss of structural integrity. Previous studies had shown that grafts thicker than 5 mm. underwent disintegration (Papp and Krompecher). In general the larger the graft used, the

greater the incidence of degenerative changes and failure. Thus osteo-chondral grafts have a better survival than half joints which in turn have a better survival than whole joints.

2 The Experiment of Herndon and Chase

The work of Herndon and Chase has already been referred to and because we are also transplanting whole knee joints in dogs, a more detailed description of their method, results and conclusions will be discussed.

Over a period of 4 years they performed whole knee transplantation in dogs in a manner similar to the one to be described in detail later. A plaster hip spica was removed at 2 weeks and the dogs allowed to roam at will. They noted that at least 2 years had to elapse before end evaluation. The dogs were divided into the following groups:

- 1 A control group in which the knee was removed and then immediately put back into its bed and fixed. This was therefore a reimplantation.
- 2 A group in which the knee joint had been stored at -20°C from one to several weeks.
- 3 A third group in which the knee joint was transplanted directly from one dog to another.

Out of 129 dogs operated upon, 56 were available for study. Many animals were lost because of distemper, diarrhea, and any animals in

which wound infection developed or fracture occurred. There were 19 animals in each group, except the first one which had 18. X-rays were taken at regular intervals and the animals were sacrificed at intervals from one to two years.

In the control group the functional results were noted to be excellent. The dogs would begin to bear weight at 4 to 6 weeks. X-rays showed the step-cut to unite in 4 to 6 weeks. Over the 2 years that the animals were followed, the joint space was well maintained and only mild arthritic changes were noted. Gross examination of the joint showed it to have excellent preservation of articular surfaces, menisci and ligaments. Arthritic changes were minimal.

Histologically, the sequence of creeping substitution of the avascular bone could be seen. By 4 weeks, fibrous replacement of the marrow cavity was complete and this was gradually replaced by marrow cells. Appositional new bone formation appeared in 3 to 4 weeks and by 6 months for the tibia, 9 months for the femur, the creeping substitution was complete. The articular cartilage remained normal for 14 days and then necrosis from superficial and peripheral areas to the deeper and weight bearing portions, occurred. Necrotic areas were eventually replaced by fibrocartilage while surviving islands of cartilage cells proliferated and contributed to the replacement;

the menisci were also eventually transformed to new fibrocartilage. In the region of the step-cuts, union was present by 1 month and the step-cut site completely obliterated by 6 to 9 months.

The group with the delayed homologous grafts were also followed for 2 years. The early clinical results were encouraging but longer follow-up revealed certain differences from the behaviour of the autologous grafts. In general, there was no appreciable differences in function or in x-ray findings until the fifth or sixth month. After this time, although joint function was satisfactory the x-ray showed increased density in the subchondral area followed by degeneration, collapse, and osteoarthritic change. Grossly the findings paralleled the x-ray appearance. Six to eight months post-operatively there was marginal articular erosions, degeneration and increased fibrous tissue formation. This led to complete joint destruction. Histologically a similar picture was seen but the timing and process of repair were different. The marrow was not replaced until after 2 to 3 months with rapid destruction of the cartilage after 1 month. Replacement was by pannus. Creeping substitution occurred but at a much slower rate and was not complete even after 2 years in some animals. Bony union was also much slower.

A third group in which direct homologous grafts were done, was designed to indicate whether the method of preservation was responsible for the degenerative changes noted or whether it was due

to an antigenic response of the host to foreign tissue. Results were closely parallel with those of the delayed homologous grafts clinically, roentgraphically and histologically. Thus the method of preservation was not the important factor in the degenerative changes seen. The factor or factors producing these changes were not determined in this experiment, but the authors thought that it may be related to "a type specific physiochemical structure of bone".

That the degenerative changes seen in homotransplants, both direct and delayed, are due primarily to immunological reactions between the host and recipient has yet to be determined. It was for this reason that this investigation into joint transplantation was initiated and has continued for the past 2 years.

3 The Immunology of Skeletal Tissue

a) General

It is not the purpose of this paper to pursue the immunological problem related to the transplantation of tissues. Nevertheless, a few basic facts should be emphasized. In their monumental review of tissue transplantation Russell and Monaco (1964) noted:

- 1 - Homograft rejection was a cell mediated phenomenon and that close contact between reactive and graft cells was necessary. These reactive cells were lymph node cells.

- 2 - Conventional circulating antibody was not an obligatory participant in the rejection of solid homografts.
- 3 - The weaker the histocompatibility antigens the more sensitive they were to irradiation; the dose depending on the type of tissue.
- 4 - A typical inflammatory cellular infiltrate was regularly associated with the rejection process.

b) Bone Immunology

That bone is antigenic was first demonstrated with any degree of certainty by Chalmers (1959). He showed that a second set phenomenon occurred when bone grafting with fresh homologous iliac bone was followed, 3 weeks later, by skin grafting from the same animal. Later work by Burwell (1964,1966) and Brooks (1963) showed that the antigenic component lay mainly but not completely in the red marrow and, in the case of cortical bone, within the bone itself. Whether the transplantation antigens lay in the osteocytes, matrix or both was not known but they could be reduced markedly in their activity by freezing, freeze-drying or by irradiation. Nevertheless certain transplantation antigens may persist but are usually so weak that they do not induce transplantation immunity.

The antigenic and osteogenic potency of bone seem to be related

since both lie within the organic material of grafted bone. (Urist, 1967). [It should be recalled that 35% of the dry fat free weight of bone is organic material and is composed of collagen, protein, polysaccharide and cellular material. Collagen constitutes 90 to 96% of this organic matrix (McLean and Urist, 1968)]. Urist calls this osteogenic potency the bone induction principle or B. I. P. In an elaborate set of experiments, using lyophilized decalcified rabbit bone, they noted that B. I. P. , although its composition could not be determined was derived from the organic matrix and was associated with, but not inseparable from the antigenic activity of bone. Very high doses of irradiation, 2 to 4 million r., destroyed B. I. P. Antibiotics had little effect on it but 6-MP did. This drug delayed new bone formation but whether this was due to inhibition of cell division, matrix production or immunosuppression was not known. If bone was rendered anorganic its osteogenic as well as its antigenic potency markedly fell. By means of the physical procedures previously mentioned (freezing, freeze-drying, low voltage irradiation) the antigenicity alone can be reduced without significantly affecting the other.

In a recent review Burwell (1968) made the following observations with respect to autologous, homologous, and heterologous cortical and cancellous bone grafts. Cortical autografts showed survival of

the outermost surface of osteocytes (0.3 mm. depth). However, these did not contribute to osteogenesis; only the periosteal and endosteal osteoblasts did. Bohr and his co-workers (1968), using a tetracycline double labelling technique, noted that the osteogenic cells of fresh cancellous autograft when transplanted under favourable conditions ie: immediately or stored in saline for less than 1 hour, would survive and produce new bone when transplanted orthotopically. Exposure of the graft to air for one hour markedly decreased its inductive as well as its osteogenic ability.

Cancellous autografts are best because:

- 1 there is a large surface area of osteoblasts which can contribute to osteogenesis.
- 2 the graft contains marrow which has a high osteogenic capacity and
- 3 the graft allows for vascular invasion.

When homografts were tested for antigenicity, vascular invasion, osteogenic potential and remodelling, Burwell found the marrow free freeze-dried cancellous bone was second only to autologous cancellous bone. Heterografts were not recommended.

Heiple et al. (1967) critically examined processed calf bone (Boplant, Squibb) and also came to the same conclusion. They found that heterograft bone behaved like a foreign body, inciting an acute inflammatory response. It also had very little bone induction potential and rated poorest among all the systems tested. These

included, in decreasing order of inductive potential homografts which had undergone a) freeze drying; b) freezing; c) decalcification; d) freezing and irradiation; e) freeze drying and irradiation; f) no treatment ie: fresh; g) deproteinization. Deproteinized heterografts also did very poorly.

Kingma and Hampf (1964) studied the behaviour of blood vessels after experimental transplantation of fresh cortical autografts and frozen cortical homo and heterografts into the iliac bone of rabbits. They observed that vascularization and replacement by new bone is much slower in the homotransplant (10 and 50 weeks respectively) than in the auto transplants (3 and 25 weeks respectively). Heterografts remained as a dead foreign body inciting an acute inflammatory response followed either by complete resorption or fibrous tissue encapsulation.

However, the osteogenic potency of the marrow cannot be underestimated. In fact Burwell found that previously stored frozen composite homografts impregnated with autogenous red marrow induced bone formation nearly as well as autogenous grafts. Fresh marrow free homologous grafts had little osteogenic potency. Thus it can be said of bone that immunological mechanisms are not important with respect to using homologous bone in bone grafting, since its antigenicity can be markedly reduced. The inductive potential

of the bone graft is the most important criterion in using treated foreign bone and when treated as mentioned previously and transplanted into marrow closely associated with it, it will induce new bone formation. Its effectiveness is increased if all soft tissues are cleaned from the bony spaces of the graft.

c) Cartilage Immunology

Cartilage has been shown to be mildly antigenic but all references regarding its antigenicity are to the chondrocytes (Craigmyle 1955, 1958, 1968 and Peacock 1957). Homologous transplants of cartilage survive longer than any tissue except cornea (upwards to 2 years). This long survival is attributed to two facts:

- 1 - the matrix tends to act like a semipermeable diffusion chamber and although not preventing release of antigen from the graft, it does prevent antibody from reaching the cartilage cell;
- 2 - the degree of antigenicity is very low because of the low cell population.

Peacock was unable to induce a second set phenomenon using cartilage homografts and skin from the same donor. However, the chondrocytes of heterografts of cartilage have been reported to die soon after grafting and the inert matrix eventually disappears over a period of 2 years. Gibson (1953). Within the last decade, there have been a number of papers in the literature implicating the matrix as being

antigenic also. Lowei and Muir (1963) Di Ferante (1964), Sandson (1966).

Before discussing this however, the general structure of cartilage should be mentioned briefly. Cartilage is avascular, composed of a firm, turgid, water-rich gel. Strands of fibrous protein are dispersed throughout the gel binding its mass to various structures. Anderson (1962) Embedded in the gel are chondrocytes which secrete the cartilage matrix and regulate its consistency. Curran (1956) Schallen (1958) Spicer (1967) Dziewia (1954). Its composition can vary, but in general 50% of the dry weight is collagen and 50% is sulphated mucopolysaccharide (MPS) or chondromucoprotein (CMP), lipids and glycogen. CMP is composed of a rod like or branched protein molecule to which aggregates of sulphated sugar polymers or polysaccharides are attached. The polysaccharide can be chondroitin sulphate A & C, or keratin sulphate. Most of it is chondroitin sulphate A and the protein moiety is not collagen. It contains no hydroxy proline and very little proline. The principle fibrous protein is collagen. The protein polysaccharide or PP complex can be fractionated by ultra-centrifugation into two products, a light fraction PPL and a heavy fraction PPH. Both these products contain chondroitin sulphate and keratin sulphate and are firmly bound to protein. Furthermore, these PPL complexes are antigenic and it is the protein part which is antigenic.

This PPL has 2 antigenic determinants, one being species specific and therefore similar to an isoantibody, the other being common to several species (human, beef, pork). Sandson (1968) noted that the hyaluronate of the streptococcus, the PPL from human cartilage, and the hyaluronate protein from synovial fluid all share a similar antigenic determinant. This common determinant is not limited to the joint but is present in the heart and other tissues, for example, the kidney. However, there have been no studies to date to determine whether PPL from an animal of one species would be antigenic when injected into another animal of the same species. Sandson (1969), attempted this in rabbits but his results were equivocal. No antibody against chondroitin sulphate has been found and collagen has a very low antigenicity.

The antigenicity of cartilage can be altered in a manner similar to bone. Thus Khvoroslukkin (1958) could diminish the antigenic properties of cartilage by storage at 0°C in serum for 7 days. Depalma showed that homologous grafts stored in 20% pooled dog plasma at 5°C for no longer than 30 days would survive much better than fresh homografts. However, cartilage which is frozen results in total cell death. Braid (1966). Viable homografts maintain their structural integrity and resist vascular invasion to a far greater extent than non viable grafts. Hagarty (1960). Chesterman et al. (1968) transplanted homografts of intact and

papain treated hyaline cartilage and isolated chondrocytes into cancellous bone beds in rabbits. They noted that so long as the cells remained viable, new matrix was formed and the grafts were not replaced by bone. Dead grafts were eventually replaced by bone. At no time was there histological evidence of any immunological reaction. Cartilage which had been cooled to -79°C in dimethyl sulphoxide and then thawed survived whereas, with no protection it died. Hence the authors stressed the importance of chondrocyte survival in the maintenance of cartilage grafts.

Immunofluorescent staining techniques can be used to detect antibody to PPL. However, a brief description of the technique is warranted before applying it specifically to cartilage.

4 The Principles of Immunofluorescence

Fluorescent antibody technique depends on the fact that dye molecules can be attached to a protein without impairment of its function as an antibody. The essential feature of the fluorescent antibody method thus developed is the precipitation of labelled antibodies from a concentrated solution placed on a tissue section containing the antigen in reactive form. The labelled antibody becomes firmly attached only to the sites containing reactive antigen. The excess of labelled antibody is washed away before examining the section with the fluorescent microscope.

a) Technique

In general there are two techniques available for the localization of antigen or antibody. These are the direct and indirect methods. The former depends on the attachment of ambient antibody to a fixed location antigen. However, this method is incapable of localizing tissue antibody. To serve this and other purposes the indirect multiple layer or sandwich technique was evolved. The simplest of these methods made use of the interposition of a layer of antigen between the tissue antibody (fixed location) and the same antibody coupled in vitro with fluorescein isocyanate. Coons (1955).

An alternative application of the sandwich technique is to use fluorescent antiglobulin for the detection of layered specific antibody attached to the antigen in or on the cell. This technique differs from the other in the fact that the intermediate layer is acting as an antibody on the one hand but as an antigen on the other. (Figure 1). This variety of the sandwich technique is especially useful where antigen is difficult to obtain and the production of specific fluorescent antibody thus difficult to achieve. Many fluorescent labelled anti-antibodies can be attached to a single antigen via the antibody (gamma globulin), and thus in comparison with the direct technique, the indirect approach provides an increased final fluorescence which may be of the order of 3 or 4 times.(Figure 2).

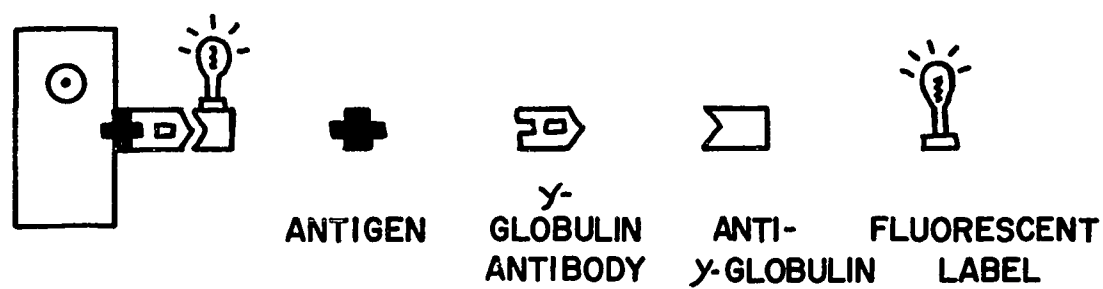


Figure (1)

The Indirect Fluorescent Antibody Technique
Indirect Localization of Antigen

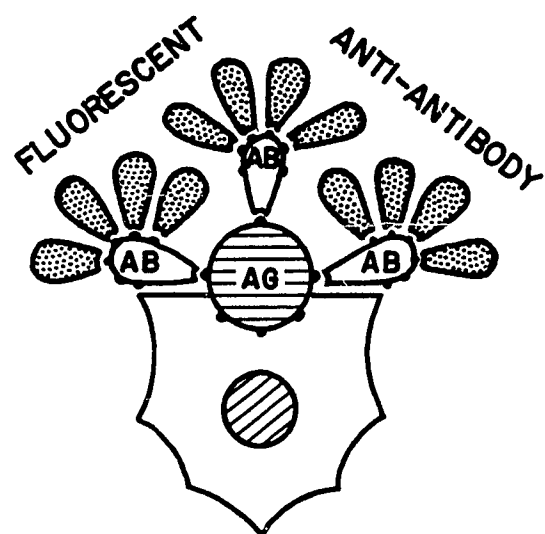


Figure (2)

The Indirect Fluorescent Antibody Technique:
Antigen - Antibody - Antigen Complex

AG = Antigen

AB = Antibody

b) Controls for Specificity

Before accepting any localization of protein obtained by immunofluorescence it is necessary to apply controls for specificity. Of the various control methods employed, the so called Blocking Test is often regarded as an absolute test. In this test staining should be inhibited by pre-treatment of the tissue section with unconjugated specific immune serum but not inhibited by non immune serum.

c) Non Specific Staining

This has been found to be mainly due to the non immunological staining of tissues with immunologically active conjugate - "protein-protein" reaction or interaction. This can be brought to a minimum by first, reduction of tissue non specific reactive sites (basic groups) by denaturization, fixation or other special procedure, second, by preparation of a correctly coupled conjugate and thirdly efficient ultraviolet microscopy in order to allow the lowest possible level of labelling to be correct. The greatest degree of non specific staining is found in fresh frozen cryostat sections even after the usual post fixation in alcohol and acetone.

d) Preparation of Tissues

Two types of tissue sections have mainly been used for fluorescent antibody studies, freeze dried and fresh frozen sections.

In both cases a necessary prerequisite is satisfied that the tissue protein (antigen or antibody) remain substantially unaltered as far as their immunological properties are concerned. There is no doubt however, that the physical properties of many proteins are radically altered by freezing and drying.

The most popular method of fixation for both freeze dried and cold microtome or cold knife sections has been 85 to 95% ethanol or absolute methanol. However, when a new problem is being considered the correct course must be to test the preservation of the antigen or the antibody concerned by using the most suitable methods, some of which are listed here:

- 1 - freeze drying, acetone post-fixation
- 2 - fresh frozen sections, acetone post-fixation
- 3 - ethanol fixation, paraffin embedding
- 4 - formalin fixation, paraffin embedding
- 5 - freeze substitution, paraffin embedding
- 6 - freeze drying, ethanol vapour fixation. (block)

e) Applications

Briefly, fluorescent antibody technique can be used for the detection of foreign antigens, nuclear fluorescence, detection of native antigen, detection of antibodies to foreign antigens and determination of immunoglobulin structure.

With respect to cartilage immunofluorescence Barland (1966) observed the normal human articular cartilage which had been reacted with antisera to human PPL revealed localized fluorescent staining largely confined to the chondrocyte cytoplasm, the lacunae, and relatively small areas of perilacunae matrix. In the superficial zone the perilacunar matrix staining assumed spindle shaped forms. In the middle and deeper zones, the matrix showed only small halos of granular fluorescence, immediately surrounding the lacunae.

5 Cartilage

a) Histology

A brief review of the structure of normal articular cartilage both from a light and electron microscopic view will enable us to understand its response to various stimuli a little better. Mankin (1967) describes 4 layers in normal articular cartilage. (Figure 3).

- 1 - The cartilage surface in which the cells are flattened, and small with their long axis parallel to the surface. This is the gliding area or tangential zone.
- 2 - The superficial third or transitional intermediate zone in which the cells are somewhat larger and nearly round and disposed at random.

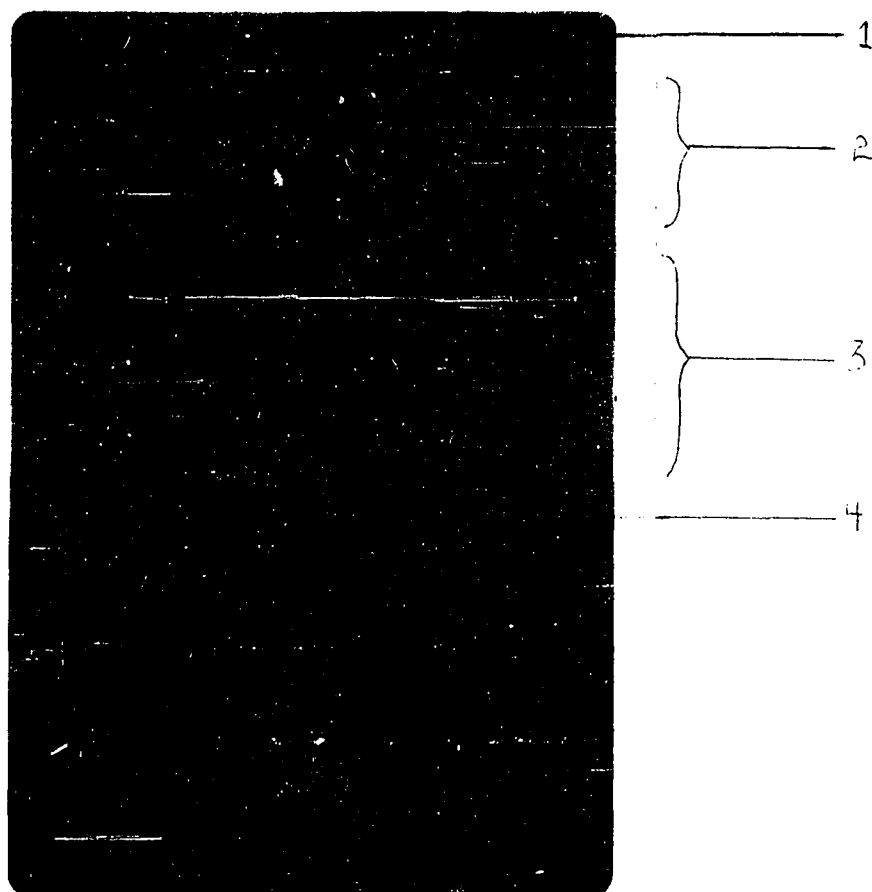


Figure (3)
Hematoxylin and Eosin Stain

The Normal Histology of Articular Cartilage of Bone x 10

Layer 1	Tangential Zone	Layer 3	Radial Zone
Layer 2	Transitional Zone	Layer 4	Tide Mark

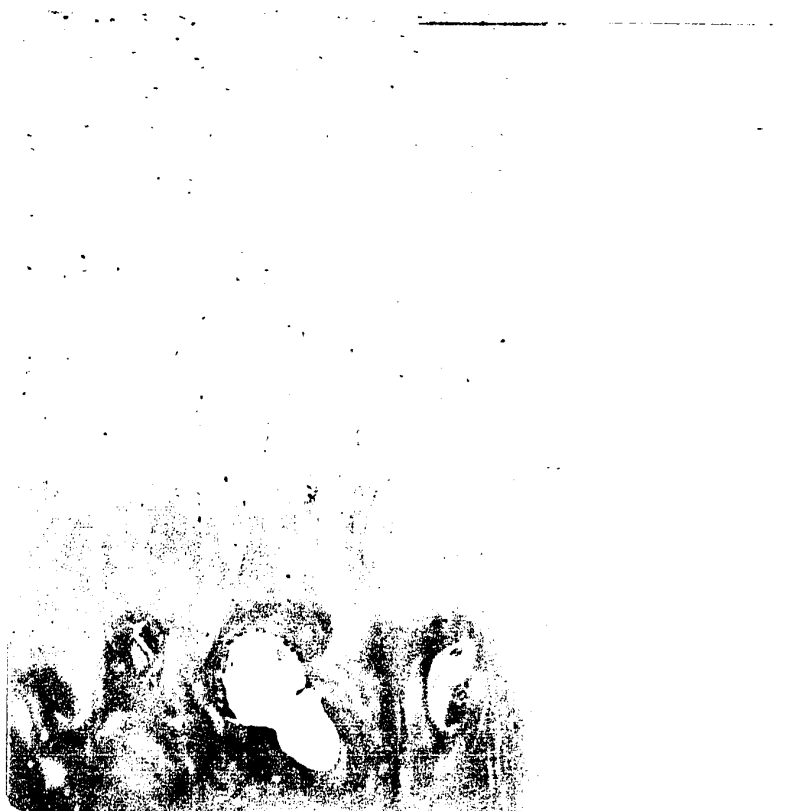


Figure (3)
Hematoxylin and Eosin Stain

The Normal Histology of Articular Cartilage of Bone x 10

Layer 1	Tangential Zone	Layer 3	Radial Zone
Layer 2	Transitional Zone	Layer 4	Tide Mark

3 - the radial zone, composed of large round cells arranged in short irregular columns.

4 - the layer of calcified cartilage-the tide mark- which is complete in the adult.

The first layer contains little polysaccharide as indicated by metachromatic staining (Figure 4). Also the cells here synthesize little or no sulphated mucopolysaccharide. At the cartilage surface is a thin, bright line which is devoid of collagen fibre and is called the lamina splendens. In the second and third layers active synthesis of mucopolysaccharide is occurring.

Electron microscopic studies show that the first layer contains typical collagen bundles running parallel to the surface at oblique and right angles to each other. Consequently there is very little matrix between the bundles; hence the lack of metachromasia. In the deeper part of this zone the collagen bundles are smaller and more ground substance is found as well as more fibrils. In the second and third layers the collagen is more randomly orientated and contains more ground substance. The lamina splendens is composed of fine fibrils only. (Weiss, 1968).

Bullough (1968) showed by light polarized and electron microscopy that the collagen fibres at the surface of the articular cartilage are directed, not by joint movement, but by paths along

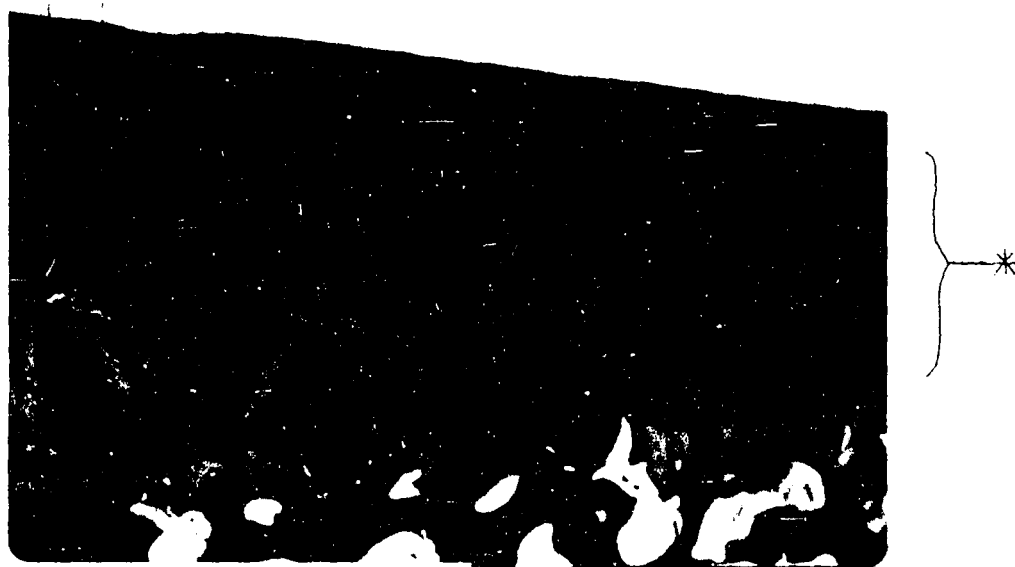


Figure (4)
Toluidine Blue Stain

The normal Histology of articular cartilage and Bone x 2.5

* positive metachromasia

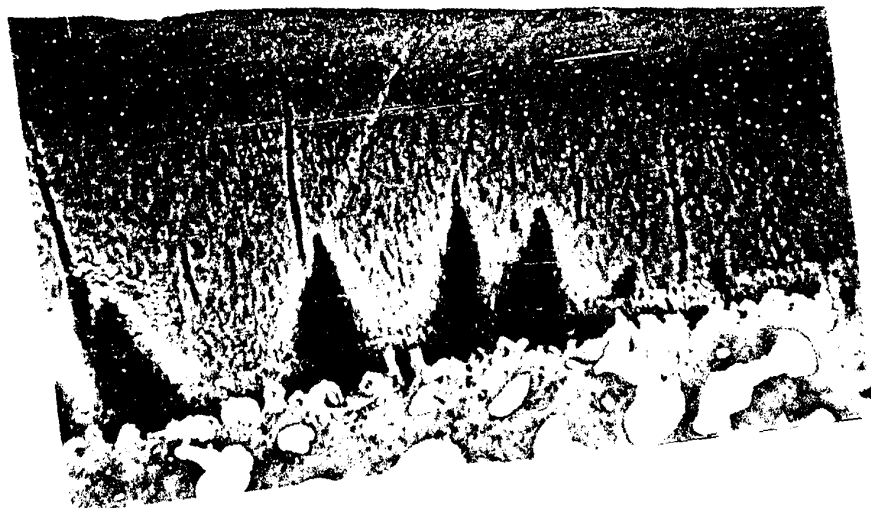


Figure (4)
Toluidine Blue Stain
The normal histology of articular cartilage and bone $\times 2.5$
* positive metachromasia

which tension or load distribution occurs. These tension lines can be detected grossly by inserting pins into the articular cartilage, staining the surface with india ink and then noting which way the cartilage has "split" after insertion of the pin. These split lines are not seen if the surface layer of cartilage is removed. This corresponds to the different direction of the collagen fibres in the deeper zones.

b) Metabolism

Chondrocytes synthesize mucopolysaccharide and the latter is necessary to maintain the structural integrity of cartilage. Mucopolysaccharide metabolism and therefore cartilage viability can be demonstrated by the use of radioactive S^{35} uptake. There have been numerous studies to confirm the fact that uptake of S^{35} is proof of viability of chondrocytes. Curran (1956), Dziwiewia (1954) and Schallen (1958). It has been shown that the S^{35} is at first taken up by the Golgi complex within the chondrocyte. Pelc (1955) Spicer (1967). It is seen in the cytoplasm of the chondrocyte within 2 hours after administration and by 24 hours the radioactivity diminishes over the cell and increases over the matrix surrounding the cell. (Figures 5, 6 and 7). However, there is little S^{35} uptake in the superficial layers; most is taken up by the deeper cells, Meachim (1967). Adult cartilage also takes up S^{35} avidly and

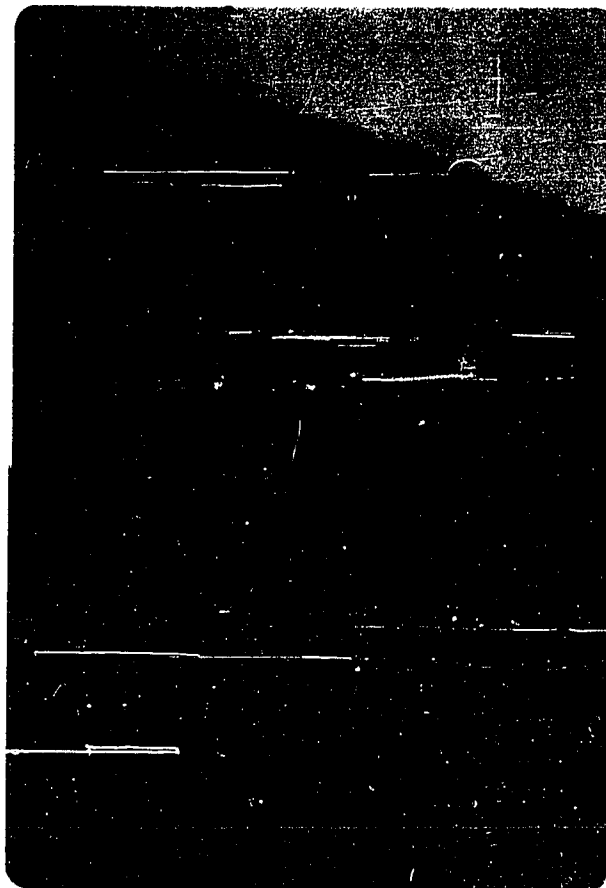


Figure (5)
Radiosulphate in Normal Cartilage (Low Power) x 10
Note relative absence of radiosulphate uptake in upper zones and
greater uptake in deeper zones



Figure (5)
Radiosulphate in Normal Cartilage (Low Power) $\times 10$
Note relative absence of radiosulphate uptake in upper zones and
greater uptake in deeper zones

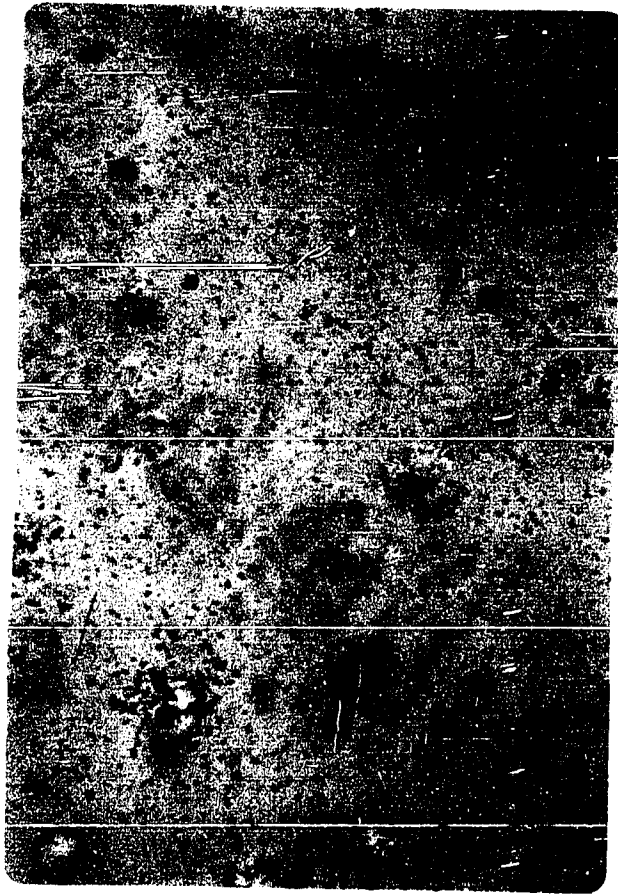


Figure (6)

Radiosulphate in Normal Cartilage (High Power) x 25
Cartilage Upper Zones and Cartilage Surface

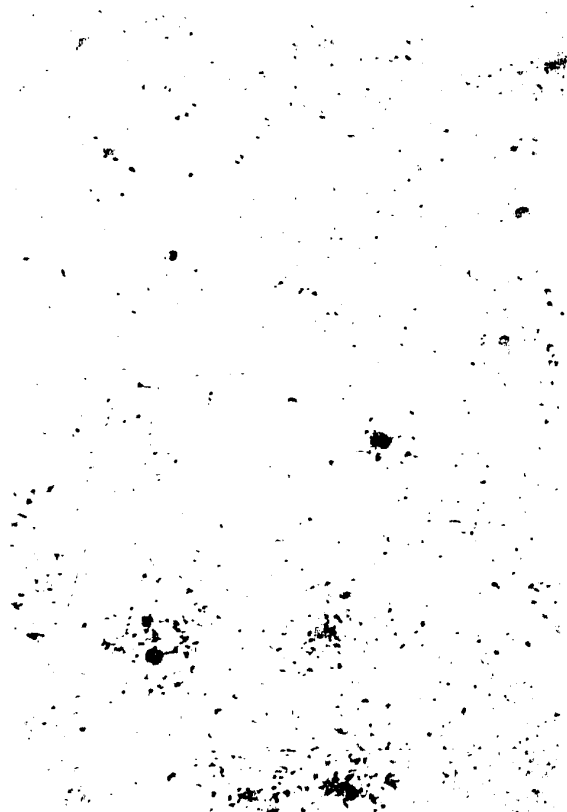


Figure (6)

Radiosulphate in Normal Cartilage (High Power) x 25
Cartilage Upper Zones and Cartilage Surface



Figure (7)
Radiosulphate in Normal Cartilage (High Power) Cartilage Bone
Junction
and deeper zones of cartilage x 25



Figure (7)
Radiosulphate in Normal Cartilage (High Power) Cartilage Bone
Junction
and deeper zones of cartilage x 25

chondroitin sulphate is therefore constantly being renewed and structural modifications are constantly occurring within the cartilage. Gibson (1953), Bostrom (1953) and Pelc (1955). Mankin (1967) has shown that there is very little collagen turnover in cartilage whereas the protein polysaccharide turnover is much more rapid, its half life being about 15 days while that of collagen is about 90 days.

c) Nutrition

This subject has been somewhat controversial until very recently. The controversy centered around whether articular cartilage received its nutrition from the underlying subchondral blood vessels, the synovial fluid or both. Ekholm in 1953 concluded from his studies that both sources were important, especially the former one. However, he had used immature animals whose epiphyses were still open and who did not have a tide-mark as seen in mature animals. The controversy has most likely been resolved if one realizes that the source of nutrition of articular cartilage in mature animals differs from that in immature animals. Maroudas (1968), Maroudas et al. (1968) and Hodge (1969) have shown that in immature animals both sources of nutrition prevail, whereas in mature animals, the presence of the tide-mark blocks diffusion of any nutrients from the subchondral blood vessels and all nutrition is

via the synovial fluid. Neither glucose nor water was shown to cross the tide-mark. The transport of materials into cartilage depended on diffusion alone. There is no active transport mechanism demonstrated which depend on living cells except the active uptake of S^{35} . The rate of diffusion was significantly lower in the immobilized joints. However, joint movement ie: alternate compression and relaxation did not increase the rate of diffusion but aided cartilage nutrition by the alternate pumping action of compression and relaxation. These workers showed both mathematically and histologically that, because cartilage nutrition is by diffusion only, it could not be thicker than 3 mm. and if the joint were immobilized, then the cartilage could be no thicker than 1.5 mm.

d) The Enzymes of Cartilage

Cathepsin D activity has been found in normal and chondromalacic cartilage and is increased in the latter. Chrisman (1967). This enzyme can split the protein polysaccharide bonds and its activity is found in the halo area where Barland has found immunofluorescence indicative of just this type of matrix degradation.

Regarding hemarthrosis and the presence of plasmin and hyaluronidase in synovial fluid and their ability to attack chondroitin sulphate, Chrisman observed that it was unlikely that these two

extrinsic enzymes could pierce the armour coat of intact surface collagen. Cartilage degeneration is most likely initiated by enzymes which lie within the cartilage itself and then the extrinsic enzymes may secondarily become involved. Cathepsins are also present within the lysosomes of the chondrocytes but lysosomal disruption is not necessary for the enzymes to become active because catheptic activity is found also in the unstructured areas of the cell and in the matrix surrounding it. It should be pointed out also that synovial lining cells contain lysosomes which in turn can release their enzymes and breakdown the protein polysaccharide complexes of cartilage. This is what most likely occurs in rheumatoid and tuberculous arthritis in which a pannus extends over the underlying cartilage.

The other enzyme capable of breakdown of protein polysaccharide is the protease from polymorphonuclear leukocytes. Curtiss and Klein showed that when an acute staphylococcal pyogenic arthritis was induced in dogs there was a 40% loss of hexosamine with no apparent loss of hydroxy proline ie: there was loss of chondroitin sulphate but not collagen. Histochemically there was a loss of metachromasia corresponding closely to the decrease in hexosamine and sulphate but macroscopically there was no gross cartilage destruction. In short, the enzymes which degrade cartilage can

therefore be divided into exogenous and endogenous groups.

The former includes 1. the protease from polymorphonuclear leukocytes, 2. the enzymes from synovial tissue and 3. plasmin; in the endogenous group there is cathepsin D from chondrocytes. Lack (1967).

e) Histochemistry

Metachromasia is closely linked with mucopolysaccharide metabolism and such staining is indicative of the presence of mucopolysaccharide. More specifically metachromasia depends on the presence of acid radicals (sulfhydryl and carboxyl) and, according to Pearse, their spatial relationship and orientation. Mucopolysaccharide can be altered in several ways either by substitution or by depolymerization:

- 1 - protein may mask metachromasia by disassociation in an acid medium and subsequent competition with the dye cations for the chromotrope.
- 2 - the release of polysaccharide from its combination with other tissue substances.
- 3 - the action of a specific chondroitin depolymerase

If there is increased breakdown of mucopolysaccharide as would occur with enzymatic breakdown of cartilage, then metachromasia would decrease unlike S^{35} which may remain the same or even increase.

On the other hand if S^{35} uptake and metachromasia diminish then chondrocyte damage and death may be occurring as in an immune reaction, or may be due to ischemic death of cartilage. Cruess (1967).

THE EXPERIMENT

1) Purpose

In order to investigate the role of immunology and its effects if any on the late degenerative phenomena noted by Herndon and Chase, an experiment was set up along similar lines but with modifications.

2) Materials and Methods

a) Selection and Grouping of Animals

In the initial stages of the experiment mature rabbits had been used, but because of the technical difficulties involved (anesthetic deaths, the difficulty of adequate internal fixation, and application of plaster post-operatively in such small animals) they were soon replaced with larger animals. Thus mature female dogs whose epiphyseal plates were closed were used. It was found in animals whose epiphyses were still open that disintegration of and/or slipping of the epiphysis would occur soon after the operation. Secondly, it has been reported by Erdelyi that changes secondary to a disturbance of endochondral ossification beneath the articular cartilage could effect this cartilage and cause deformity in a growing animal. Thirdly, the epiphyseal plate could act as a barrier to creeping substitution. Male animals could not be used because of the difficulty in applying a hip spica

near the genital area. Almost invariably the males developed severe skin problems in and around the scrotum and penis which necessitated sacrificing the animals.

The animals had their knee x-rayed to ensure that their epiphyseal plates were closed and they were then isolated in special pens for 10 to 14 days during which time they received a single dose of distemper vaccine and a course of tetracycline for 3 days. In this way only healthy, mature animals were operated upon.

The animals were then divided into the following 4 groups:

1 The control group which in reality underwent a reimplantation rather than a transplantation. There were 13 animals in this group and the longest survivor was 18 months.

2 The frozen homotransplant group, in which the knee joint was stored at -20°C under sterile conditions for 1-4 weeks and then transplanted. There were 9 dogs in this group and the longest follow-up was 16 months.

3 The frozen irradiated group, in which the knee joint, in addition to storage at -20°C received 125 rads of x-ray irradiation. There were 9 dogs here, and the longest follow-up was 14 months.

4 The Imuran group, in which the animals received a frozen homotransplanted joint as in group 2, but in addition were put on

Imuran 4 mg/kilo/day which was tapered to 2 mg/kilo/day after the first week post-operatively. There were 5 animals in this group, and the longest follow-up was 12 months.

At no time was any knee stored longer than 4 weeks and all had been removed under sterile conditions and placed in sterile bottles whose tops were then screwed tightly and then sealed with adhesive.

b) Operative Technique

Under general pentobarbital anesthesia and sterile conditions, the knee joint of the animal was exposed, using a curvilinear medial parapatellar incision from just proximal to the medial malleolus to the upper lateral aspect of the thigh (Figure 8): soft tissue dissection was carried down to the lateral border of sartorius. The deep fascia was incised at this point and the sartorius was reflected medially, thus exposing the underlying quadriceps femoris. Its fascia was incised and the cleavage plane developed between the vastus lateralis and rectus femoris. Hemostasis was secured. This exposed the vastus intermedius.

The tibia was then exposed subperiosteally by first incising the periosteum along its medial surface; thence, the incision was carried to a point just distal to the tibial tuberosity. The

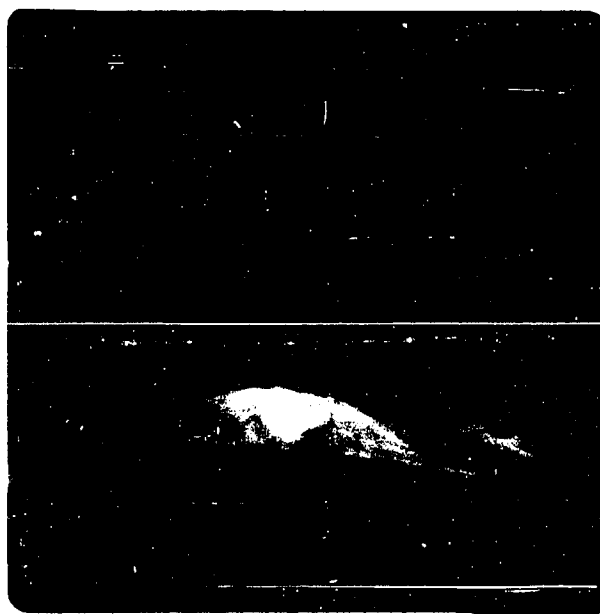


Figure (8)
Operative Technique - Incision



Figure (8)
Operative Technique - Incision

deep fascia over the anterior compartment muscles was then incised close to the tibial tuberosity and was continuous with the incision of periosteum on the tibia. This incision was such that the deep fascia could be sutured to the soft tissue still attached to the tibial tuberosity during closure. Subperiosteal dissection of muscle was then carried out. In the process, the nutrient artery to the tibia was always severed. The joint capsule and synovium were then incised in line with the incision lateral to the tibial tuberosity and was continued up across the insertion of the rectus femoris, thus enabling one to displace the patella medially. After having dissected the fat pad free from the patella tendon, the tibial tuberosity with its attached tendon was removed using an osteotome and hammer. This exposed the knee joint completely.

The femoral periosteum and vastus intermedius were then excised anteriorly and then subperiosteal dissection of all soft tissue and muscle attachments was carried out. In the process the nutrient vessels to the femur were almost always retained. The joint was now cleared of its soft tissue attachments as follows: beginning laterally the tendons of the extensor hallucis longus and popliteus were identified, tagged and cut. The origin of the lateral head of the gastrocnemius was severed as was the lateral collateral ligament. By using the lateral ligament as a

guide to the proximal end of the fibula, we could identify and open into the proximal tibiofibular joint and thus sever the attachments of the proximal end of the fibula to the tibia. The lateral popliteal nerve was left undisturbed. This allowed relatively easy access to the popliteal space which was approached from the lateral side. By careful dissection, the popliteal vessels and the genicular branches were dissected from the posterior surface of the knee joint as far medially as possible.

On the medial side, the collateral ligament was preserved and the medial head of the gastrocnemius was divided. The tendon of the semi tendinosis was identified, tagged and severed. Any remaining soft tissue attachments to the postero-medial aspect of the joint were now dissected carefully and this left the knee joint with the distal 2/3's of the femur and the proximal 2/3's of the tibia exposed. (Figure 9). Hemostasis was secured.

An oblique step-cut osteotomy was then done with a distance of about 5-6 cms. between each step and the knee joint removed. Its marrow cavity was then curetted and all soft tissue except the collateral ligaments, tendons and menisci were removed. (Figure 10). A synovectomy was not done.

Then depending on the group to which the animal belonged, a



Figure (9)
Exposure of knee joint



Figure (9)
Exposure of knee joint



Figure (10)
Osteotomy Cut

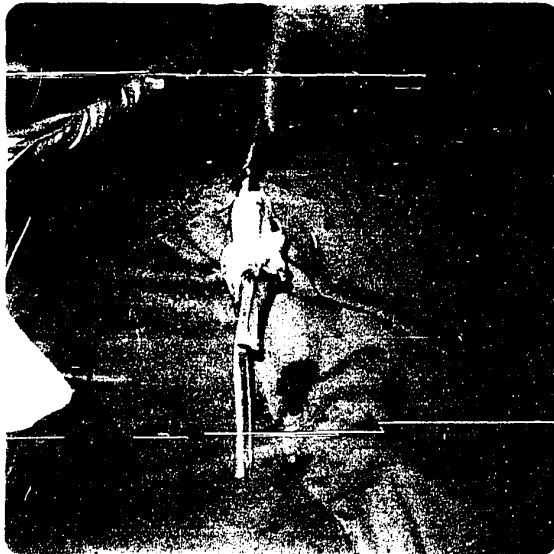


Figure (10)
Osteotomy Cut

knee joint of similar size was transplanted to the site of the space previously occupied by the original knee and secured in place by 2 screws and 4 circumferential wires; 2 in each bone (Figure 11). This ensured firm fixation. Drill holes using a 7/64 inch drill were then placed through the tibial tuberosity and at the attachments of the gastrocnemius, so as to facilitate re-attachment of that muscle.

After washing the wound with 1/2% neomycin solution the wound was closed. The two heads of gastrocnemius were reattached to their original site and then the tendon of the popliteus, fibular collateral ligament, the tendons of the extensor hallucis longus and semi tendinosus were resutured in that order with #2 chromic. The muscle and periosteum of the femur were then re-opposed and the insertion of the rectus femoris into the patella was reattached and the patella relocated. Then, by means of a long screw through the tibial tuberosity, the patella tendon was reattached to the tibia and this was reinforced with a stout wire through the tibia at a site just distal to the screw.

The periosteum of the tibia was sutured as well as the joint capsule using interrupted #1 chromic.

#2 chromic was used for the subcutaneous layer and interrupted running 2-0 for skin.

The hip, knee and ankle were then gently flexed to 90° and a hip spica applied (Figure 12). Figure 13 is the radiological appearance of the transplanted whole joint.



Figure (11)
Transplantation Complete



Figure (11)
Transplantation Complete



Figure (12)
Application of Hip Spica



Figure (12)
Application of Hip Spica

The animals were immobilized post-operatively for 3 weeks and then allowed full activity. Clinical and radiological assessments were made at regular intervals, and, 12 to 16 hours prior to sacrifice, 100 microcuries of radioactive S^{35} * were injected into both knee joints of the animal. At sacrifice, the joint was photographed, slices of fresh cartilage were quick frozen for later immunofluorescent studies, and sections of patella and both femoral and tibial articular surfaces were taken from both knees. These were fixed in 10% formalin and decalcified**. Tissue sections were then stained with hematoxylin and eosin and toluidine blue. As well autoradiographic slides were prepared by the method of Mankin using NTB-3 Kodak Nuclear Tract Emulsion and developed according to the method of Kopriwa and Leblond.

Animals which developed infection or had loss of fixation with gross angulation at the osteotomy site were not included in the study. Animals having loss of fixation with less than 5% angulation were included.

*Supplied as sterile sodium sulphate³⁵ 200 microcuries per cc.
Charles Frosst Co. Ltd.

**A special decalcifying ion exchange solution supplied by
Mr. Fotheringham, Department of Pathology, Montreal General
Hospital.

c) Immunological Investigations

Both serum and cell antibodies to cartilage were sought for by the following methods:

To detect any circulating antibodies to cartilage and specifically to PPL, weekly serial blood samples were withdrawn from recipient animals of all groups and tested for the presence of any anti PPL antibodies using lyophilized bovine PPL antigen and agar double diffusion studies as described by Ouchterlony (1958).

Immunofluorescent staining techniques were employed to detect the presence of any cell bound anti PPL antibodies. Because there was much difficulty in applying this technique to cartilage, a detailed description of the method follows:

Fresh cartilage was quick frozen to -70°C in dry ice and then stored at -20°C in 2 methyl butane surrounded by dry ice. Frozen sections were then prepared in an Internal Cryostat and mounted on a slide. The slide was air dried for 3 minutes, following which one drop of formalin was applied and the section then allowed to dry. The section was then washed for ten minutes with buffered saline pH 7.2 and then layered with rabbit antidog gamma globulin serum conjugated with fluorescein isothiocyanate for 30 minutes at 37°C . Following this, it was washed thoroughly with phosphate buffered saline at pH of 7.3 three times; the first, second and third wash took 5, 10 and 15 minutes respectively. They were then mounted in glycerin on glass slides and

observed immediately with the Zeiss fluorescence microscope illuminated by Osram HB200 mercury vapour lamp, UG2 plus BG12 exciter filters, a dark field condensor and 41/65 barrier filters. A blocking control was also done for each specimen by following the same procedure as described except that unconjugated rabbit anti-dog gamma globulin serum was employed. Then, after washing, the conjugated anti gamma globulin serum was added. In this way non specific fluorescence could be determined.

RESULTS

One hundred and fifty animals have been operated upon and of this, 36 are available for review. The high mortality rate was due to canine distemper, diarrhea, loss of fixation and ulcers from plaster and infection. These events would almost always occur during the first 3-4 weeks following operation.

When each group was examined radiologically and clinically, and the gross appearance of the joint noted at the time of sacrifice the control group, group 1, did the best, followed in turn by the irradiated group, group 3, the Imuran group, group 4 and lastly the frozen homotransplanted group, group 2.

Radiological Observations

Five animals in the control group were followed for longer than one year and all showed, on radiological examination that the cartilage space was well maintained, the osteotomy site united (Fig. 13 & 14) and the degree of graft resorption and consequently the joint architecture was well preserved. Fig. 15 shows the radiological appearance of a control group animal at 18 months. It can be seen that the cartilage interval is well preserved, there is minimal graft resorption and joint alignment is well maintained. The lateral view of another animal also at 18 months (Fig. 16) shows that the patello-femoral cartilage interval is well maintained and no osteophyte formation can be seen.



Figure (13)
Control Group Animal at 6 weeks showing x-ray evidence
of union and also post-operative appearance, lateral view



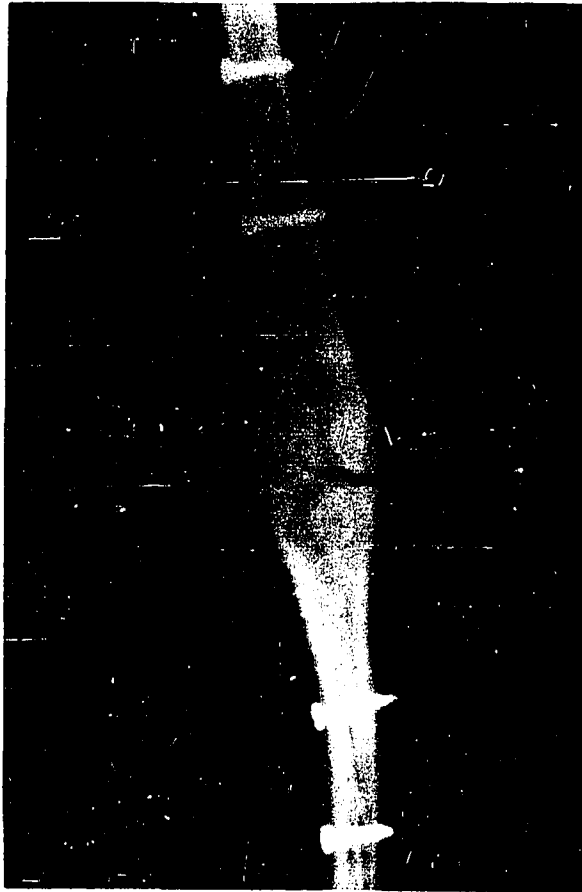


Figure (14)
Control Group Animal at 6 weeks post-operatively
A P View

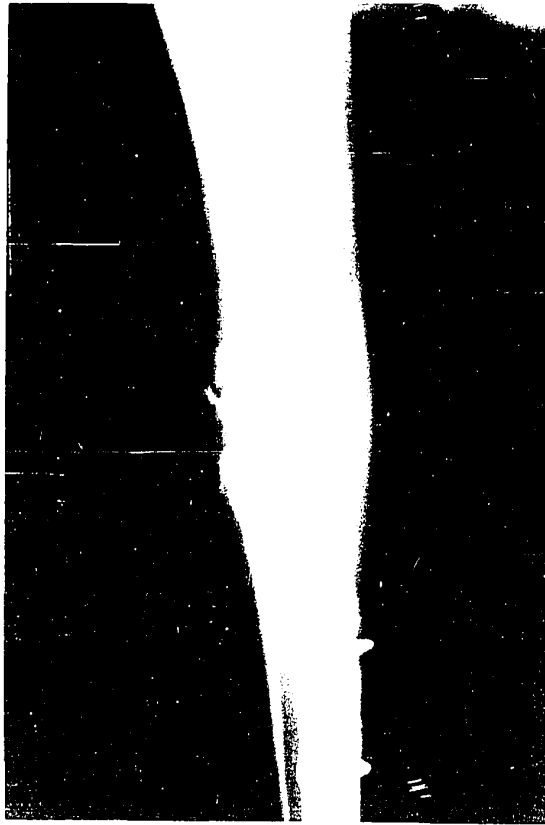




Figure (15)

Control Group Animal at 18 months showing well preserved cartilage interval, minimal graft resorption and maintenance of joint alignment



Figure (15)

Control Group Animal at 18 months showing well preserved cartilage interval, minimal graft resorption and maintenance of joint alignment



Figure (16)

Control Group Animal at 18 months showing patello-femoral joint is well maintained and no osteophyte formation can be seen



Figure (16)

Control Group Animal at 18 months showing patello-femoral joint is well maintained and no osteophyte formation can be seen

In contrast, in 3 out of 4 animals of the frozen homotransplant group whose follow-up was also more than one year, there was graft resorption as early as 6 weeks (Figures 17 & 18). This shows beginning patchy resorption in the lateral femoral and tibial condyles and the femoral shaft. There is also marked periosteal new bone formation and the osteotomy site is still easily visualized. Eventual collapse of the subchondral bone plate and articular cartilage became more evident with time. (Figures 13 & 14 show lateral and antero-posterior radiographs respectively, of a control group animal's knee joint at 6 weeks. As compared to Figures 17 & 18, the osteotomy site is not as apparent and there is no evidence of graft resorption). By 12 months there was complete disorganization and collapse of the joint architecture (Figures 19 through 23). These photos show the progressive changes seen in one animal from 6 weeks to 12 months post-operatively. The one animal whose follow-up was 16 months showed fair maintenance of the cartilage interval with relatively good preservation of the subchondral bone architecture (Figures 24 & 25). However, patchy resorption is evident, especially in the lateral radiograph (Figure 25) and almost complete separation of the lateral femoral condyle has occurred. (Figure 24).

In the frozen irradiated group, 3 animals have been followed for one year or longer and all showed a well maintained cartilage

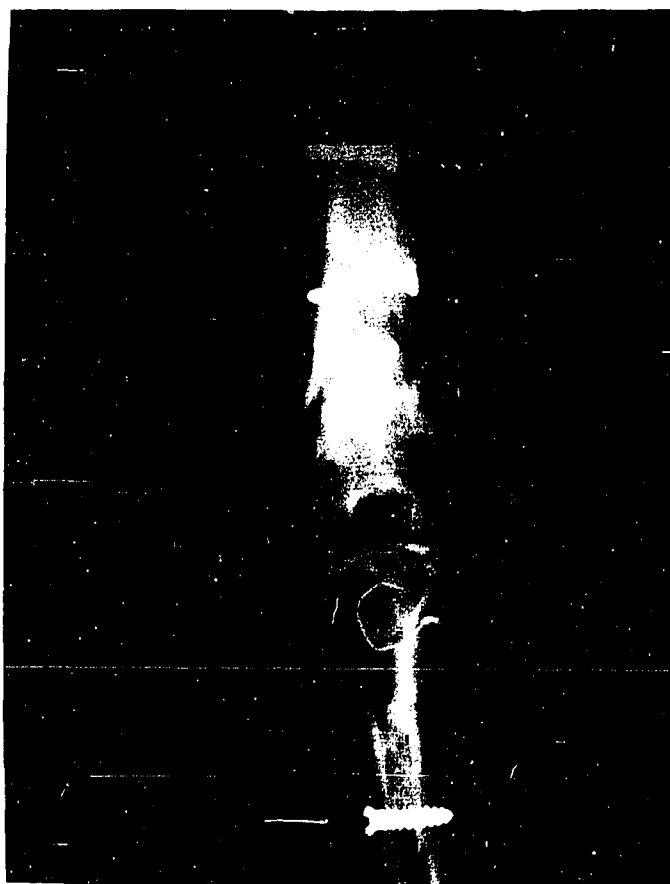


Figure (17)

Frozen homotransplant animal A P view at 6 weeks. This shows beginning patchy resorption in the lateral femoral and tibial condyles and the femoral shaft shows marked periosteal new bone





Figure (18)
Lateral view of figure 17





Figure (19)

Frozen homotransplant animal at 6 weeks showing progressive degeneration, disorganization and collapse of the joint.
A P View.



Figure (10)

exposed to ultraviolet and infrared radiation and relative
 as well as the organization and collapse of the joint.

(10/10)

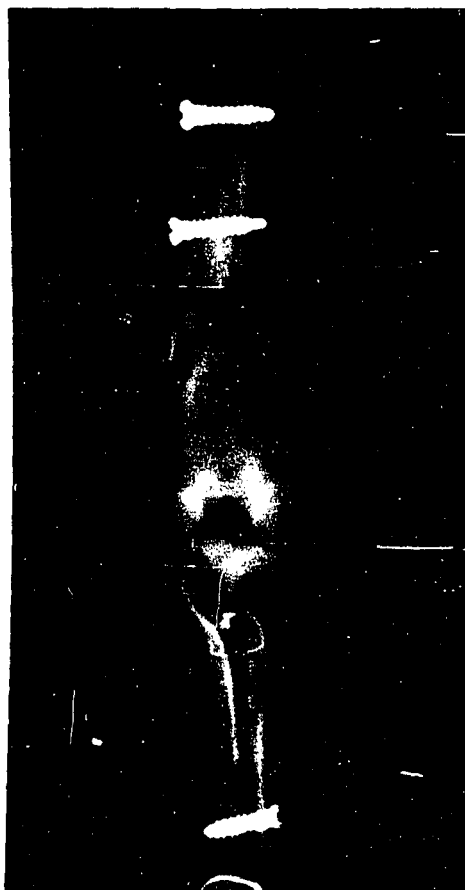


Figure (20)
Same animal as figure 19, but at 3 months. A P view.

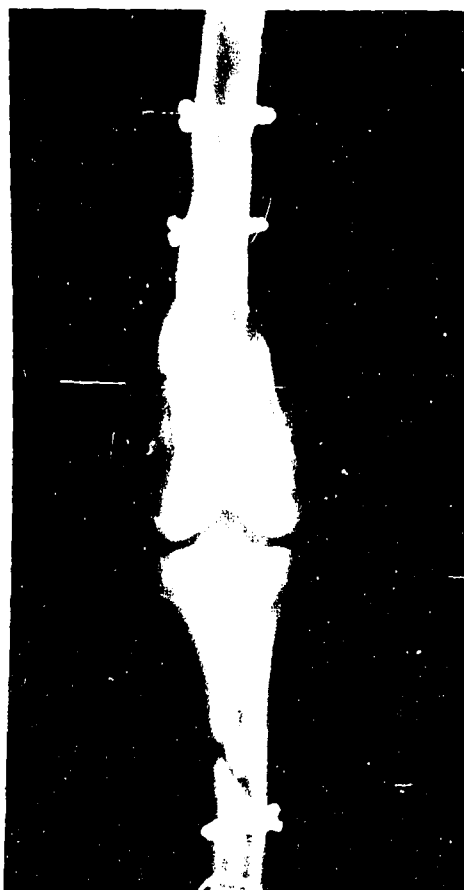


Figure (20)

Same animal as figure 19, but at 3 months A P view

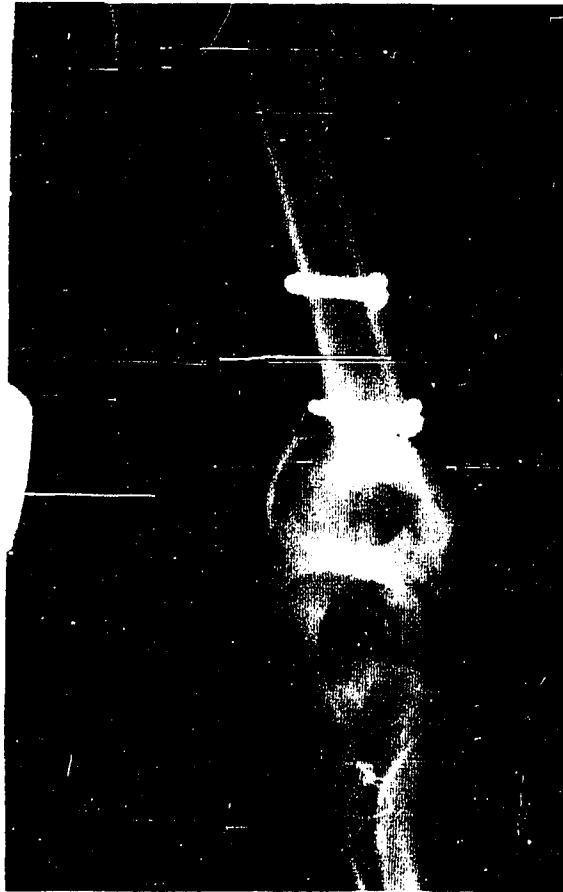


Figure (21)

Same animal as figure 19, but at 9 months, A P view.



Figure (21)

Close-up of face of person in Figure 19, but not wearing mask.

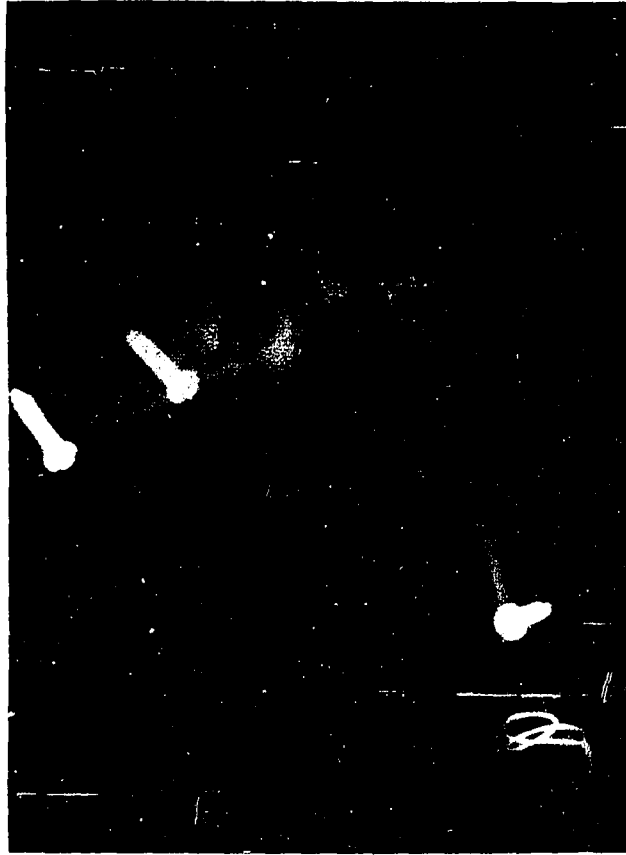


Figure (22)

Same animal as in figure 19, but at 9 months, lateral view





Figure (23)

Same animal as in figure 19 but at 12 months A P view

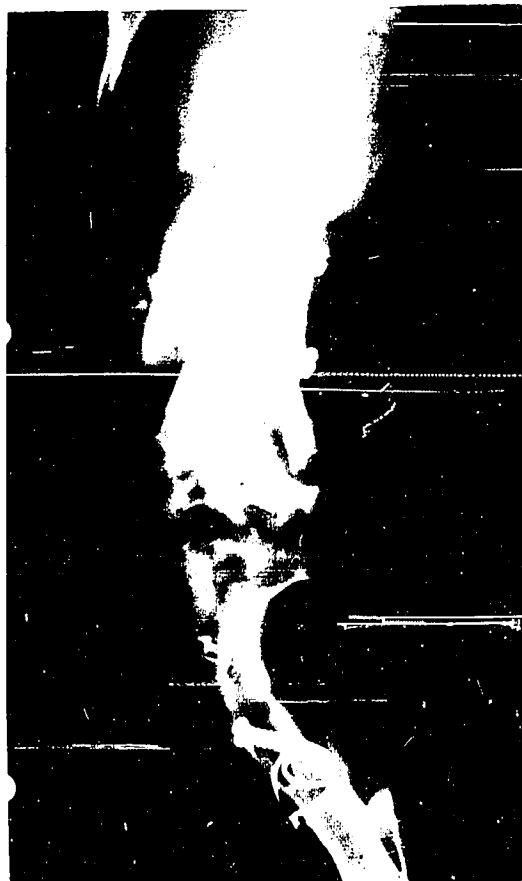


Figure (73)

Figure (73) is the same as Figure 10 but at 12 months of age.

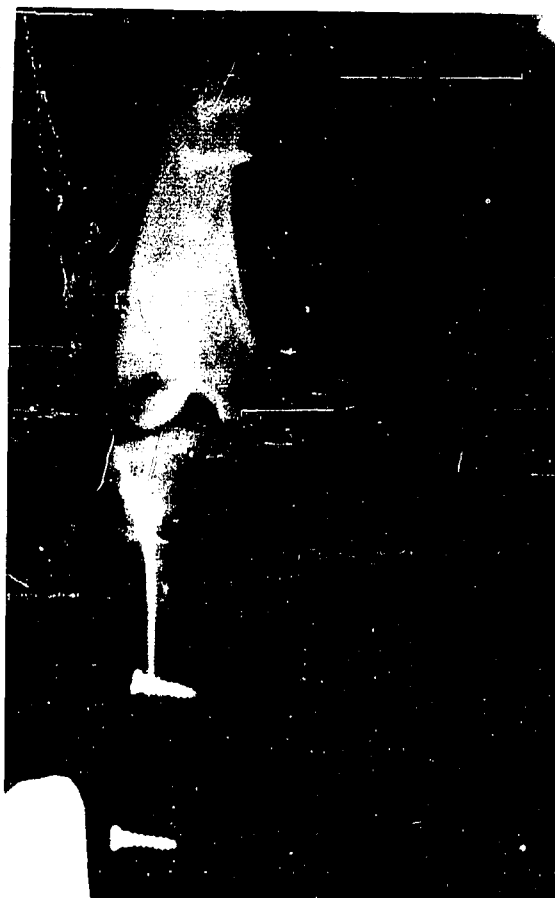


Figure (24)

Frozen homotransplant animal at 16 months showing fair maintenance of the cartilage interval and relatively good preservation of the subchondral bone architecture A P view

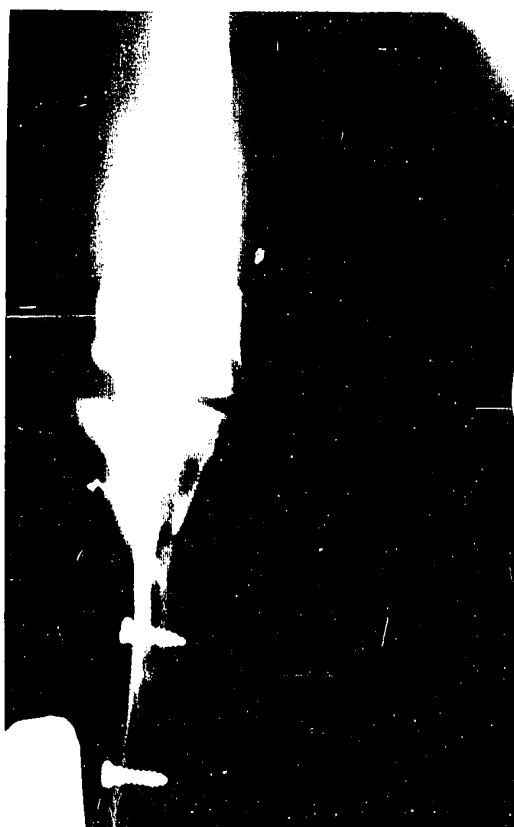


Figure (24)

in zone of transplant and at 16 months, showing fair
distances of the cartilage interval and relatively good
preservation of the functional bone architecture. A-L view.



Figure (25)

Same animal as in figure 24. Marked patchy resorption of bone is evident.



interval (Figures 26, 27, 28). In Figure 26, there is moderate collapse of the lateral tibial condyle which was present since the third month post-operatively but it had not progressed to any significant degree since.

In the Imuran group only 2 animals have been followed for one year, but both have shown early and progressive resorption, bone deformity, collapse of the subchondral bone plate and eventual joint destruction (Figures 29 & 30). However, radiological evidence of bone resorption was not evident until 3 months following operation (compared to 6 weeks in group 2 - see Figures 17 & 18) and never became as severe as in group 2.

Clinical Observations

Clinically the animals in all 4 groups were weight bearing by 6 to 8 weeks and continued to do so until they were sacrificed. This was true even in those animals whose radiographs showed severe joint destruction (Figures 19 to 23). However, the range of joint motion at the time of sacrifice was significantly better in the control group than in the frozen homotransplant group (see Table 1). In the normal dog, the knee can be flexed to about 170° . In the control group at the time of sacrifice, the average range of motion was flexion to 120° and extension to 45° .

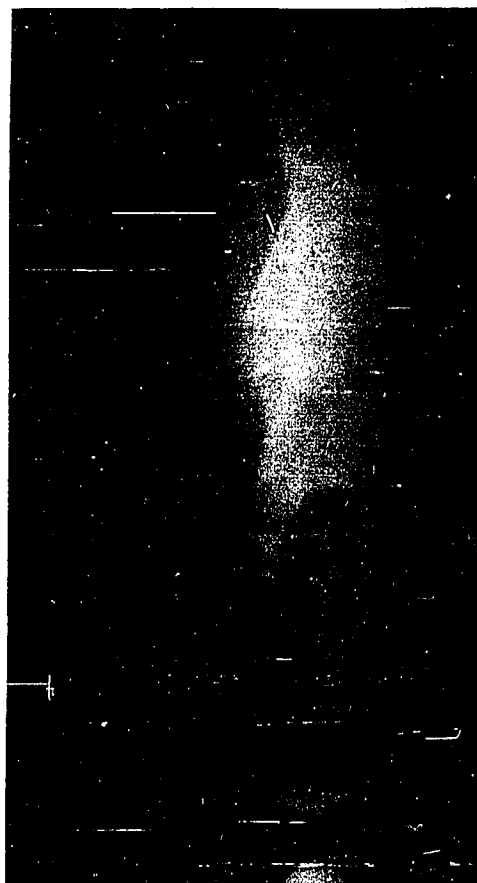


Figure (26)

Frozen irradiated animal at 12 months A P view, showing cartilage interval well maintained but moderate collapse of the lateral femoral condyle is evident.



Figure (26)

Frozen irradiated animal at 12 months A P view, showing cartilage interval well maintained but moderate collapse of the lateral femoral condyle is evident.



Figure (27)

Frozen irradiated animal at 13 months A P view. The cartilage interval is well maintained.



Figure (27)

Frozen irradiated animal at 13 months A P view. The cartilage interval is well maintained.



Figure (28)

Frozen irradiated animal at 14 months A P view. The cartilage interval is well maintained.



Figure (2B)

Frozen irradiated animal at 14 months A P view. The cartilage interval is well maintained.

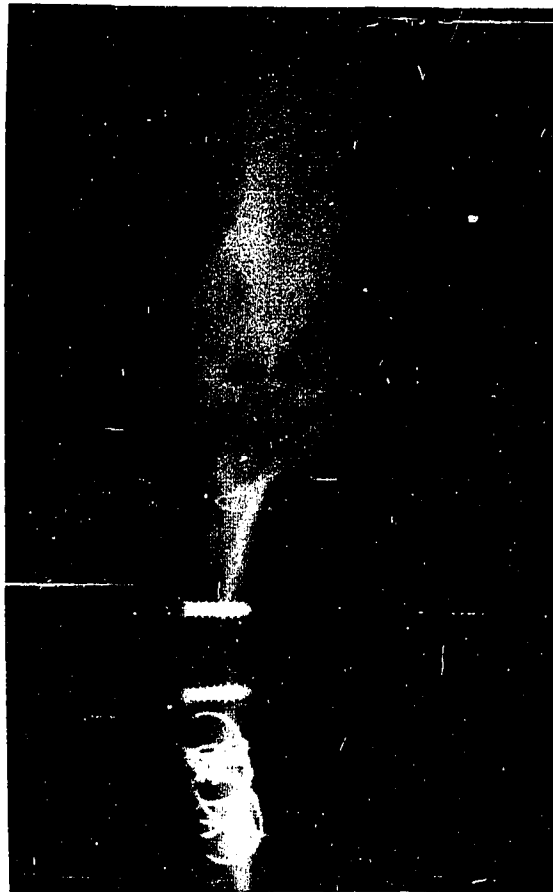


Figure (29)

Imuran group animal at 12 months showing progressive resorption of bone, deformity, collapse of bone plate and eventual joint destruction



Figure (29)

Deuran group animal at 12 months showing progressive resorption of bone, deformity, collapse of bone plate and eventual joint destruction



Figure (30)

Imuran group animal at 12 months A P view, showing progressive resorption of bone, deformity, collapse of bone plate and eventual joint destruction

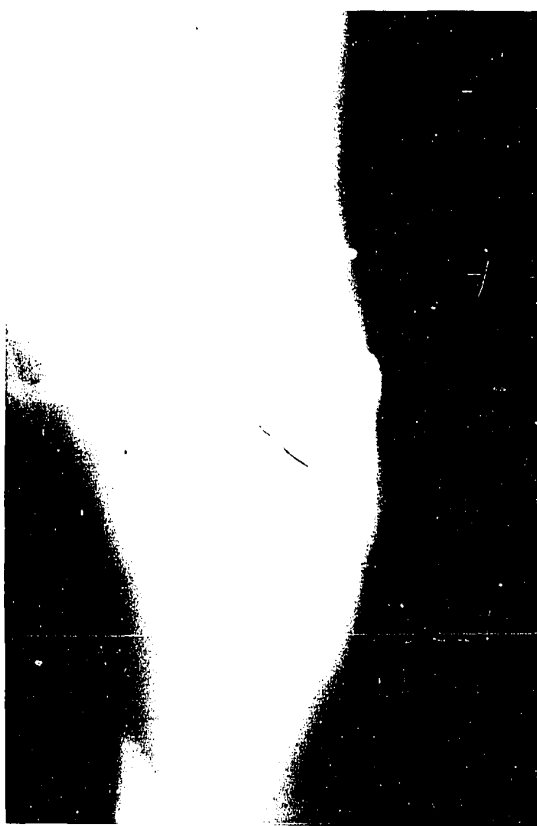


Figure (30)

Imuran group animal at 12 months A P view, showing progressive resorption of bone, deformity, collapse of bone plate and eventual joint destruction

and in the frozen homotransplant group, flexion was to 90° and extension to 30° . In groups 3 and 4, the range of motion was 130° to 45° and 90° to 75° respectively. From about 4 months following transplantation, until sacrifice, the range of motion in both flexion and extension increased in the control group (flexion by 30° and extension by 15°). In the other 3 groups, while extension stayed essentially the same, flexion gradually diminished, especially in group 2 and 4 (the decrease being 50° and 70° respectively). Group 3 had the least loss of joint motion excluding the control group. Thus the range of motion correlated fairly well with the radiological findings.

Gross Observations

On gross examination of the joint at the time of sacrifice, a normal amount of synovial fluid was present. Its appearance varied from normal to turbid yellow, to sanguinous, depending on the time of sacrifice post-operatively. No investigations of the synovial fluid other than its approximate amount and appearance were performed. Figures 31 and 32 are photographs of a dog's normal femoral and tibial articular cartilage respectively and these can be used for reference when comparing the gross changes seen.

The control group showed the least degenerative changes even after 18 months (Figure 33). This shows the femoral condylar surface on an

TABLE (1)

RANGE OF KNEE MOTION AT 4 MONTHS AND AT TIME OF SACRIFICE

GROUP	FLEXION			EXTENSION		
	@ 4 mos.	@ sacrifice	loss(-) or gain(+) of motion	@ 4 mos.	@ sacrifice	Degree of loss(-) or gain(+) of motion
NORMAL	170 ⁰	170 ⁰	--	10 ⁰	10 ⁰	--
1	90 ⁰	120 ⁰	+30	60 ⁰	45 ⁰	+15
2	140 ⁰	90 ⁰	-50	30 ⁰	30 ⁰	0
3	150 ⁰	130 ⁰	-20	40 ⁰	45 ⁰	- 5
4	160 ⁰	90 ⁰	-70	60 ⁰	65 ⁰	- 5



Figure (31)
Normal articular cartilage of a dog's femur

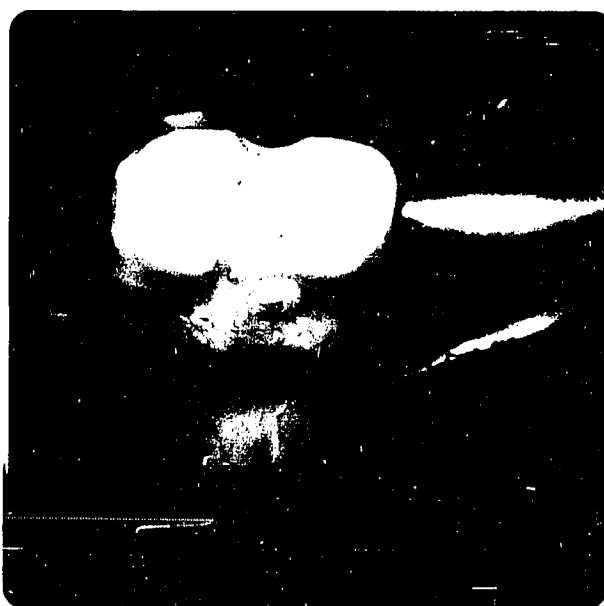


Figure (31)
Normal articular cartilage of a dog's femur



Figure (32)
Normal articular cartilage of a dog's tibia



Figure (32)
Normal articular cartilage of a dog's tibia

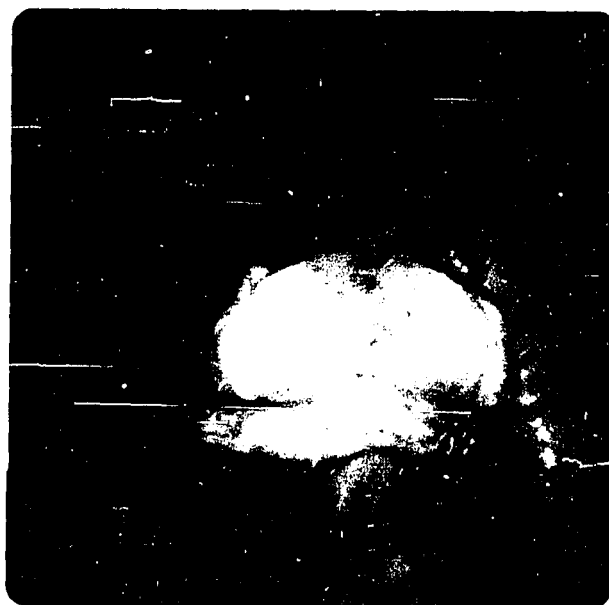


Figure (33)

Femoral condylar surface of a control group animal at 18 months
Note the focal minimal pitting and softening of the
articular cartilage



Figure (33)

Femoral condylar surface of a control group animal at 18 months
Note the focal minimal pitting and softening of the
articular cartilage

18 month animal. Only focal, minimal pitting and softening of the articular cartilage can be seen. The tibial articular surface showed the protective function of the menisci quite clearly. In the "central unprotected area", ie: the cartilage not covered by the meniscus, slight fibrillation, softening and discoloration was seen, (Figure 34). These degenerative changes were seen after 12 months and did not seem to progress. Except for moderate synovial hyperplasia and occasionally metaplastic cartilage formation within the synovium, the articular cartilage remained grossly normal. However, it tended to take on a reddish discoloration for at least 6 weeks following operation (Figure 35) and this was no doubt due to the serosanguinous synovial fluid staining the cartilage. Also, the osteotomy site was almost completely obliterated by this time.

In the frozen homotransplant group, bony union was not apparent until about 3 months. The graft bone of this group was much softer, and even at 6 weeks, cut with relative ease when sections were taken for microscopic section. With the early weakening of the underlying bony structure and subchondral bone-plate, osteochondral fractures were common and were seen as early as 6 weeks (Figure 36). This shows a fracture of the lateral tibial condyle with minimal displacement. In time, these fractures became completely separated from the rest of the graft bone and could be



Figure (34)

Tibial articular surface of a control group animal at 18 months showing slight fibrillation, softening and discolouration in the articular cartilage not covered by the meniscus.



Figure (34)

Tibial articular surface of a control group animal at 18 months showing slight fibrillation, softening and discolouration in the articular cartilage not covered by the meniscus.



Figure (35)

This is a control group animal at 6 weeks showing normal appearing cartilage except for the reddish discolouration



Figure (35)

This is a control group animal at 6 weeks showing normal appearing cartilage except for the reddish discolouration



Figure (36)

Frozen Homotransplant animal at 6 weeks showing an undisplaced osteochondral fracture of the lateral tibial condyle



Figure (36)

Frozen Homotransplant animal at 6 weeks showing an undisplaced osteochondral fracture of the lateral tibial condyle

removed with ease or with a scalpel. In the latter case a fibrous union existed between the loose fragment and graft bone. Not uncommonly one or both femoral condyles would be separated from the rest of the femoral shaft and these often would undergo complete collapse, leaving a large gaping defect in the articular surface (Figure 37). This is the femoral condylar surface of a 14 month animal showing complete destruction of the lateral femoral condyle. Figure 38, shows the tibial condylar surface of another animal at 16 months. Note the marked degenerative changes of the articular cartilage. Osteophyte formation of the patella was common, (Figure 39), but was usually not seen in the graft.

In group 3, the frozen irradiated group, the longest follow-up was 14 months and bony union occurred at about 3 months as in group 2, but in contrast, degenerative changes of the cartilage were much less as when compared to a group 2 animal which had been sacrificed at the same post-operative period. Figures 40 and 41 are photographs of 2 animals sacrificed at 14 months. Both show relatively normal appearing cartilage with little in the way of degenerative changes. The Imuran group was intermediate between groups 2 and 3 with respect to articular cartilage degenerative change (Figure 42). This is an animal at 12 months showing moderate degenerative changes of the femoral condyles.



Figure (37)

Frozen Homotransplant animal at 14 months showing
complete destruction of the lateral femoral condyle



Figure (37)

Frozen Homotransplant animal at 14 months showing complete destruction of the lateral femoral condyle

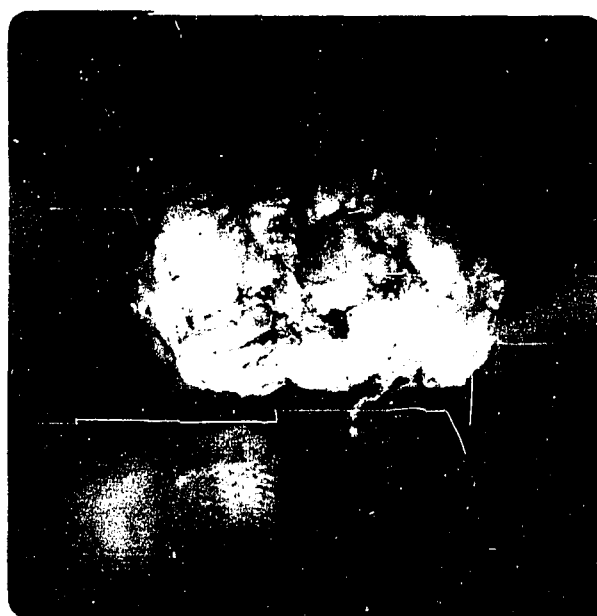


Figure (38)

Frozen Homotransplant animal at 16 months showing
marked degeneration of the articular cartilage



Figure (38)
Frozen Homotransplant animal at 16 months showing
marked degeneration of the articular cartilage



Figure (39)

Frozen Homotransplant Animal at 16 months showing marked osteophyte formation around the patella as well as other degenerative changes in the rest of the joint



Figure (39)

Frozen Homotransplant Animal at 16 months showing marked osteophyte formation around the patella as well as other degenerative changes in the rest of the joint



Figure (40)

The tibial articular surface of a frozen irradiated group at 14 months showing minimal degenerative changes in the area not covered by the menisci



Figure (40)

The tibial articular surface of a frozen irradiated group at 14 months showing minimal degenerative changes in the area not covered by the menisci

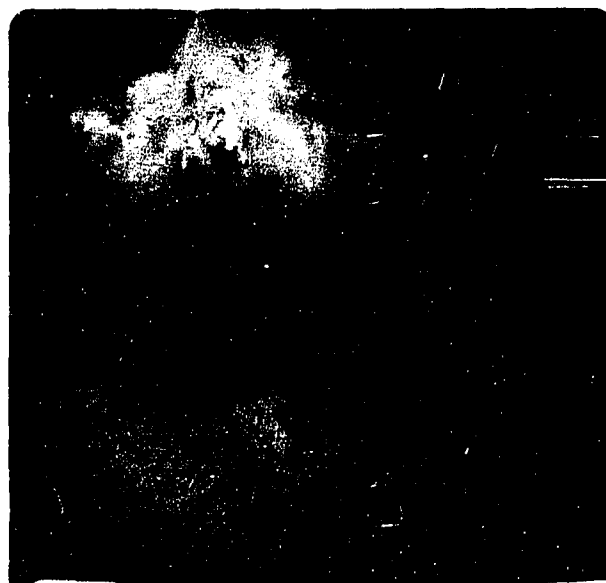


Figure (41)

Femoral condylar surface of a frozen irradiated animal at 14 months showing minimal changes in the articular cartilage



Figure (41)

Femoral condylar surface of a frozen irradiated animal at 14 months showing minimal changes in the articular cartilage



Figure (42)

Imuran group animal at 12 months showing moderate degenerative changes of the femoral condyles



Figure (42)

Imuran group animal at 12 months showing moderate degenerative changes of the femoral condyles

Histological Observations

The microscopic appearance can be summarized in Figure 43 which is a diagramatic cross section of one of the long bones of the knee. The arrows indicate the direction of creeping substitution involving not only the avascular bone but also the articular cartilage. There was no articular cartilage survival in groups 2, 3 or 4. In the control group, survival was occasionally seen and this involved the non weight bearing intercondylar femoral area in almost all cases. The dead cartilage was gradually but not completely replaced by synovial proliferation, overgrowth and subsequent metaplastic change to a fibrous type of articular cartilage. This histochemically usually behaved like true cartilage ie: it stained metachromatically and took up radiosulphate.

The control group did the best with respect to this replacement of dead bone and cartilage with living tissue, followed by the frozen irradiated group, the Imuran group and finally the frozen homotransplant group. However, the differences among these latter 3 were minimal.

In general, with respect to the reliability of the three parameters used to measure cartilage viability (H & E and Toluidine blue stain, radiosulphate uptake) there was excellent correlation only after about three months following the operation. Before this period of time, the dead cartilage could still show metachromasia and secondly, the patella of the experimental knee would often show only

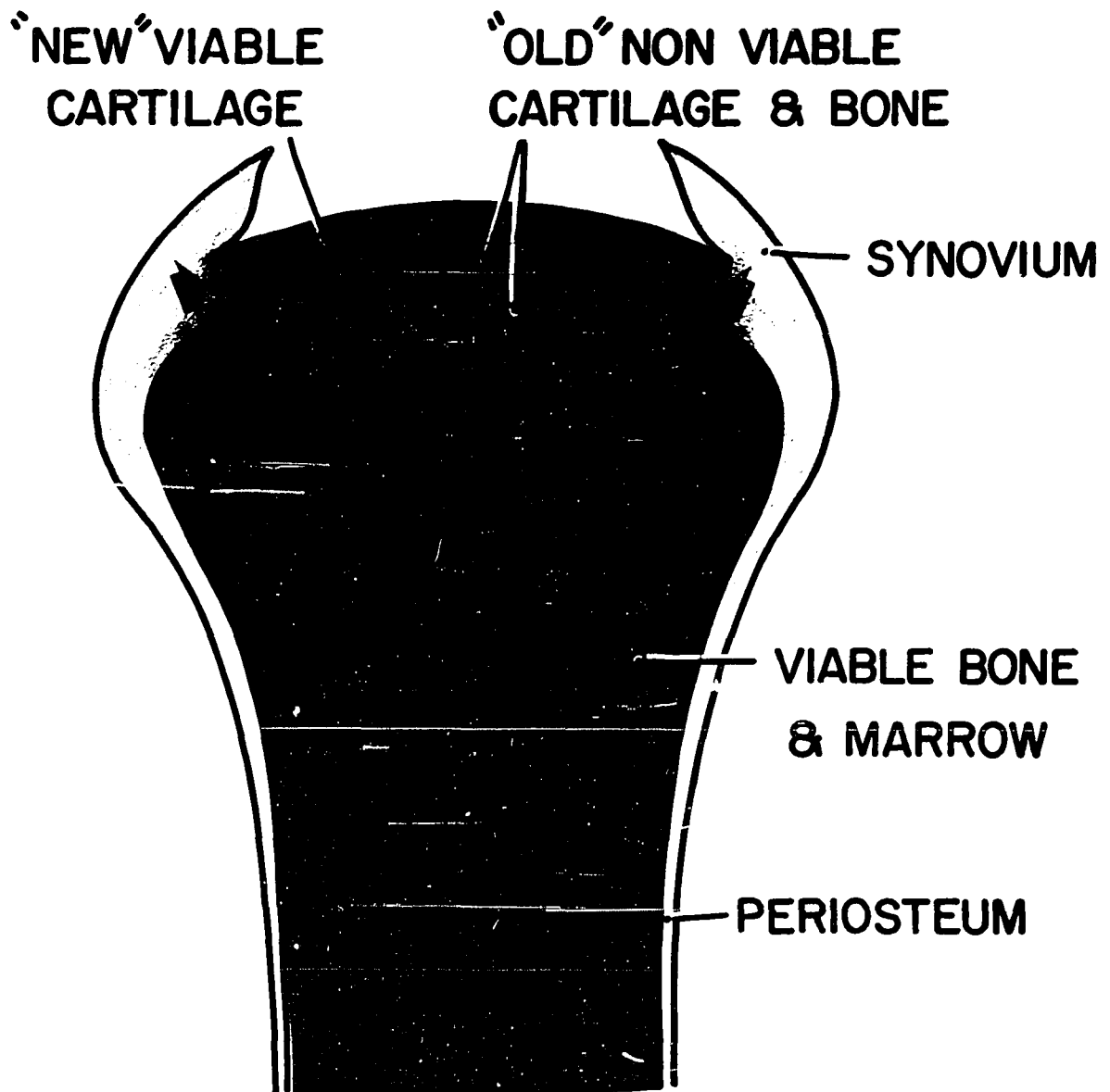


Figure (43)
Microscopic Changes - Diagramatic

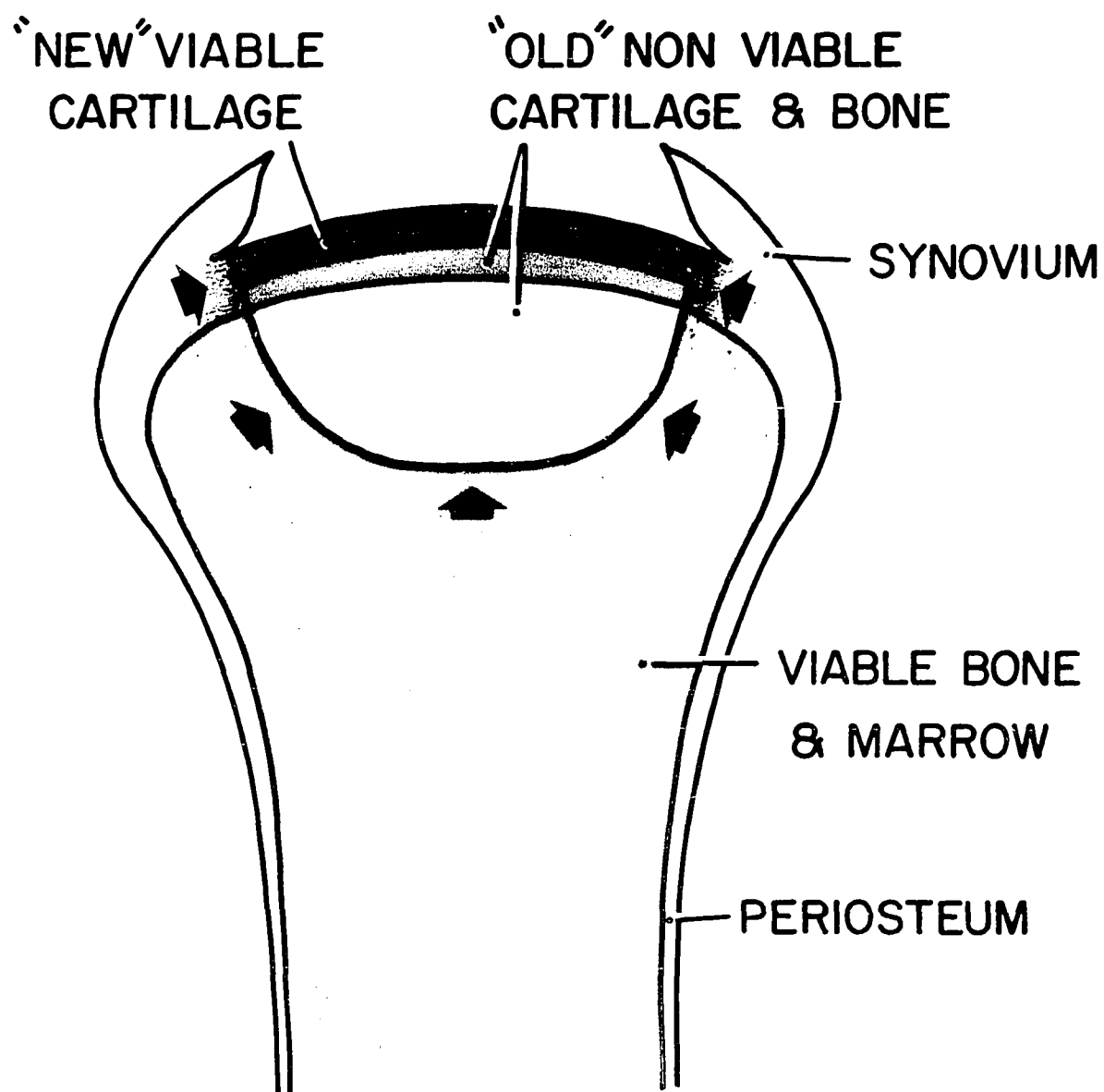


Figure (43)
Microscopic changes - Haversian

minimal uptake of radiosulphate and variable loss of metachromasia in the superficial layers (Figure 44). Therefore, although radiosulphate is probably the best means by which cartilage viability can be measured it was difficult to assess before three months in this experiment. Thus, in all groups during the first six weeks post-operatively, the articular cartilage and underlying bone trabeculae and marrow spaces became completely acellular (Figure 45) but retained their normal substructure. There was a variable loss of metachromasia, from relatively little (Figure 46) to complete (Figure 47) even though all sections showed complete absence of radiosulphate uptake.

As early as three weeks however, changes were evident that distinguished the control group from the other groups. At this time viable cells, presumably fibroblasts from the cartilage-synovial junction could be seen growing over the acellular articular cartilage (Figure 48). This overgrowth was usually more evident in the femoral non weight bearing intercondylar area (Figure 49). This fibroblastic tissue as it grew over the acellular cartilage, also invaded and replaced it (Figure 50) and by 12 months, most of the articular cartilage had been replaced. The acellular cartilage which was most remote from the cartilage synovial junction ie: the deep layers and central portions, remained and

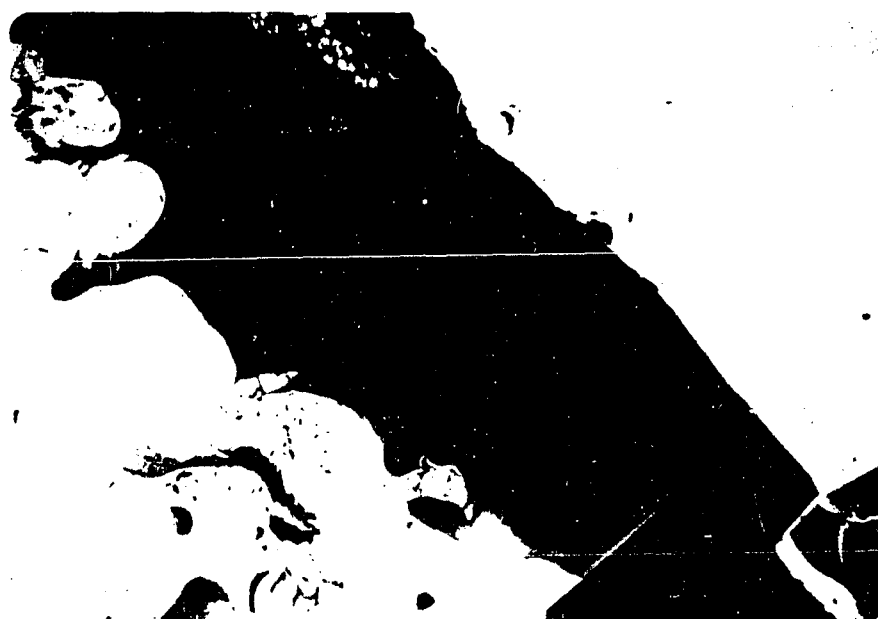


Figure (44)

Frozen irradiated animal at 6 weeks, Toluidine Blue Stain, showing the patellar cartilage with loss of metachromasia in the superficial layer x 2.5

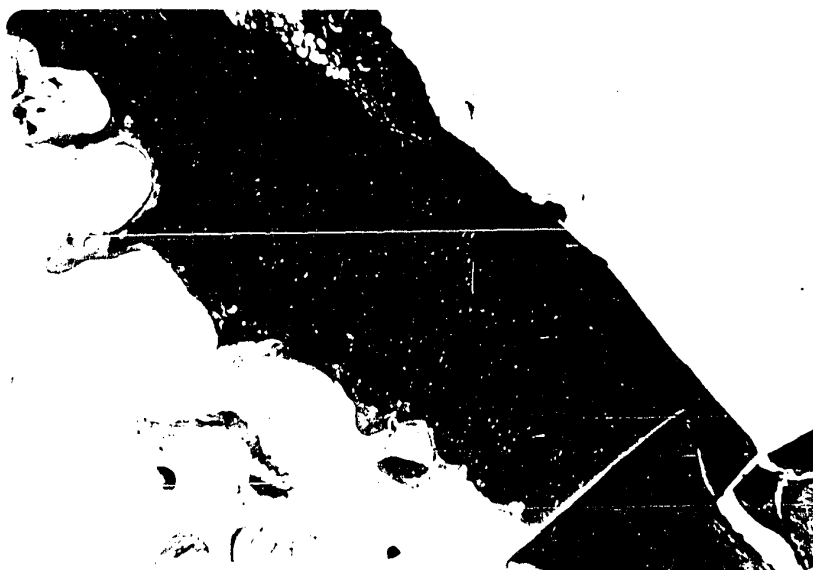


Figure (44)

Frozen irradiated animal at 6 weeks, Toluidine Blue Stain, showing the patellar cartilage with loss of metachromasia in the superficial layer x 2.5

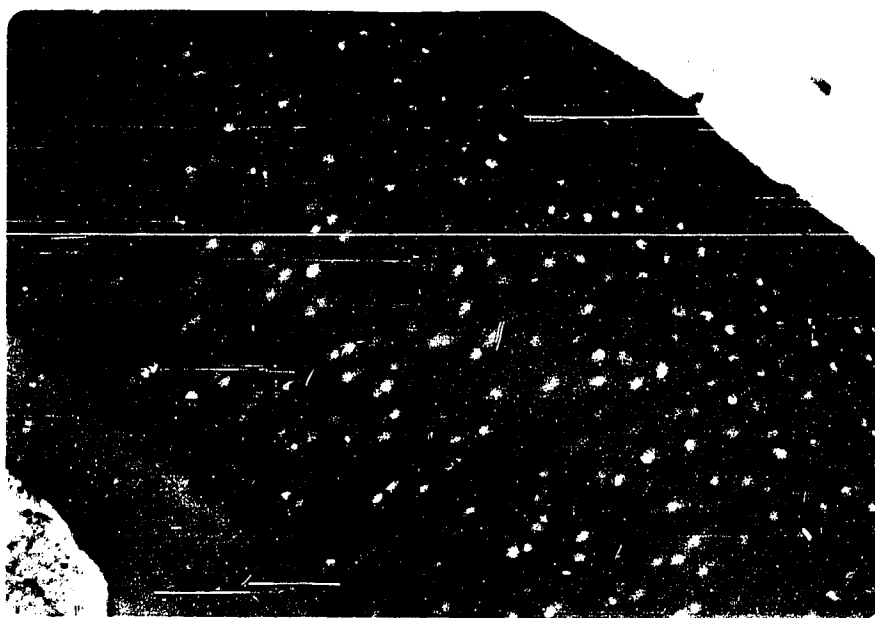


Figure (45)

Control Group Animal at 3 weeks H & E stain. There is loss of cell nuclei in the articular cartilage and underlying bone and marrow spaces. The architecture is well preserved. x 10

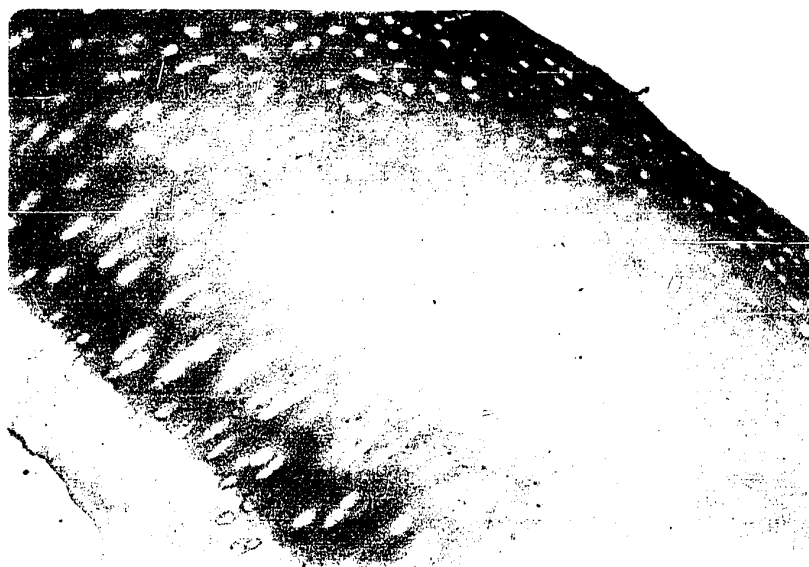


Figure (45)

Control Group Animal at 3 weeks H & E stain. There is loss of cell nuclei in the articular cartilage and underlying bone and marrow spaces. The architecture is well preserved. x 10

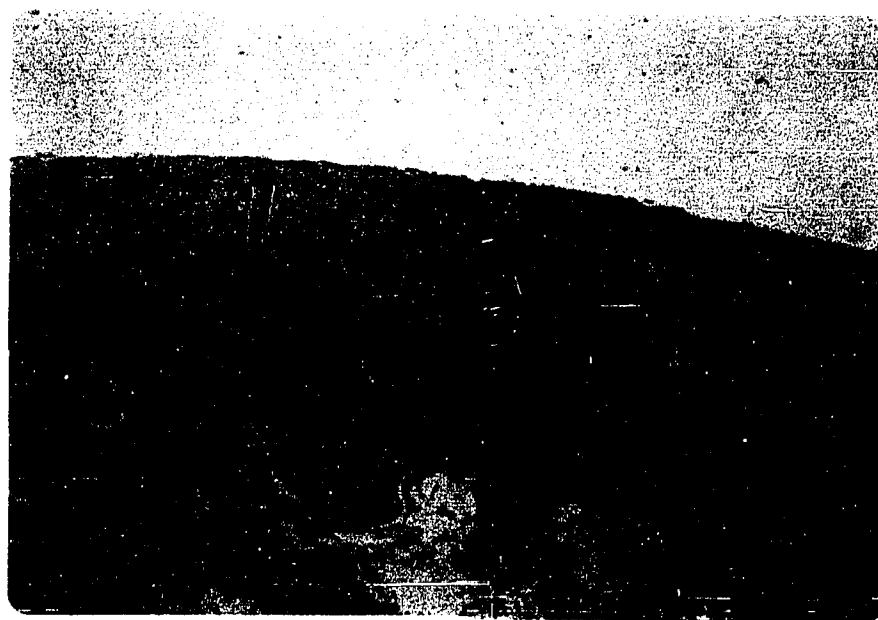


Figure (46)

Control group animal at 6 weeks Toluidine Blue Stain
There is loss of metachromasia in the superficial one half
of the cartilage x 2.5

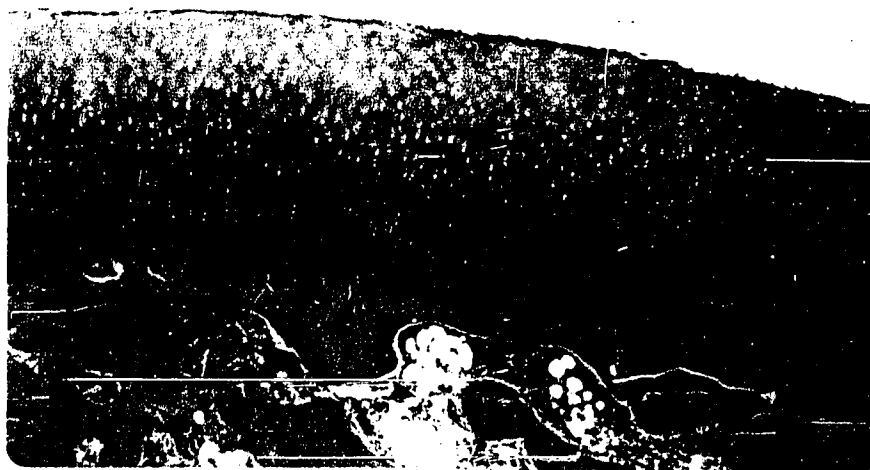


Figure (46)

Control group animal at 6 weeks Toluidine Blue Stain
There is loss of metachromasia in the superficial one half
of the cartilage x 2.5



Figure (47)

Frozen irradiated group animal at 6 weeks. Toluidine Blue Stain.
There is complete loss of metachromasia x 2.5

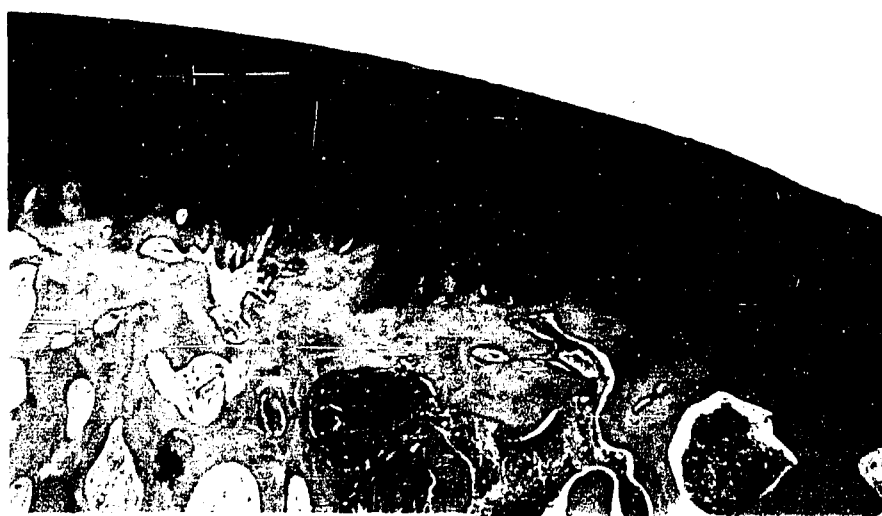


Figure (47)

Frozen irradiated group animal at 6 weeks. Toluidine Blue Stain.
There is complete loss of metachromasia x 2.5

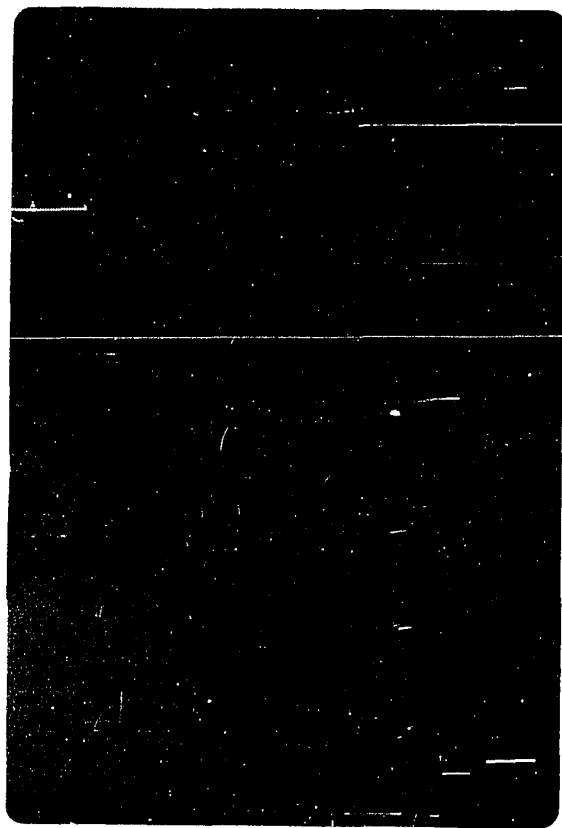


Figure (48)

Control Group Animal H & E at 3 weeks showing overgrowth of cells
from cartilage synovial junction and underlying acellular
articular cartilage, x 10



Figure (48)

Control Group Animal H & E at 3 weeks showing overgrowth of cells from cartilage synovial junction and underlying acellular articular cartilage x 10

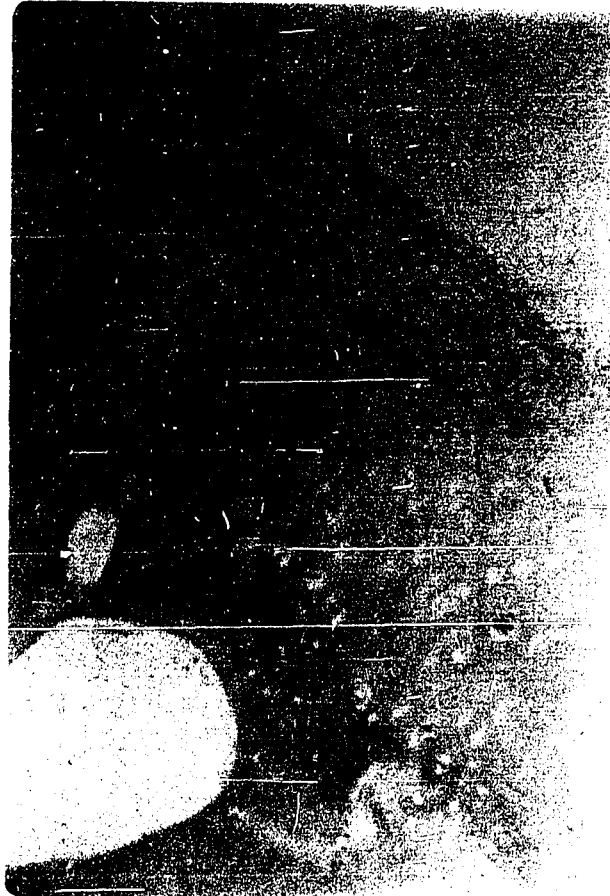


Figure (49)

Control Group Animal H & E at 6 weeks showing a thin pannus of cells growing over the surface of the cartilage in the intercondylar groove x 10

Figure (49)

Control Group Animal H & E at 6 weeks showing a thin pannus
of cells growing over the surface of the cartilage in the
intercondylar groove x 10



Figure (50)

Control Group Animal at 5 months H & E showing fibroblastic tissue growing over and replacing acellular cartilage x 10

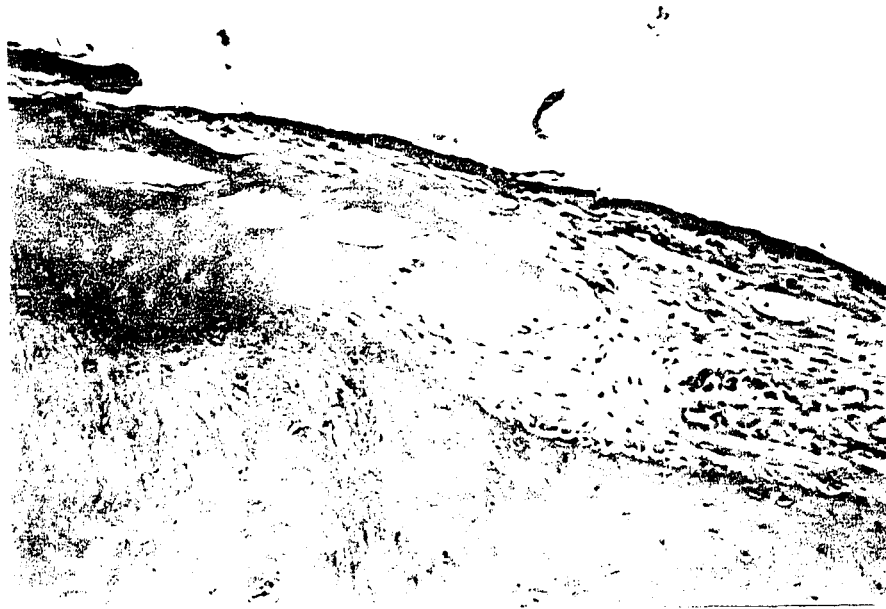


Figure (50)

Control Group Animal at 5 months H & E showing fibroblastic tissue growing over and replacing acellular cartilage x 10

had not been replaced even at 18 months following operation. This tissue underwent metaplastic change to fibrocartilage ie: it stained metachromatically and took up radiosulphate (Figures 51 to 55).

The dead cartilage underwent degenerative changes (fibrillation, thinning) much more readily than the viable metaplastic cartilage like tissue. Secondly, there was more degeneration noted in the femoral articular surfaces as compared to the tibial surfaces, and, thirdly, the non weight bearing intercondylar femoral ^{area} showed less degenerative change than elsewhere in the joint.

As mentioned previously, no cartilage survived transplantation except in the control group and, even here, this was the exception rather than the rule. The picture was that of viable chondrocytes in the superficial 1/2, usually arranged in clusters or groups. (Figures 56 and 57).

The replacement of dead cartilage by fibrocartilage as described, was seen in all groups. However, the rate at which this occurred was different in each group. In the frozen homotransplant group, overgrowth was first seen at 6 months and was not progressing to any degree even up to 16 months post-operatively. In groups 3 and 4, the changes were somewhat more advanced than in group 2, but not appreciably so.

The big difference in these groups with respect to replacement

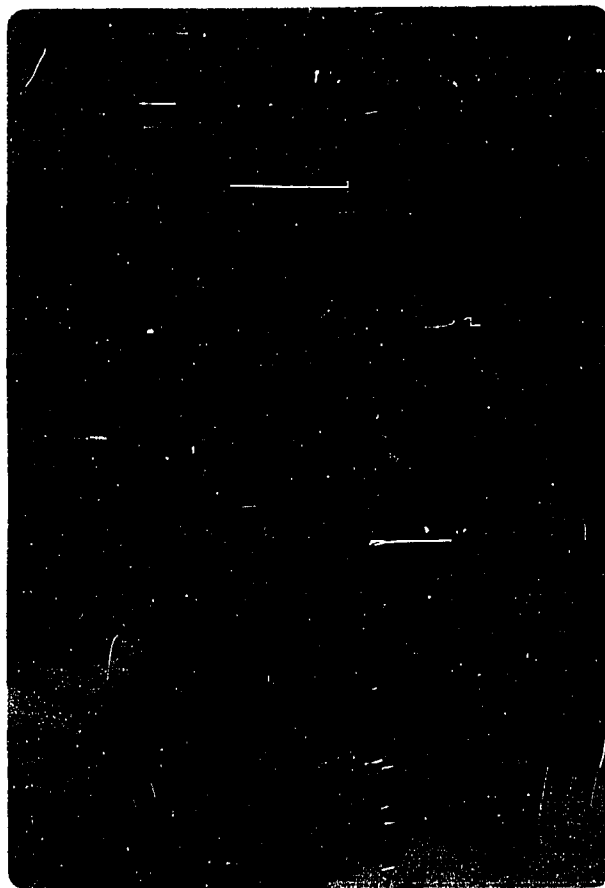


Figure (51)
Control Group Animal at 16 months, H & E. This shows
fibrocartilage like tissue in the superficial half and
deep to this, acellular cartilage x 10



Control (51)
 Control (51) at 16 months, H & E. This shows
 the cartilage in the superficial half and
 deep half of the cartilage (x 10)

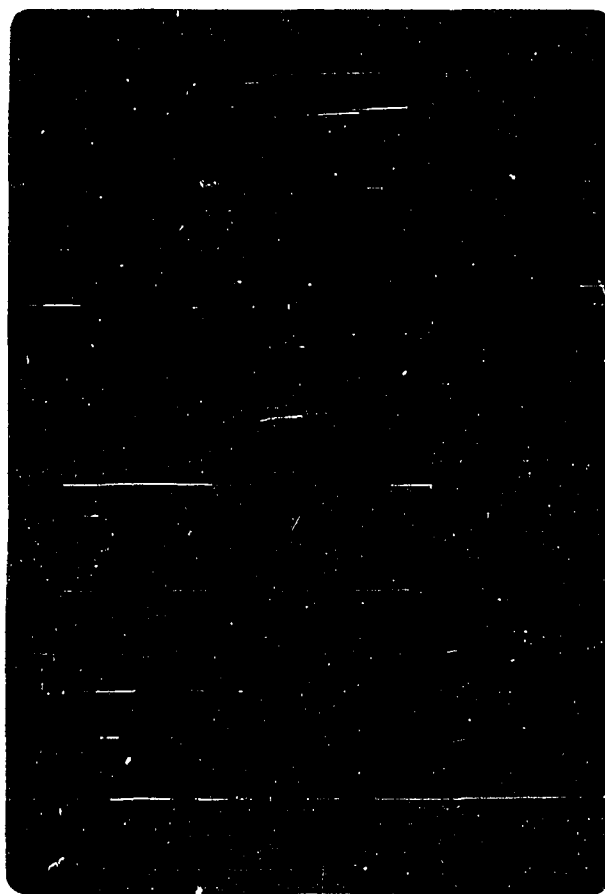


Figure (52)
Control Group Animal at 16 months - autoradiograph of
same section as in figure 51, showing radiosulphate uptake
in this tissue x 10

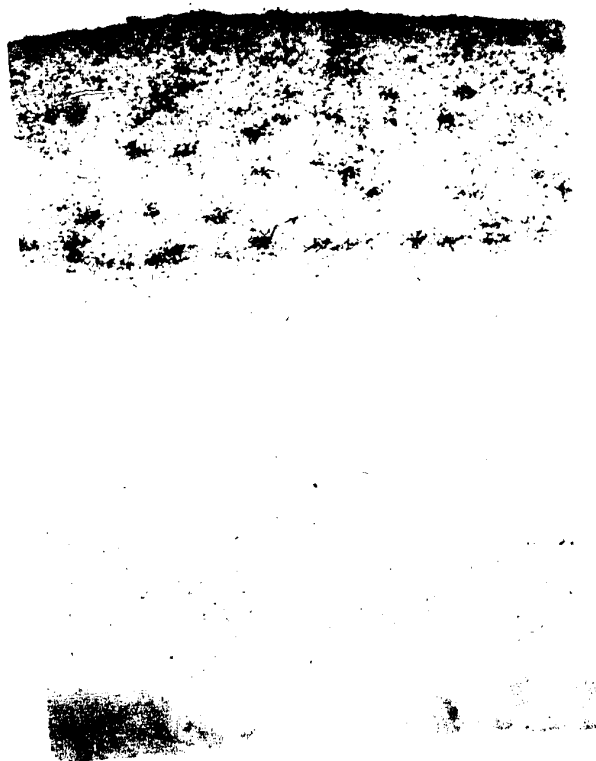


Figure (52)
Control group (animal at 16 months) - autoradiogram of
coron section as in figure 51, showing radiocarbon uptake
in this tissue (1).

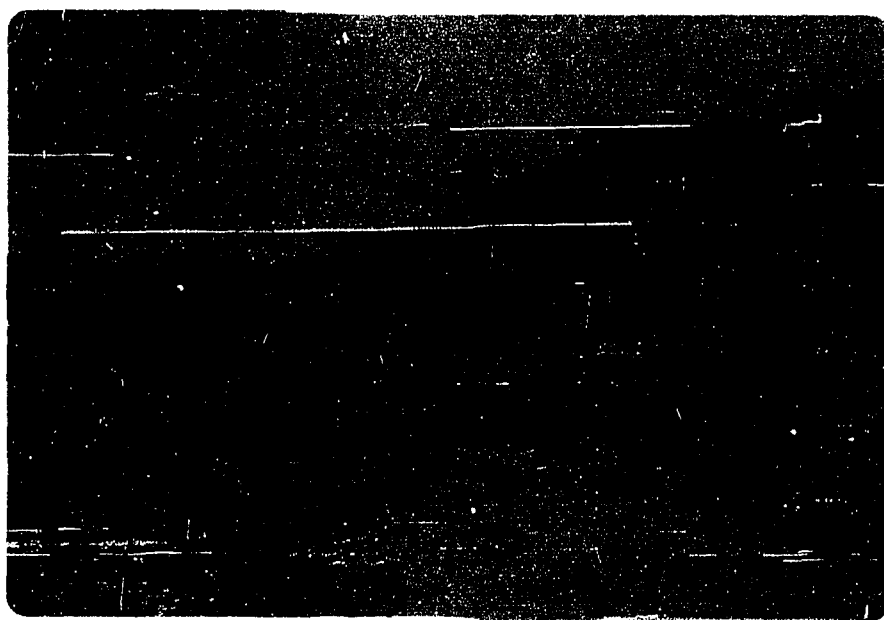


Figure (53)

Control Group Animal at 9 months. This is a section of tibial cartilage-synovial junction showing metachromasia of the synovial tissue which is proliferating and beginning to grow over the dead articular cartilage which lacks metachromasia. x 2.5



Figure (53)

Control Group Animal at 9 months. This is a section of tibial cartilage-synovial junction showing metachromasia of the synovial tissue which is proliferating and beginning to grow over the dead articular cartilage which lacks metachromasia. x 2.5

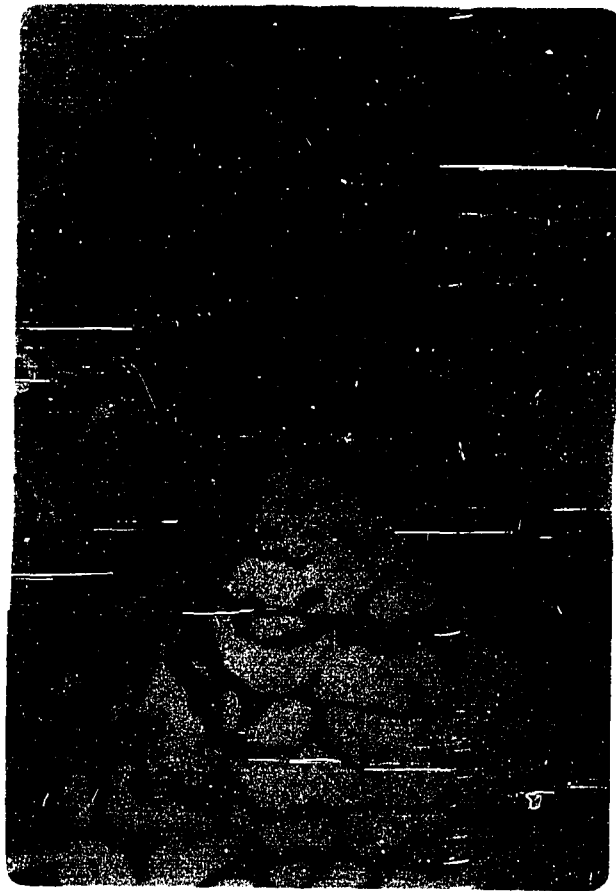


Figure (54)
Normal Cartilage synovial junction Toluidine Blue stain. x 2.5
Note the sharp distinction between the cartilage which stains
metachromatically and the synovium which does not. Compare Fig.(53)



Figure (54)
Normal Cartilage synovial junction Toluidine Blue stain x 2.5
Note the sharp distinction between the cartilage which stains
metachromatically and the synovium which does not. Compare Fig.(53)

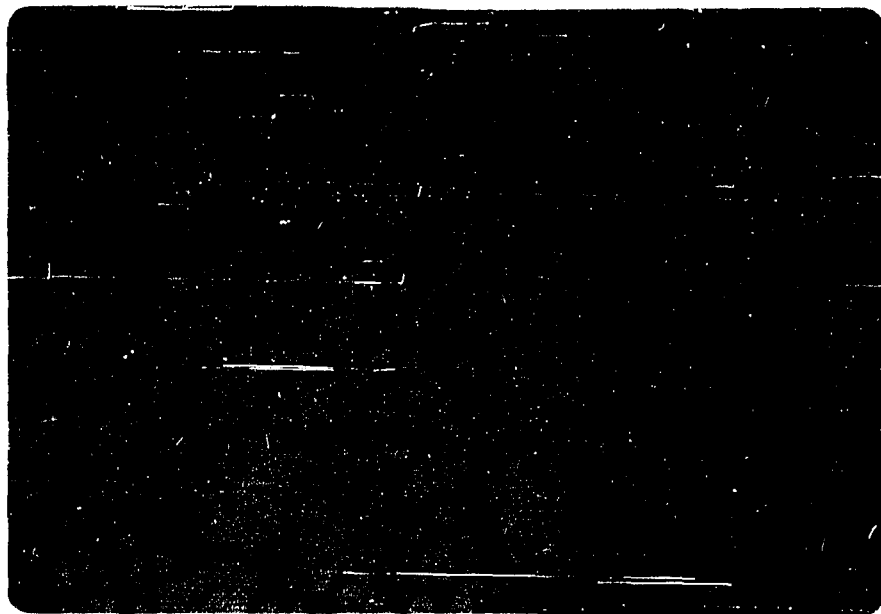


Figure (55)
Control Group Animal at 16 months. Toluidine Blue.
Metachromasia is absent in the deeper zones and present
in the upper zones x 2.5

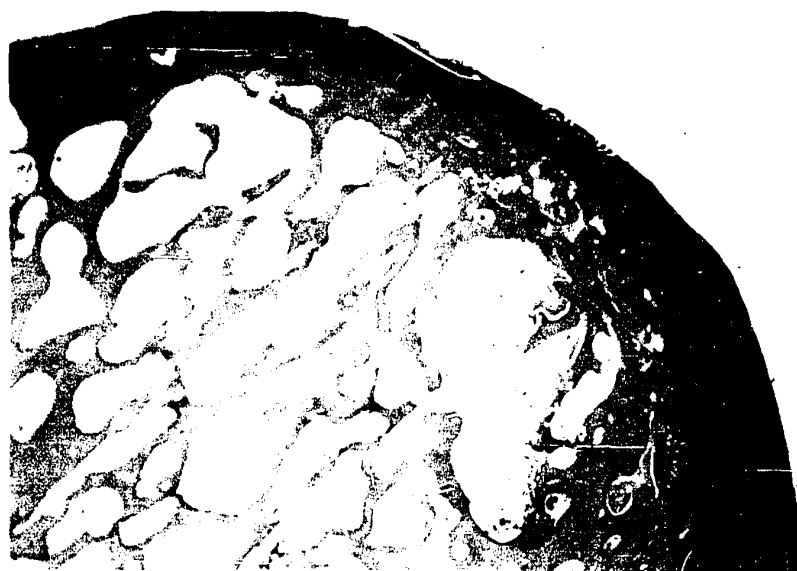


Figure (55)
Control Group Animal at 16 months. Toluidine Blue
Metachromasia is absent in the deeper zones and present
in the upper zones x 2.5



Figure-(56)
Control Group Animal at 18 months autoradiograph showing
cell nests or clusters of chondrocytes in the upper layers
of cartilage x 10



Figure (56)
Control Group Animal at 18 months autoradiograph showing
cell nests or clusters of chondrocytes in the upper layers
of cartilage x 10



Figure (57)
Control Group Animal at 18 months. H & E showing viable
appearing cells superficially and non viable acellular
lacunae deep to this. x 10



Figure (57)
 Central area. Animal at 18 months. (1 & 2) showing viable
 epithelial cells, superficially, and non viable acellular
 keratinized cells. (3) (4)

of non viable with viable tissue involved the bony trabeculae and marrow spaces. In the control group, the subchondral marrow spaces were filled with fibrous like tissue by about 3 months, and by 12 months replacement of the avascular bone with new viable bone was almost complete. At this time also, the marrow spaces were filled with normal appearing fatty marrow. In group 2 on the other hand, although active resorption of dead bone occurred as early as 6 weeks, new bone formation was considerably delayed (Figure 58); large cyst-like spaces developed within the homograft and these were filled with fibrous tissue only. The subchondral bone was therefore left with little to support it and microfracture, with collapse and disruption of the overlying cartilage often occurred. In groups 3 and 4, the disparity between bone resorption and new bone formation was not as great as in group 2 and the cyst-like spaces were not as evident. The marrow spaces in groups 2, 3 and 4, were slightly more cellular than those in the control group. There were occasional clusters of lymphocytes seen but this was unusual. Thus, histological evidence of rejection to foreign tissue ie: bone, was minimal at any stage. (Figure 58).

Immune Studies

Over 400 weekly blood samples were tested against bovine PPL antigen and all have been negative. Thus, no circulating antibodies



Figure (58)
Frozen Homotransplant Animal at 14 months showing active bone
resorption, fibrous marrow, absence of lymphocytes and no new
bone formation x 10

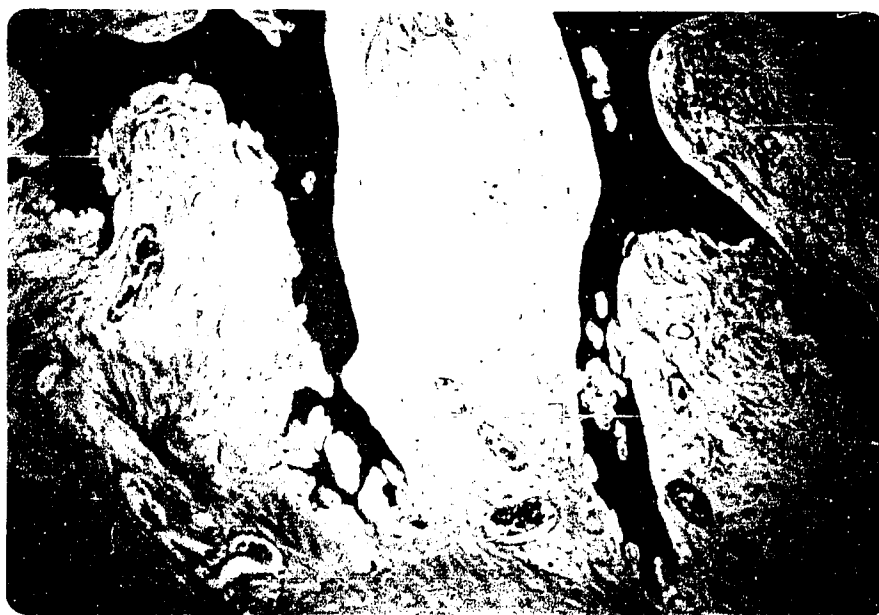


Figure (58)
Frozen Homotransplant Animal at 14 months showing active bone
resorption, fibrous marrow, absence of lymphocytes and no new
bone formation x 10

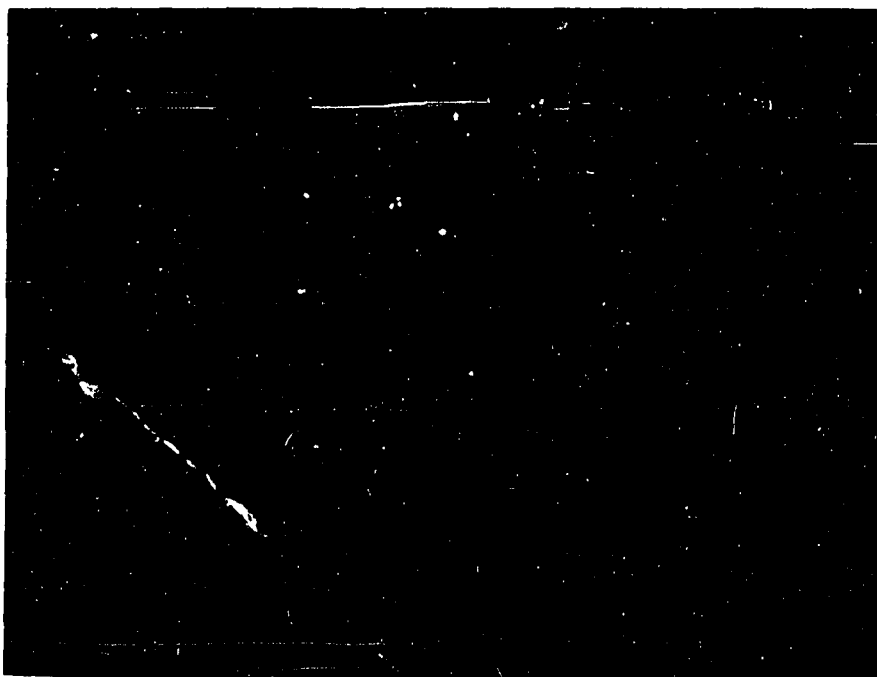


Figure (59)

Immune Studies: Fluorescein labelled articular cartilage to detect cell bound antibodies and PPL antigen. These results do not differ from the Blocking Test, figure 60.

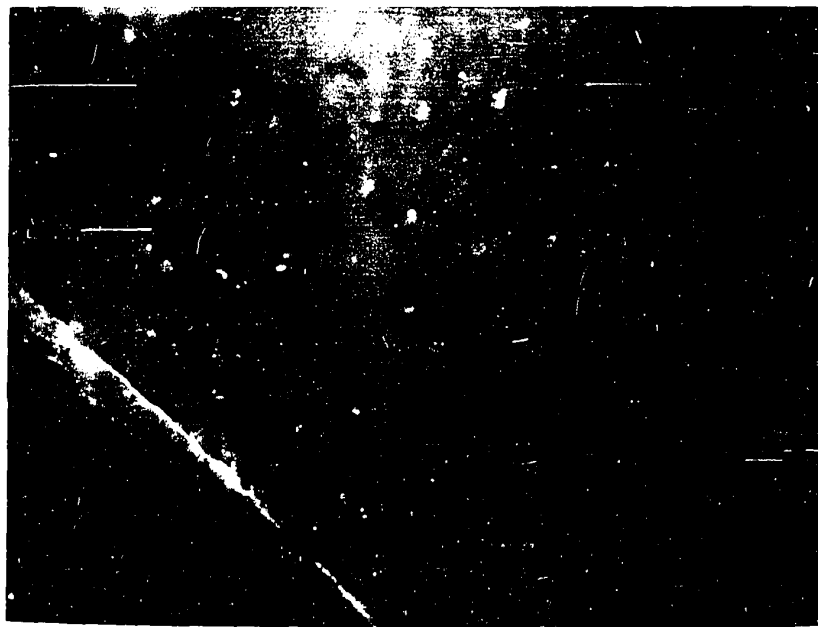


Figure (59)

Immune Studies: Fluorescein labelled articular cartilage to detect cell bound antibodies and PPL antigen. These results do not differ from the Blocking Test, figure 60.

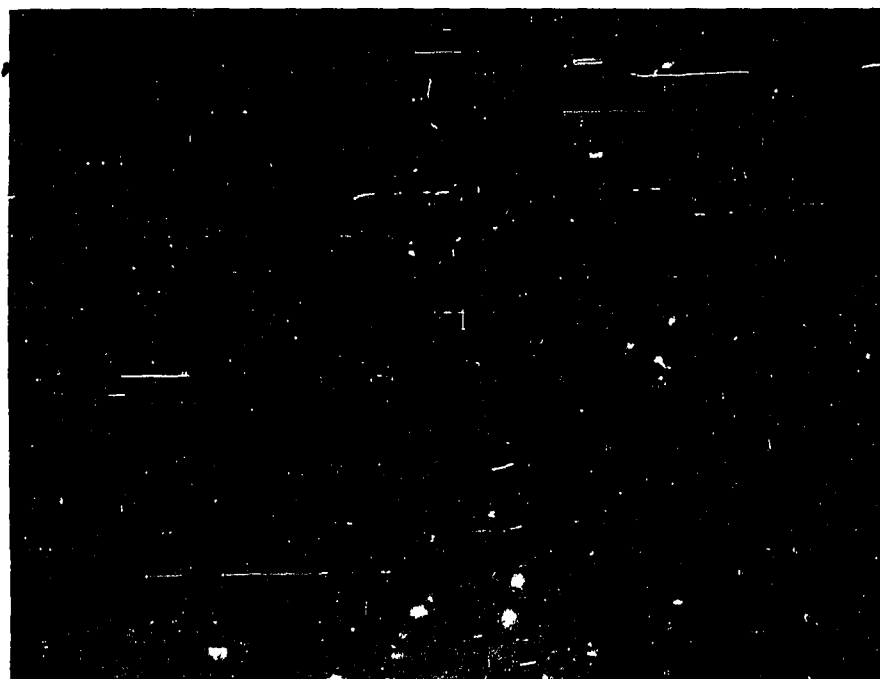


Figure (60)

Immune Studies: Blocking Test. There is no difference between this and figure 59 ie; the non specific staining is the same in both

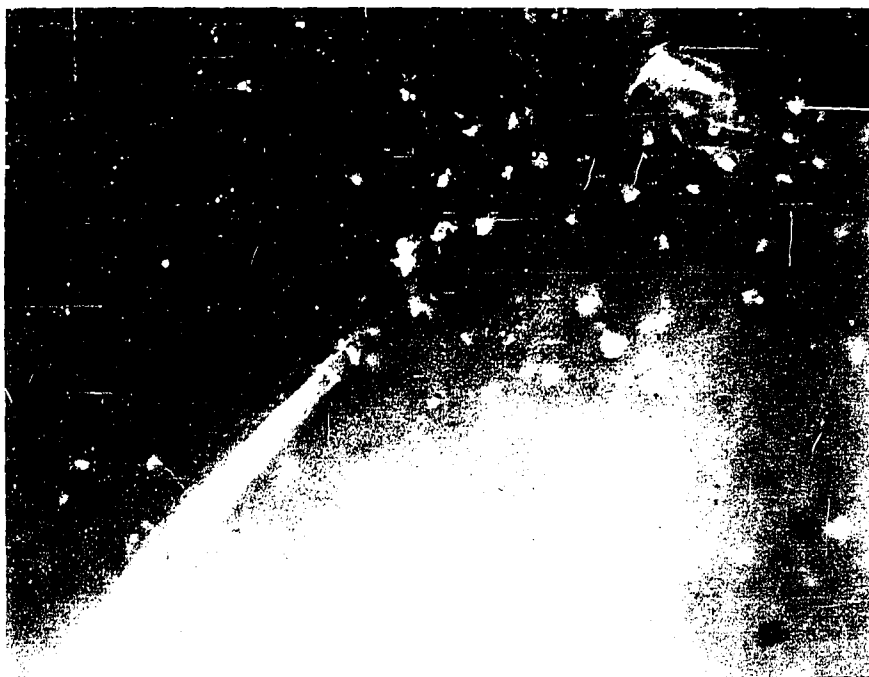


Figure 100

Figure 100 shows a bright, diagonal beam of light or smoke against a dark, textured background. The beam originates from the bottom left and extends towards the upper right, illuminating a path through a field of small, dark, irregular particles or debris. The overall effect is dramatic and abstract, possibly representing a celestial event or a microscopic view.

to cartilage were detected.

The results with the immunofluorescent stains have also proved to be negative (Figures 59 & 60). These photographs show no detectable differences between the blocking control and the experimental cartilage.

DISCUSSION

Despite the low survival rate amongst these animals (36 out of 150 or 24%) the operation is technically feasible and if done on human subjects, no doubt the survival rate would be substantially higher. However, the operation is over four and one half hours long and involves a very wide surgical exposure. Thus, the chance of infection is an ever present one. Such factors as better pre-operative preparation of the patient, a more adequate means of fixation and better post-operative care would no doubt increase the chances of survival while decreasing both morbidity and mortality. However, because most clinical studies of whole joint transplantation involving both weight bearing and non weight bearing larger joints indicate degenerative changes radiologically (Parrish, Ottolenghi) with or without clinical symptoms within 5 years following transplantation, this type of procedure will probably not replace prosthetic replacement arthroplasty in the Western hemisphere. Prosthetic appliances in the hip for example have been reported to last from 5 to 10 years and sometimes longer (McKee, Farrar) 1968. In the Eastern European countries, replacement arthroplasty is not carried out as often because of economic reasons and even under these circumstances, whole joint transfers are not usually done; osteochondal grafts, as described by Campbell, and Depalma have been used and have been shown to work. These workers listed their recommendations (see page 4) among which was the use of adults as donors and the

problems encountered if donors whose epiphyseal plates were still present were used. They also noted that the synovium was detrimental to the transferred graft but their reasons for this statement were not given. The opposite was found in this experiment and this will be discussed below.

That both bone and cartilage are antigenic and that the former is much more so has been discussed earlier. This has been borne out in this experiment. All studies to detect circulating or cell bound antibodies to cartilage PPL have been negative. However, it is known that freezing cartilage, besides killing the chondrocytes if no protective additives are used, also renders it less antigenic (Kvoroslukkin) and thus any antigenic properties which may have been present may have been at too low a level to stimulate detectable antibody. Secondly, in all reports in the literature regarding the detection of cartilage antigen the method employed has been to use animals of one species and to immunize them with the PPL from another species. No studies have been done describing the effects, if any, of immunizing an animal with PPL from another animal of the same species. Thus the negative results reported on the immunofluorescent studies may not necessarily indicate the absence of cell bound antigen-antibody complexes to cartilage. With these observations in mind it can probably be stated that the cartilage was not primarily

affected in the degenerative changes seen in the joint. The primary changes were initially in the bone. Bone itself, although more antigenic than cartilage, has a much lower antigenicity when compared to other tissues eg: the kidney. This manifested itself in the paucity of histological evidence of a rejection process in the marrow spaces. Only scattered collections of lymphocytes were found in any of the homotransplanted groups. More importantly, bone antigenicity manifested itself by affecting the graft's bone inductive properties, which were decreased. Thus bone resorption occurred but active new bone formation was delayed and consequently the underlying bony architecture was markedly affected. In the frozen homotransplant group, group 2, which had done the poorest from a radiological, clinical, gross and microscopic point of view as compared to the other groups, the first changes noted were not in the cartilage, but in the bone. Thus radiologically, patchy resorption was seen as early as 6 weeks (Figures 17 & 18) and progressed rapidly to complete disruption and disorganization of the joint by 12 months (Figures 19 to 23). However, the cartilage space was relatively well maintained until the very end when collapse of the subchondral bone plate occurred (Figures 24 & 25). Grossly, it was noted that the graft bone of this group was much softer than normal, even at 6 weeks and that osteochondral fractures were common (Figure 36).

The overlying cartilage was grossly normal at this stage despite being histologically dead. It is interesting to postulate that if more rapid replacement of the homograft bone had occurred, as compared to the control group, perhaps the cartilage would have retained its structural integrity and be replaced by metaplastic fibrocartilage more completely. By removing all marrow and cancellous bone elements of the graft and filling it with autogenous marrow as was described by Burwell in his discussion of "Composite Homografts" or by drilling multiple holes in the cancellous structure of the graft (Fiala) or also by maintaining the blood supply to the graft (Reeves and Slone) this could possibly have been achieved. In this experiment, even freezing and irradiation (group 3), although the most effective method of reducing the antigenicity of the bone was not enough to produce results comparable to the control group. The irradiation dose selected was the same as that used in irradiating donor kidneys and even though bone is much denser than kidney, the dose of 125 rads was still effective, at least when done in conjunction with freezing. It was somewhat surprising that the Imuran group did not do better since Imuran or azathioprine, an antimetabolite related to 6 mercaptopurine has been shown to be particularly effective in depressing the immune response. However, Urist did note that its analogue 6-M.P. depressed bone induction. Secondly, it may be argued that this group is too small for accurate appraisal.

Cartilage cell death per say cannot be equated with cartilage degeneration. Papp and Krompcher, Depalma, Campbell and Kosinka all note that in the homotransplantation of osteochondral grafts, if the graft bone is thicker than 5 mm then collapse of the subchondral bone plate occurs leading to degeneration of the overlying cartilage. When the underlying homograft bone was of the right thickness, the cartilage, whether viable or not, would survive structurally for at least 300 days and then undergo degeneration (Campbell). Yet, many workers have emphasized the fact that in order for articular cartilage to preserve its function, it must retain its viability (Hagarty, Chesterman). However, these workers used small osteochondral fragments or small pieces of articular cartilage for their studies and not whole joints. In this experiment, it was found that the dead cartilage was replaced by synovial overgrowth and metaplastic change to fibrocartilage. This tissue then showed all the histochemical functions of cartilage, namely metachromasia, and most importantly, radiosulphate uptake. (Figures 51-55). Synovial tissue contains primitive mesenchymal cells which can undergo metaplastic change to many mesodermal tissues, cartilage being one of them. The clinical entity of synovial chondromatosis is an excellent example of this. Synovial tissues also contain lysosomes and these have been shown to "digest" cartilage (see page 32). It is not surprising then, to see synovial tissue behave as it did. The synovium was also important with respect to cartilage nutrition since mature animals were used (see page 31). Nevertheless, even though cartilage antigenicity is low, the rate and degree of replacement by metaplastic fibrocartilage was considerably slower in the homotransplanted groups than in the control group.

The hemarthrosis produced by the trauma of the operation

probably did little with respect to cartilage degeneration. Hoagland (1967) concluded from his experiments on hemarthrosis in dogs that multiple injections of blood over a prolonged period of time (12 to 18 weeks) were required before degenerative changes were seen. However, what the presence of blood does on dead articular cartilage has not been reported. It was noted in the control group that cartilage death, rather than survival was the rule, although the latter would have been expected. The presence of blood and the relative absence of synovial fluid post-operatively may have been enough to cause chondrocyte death. Histologically it was noted that at least 3 months had to elapse before accurate conclusions regarding chondrocyte death could be made from the 3 parameters used. That the patella, which presumably contained viable cartilage, also showed changes in two of the three parameters (toluidine blue stain and radiosulphate uptake) which were very similar to those seen in the dead transplanted articular cartilage during the first three months post-operatively was not too surprising, considering the amount of trauma the patella must have received, (see below). Secondly, the synovial membrane and fluid were both severely affected by the operative procedure and reference to this and its relationship to cartilage nutrition has already been mentioned. Cruess and Mitchell (1967) noted a decrease in metachromasia in articular cartilage in rabbits following synovectomy. This reached

a maximum by 30 days and gradually returned to normal by the 45th day. This was in keeping with a return of synovial living cells. Meachim (1964) noted a marked decrease in radiosulphate uptake following moderate operative trauma to the knee joint. Despite these changes in the patellar cartilage during this period of time, it still acted as an excellent control because, if it revealed radiosulphate uptake, it would be indicative of the fact that radiosulphate had been correctly injected into the joint. Thus, the lack of uptake by the remaining joint cartilage would indicate cartilage nonviability, the exception being noted above. The patellar cartilage was important in another way. In some animals whose knee joints showed marked degenerative changes in the patellar cartilage, cell nests were very prominent, being very similar to those seen in Figure 56. Since this observation represents attempted cartilage repair ie; viability, it must be concluded that there were areas in the articular cartilage from the control group animals that did survive transplantation.

Lastly, the observations seen in this experiment should be briefly compared to those seen by Herndon & Chase. Most of the observations seen here coincided well with these authors, namely the rate of bony union, joint function and gross findings. The microscopic observations regarding cartilage replacement by fibrocartilage and proliferation of remaining chondrocytes in the

control group were also similar. The major differences involved the histological observations noted in the homotransplanted animals. These authors noted complete replacement of new bone in the control group by 9 months, whereas it was not noted to be complete until 18 months in the control group of this experiment. Secondly, Herndon & Chase noted only superficial necrosis of cartilage cells after one month and implied that the cartilage survived in all groups. However, it was noted here that the cartilage, even of the control group, did not survive and this was evident as early as seven days post-operatively. Thirdly, the "rapid destruction of articular cartilage which occurred after one month" was not observed.

Structural integrity of the cartilage (and underlying bone), although undergoing destruction at an earlier and faster rate than that seen in the control group, was well maintained for the first 6 months. When cartilage degeneration did occur, it was secondary to the disruption of the subchondral bone. Their observation that cartilage replacement by pannus and fibrocartilage was not seen until 8 months was somewhat similar to the findings in this experiment (6 months).

SUMMARY

This thesis describes whole knee transplantation in the dog. The literature is reviewed with specific reference to the work of Herndon and Chase who did similar experiments in 1952. The immunology of osteochondral tissue is discussed as well as a brief description of immunofluorescent technique and its application to cartilage. The structure and metabolism of cartilage is described.

Mature female dogs underwent total knee transplantation according to the method of Herndon and Chase, and were divided into 4 groups; a control group, group 1, a frozen homotransplant group, group 2, a frozen irradiated group, group 3, and a frozen homotransplant plus Imuran to the recipient group, group 4. The results have shown that the control group did the best radiologically, clinically, grossly and microscopically. This was followed by groups 3, 4 and lastly 2.

The synovial tissues played an important role in the replacement of the dead cartilage by metaplastic fibrocartilage. Cartilage cell death, per se cannot therefore be equated with cartilage degeneration. The latter is most likely due to the disruption of the bony architecture which occurs primarily. The delay in new bone formation, despite early active bone resorption (especially noted in group 2), was the reason for this disruption.

The delay in turn, was most likely due to the inhibitory effect of the bones' histocompatibility antigens on bone induction. The means by which this effect could be diminished is discussed.

Although both bone and cartilage are antigenic, the former is much more so. All immune studies to detect anticartilage antibody were found to be negative. The immuno fluorescent antibody technique and its possible shortcomings is discussed.

The significance of blood and its effects on nonviable cartilage is discussed.

There was excellent correlation of the 3 parameters used to determine cartilage viability only after 3 months. The patella acted as an important control in this respect. The reasons for this are discussed.

The gross and microscopic of this experiment are very similar to those noted by Herndon and Chase.

CONCLUSIONS

1. Articular cartilage did not survive homotransplantation in whole joints. The exception to this has been noted in the control group.
2. This nonviable articular cartilage is reabsorbed and replaced by metaplastic fibrocartilage from the synovium. Thus, cartilage death per se is not indicative of subsequent joint destruction.
3. Immunological factors play a role in the rate of bone replacement and subsequent preservation of joint architecture. The cartilage alone does not appear to be highly antigenic.
4. By decreasing the antigenicity of bone by freezing and irradiation, or freezing plus Imuran to the recipient, it was possible to lessen but not prevent the joint degeneration. Freezing alone did not reduce the antigenicity of bone significantly.
5. The control group did the best, followed in turn by the frozen, irradiated group, the Imuran group and lastly the frozen group.
6. There was excellent correlation of the 3 parameters used to detect cartilage viability and function only after 3 months following operation. The patellar cartilage served as an excellent control.

BIBLIOGRAPHY

1. Anderson, C.
The Structure and Function of Cartilage.
J.B.J.S. Vol 44A, 1962.
2. Axhausen G. R.,
Arch F. Klin Chir. 99, 1, 1912.
3. Barland, P. Janis, R. Sandson, J.
Immunofluorescent Studies of Human Articular Cartilage,
Ann. Rheum Dis. 25:156, 1966.
4. Bohr, H. Ravin, H. & Werner H.,
The Osteogenic Effect of Bone Transplants in Rabbits,
J.B.J.S. 50B No. 4, Nov., 1968.
5. Bostrom, H. & Mauson, B.
Ark. Kern. 6:23, 1953.
6. Braid H., Hagarty, R., Lec. W. Preservation of Cellular
Viability of Human Cartilage at Low Temperature.
Transplantation Vol. 4 No. 1, Jan. 1966.
7. Brooks, D. B. et al.
Immunological Factors in Homogenous Bone Transplantation
J.B.S. 45A, No. 8, Dec. 1963.
8. Bullough, P. Goodfellow, J.
The Significance of Fine Structure of Articular Cartilage
J.B.J.S. 50B, No. 4, Nov. 1968.
9. Bunche, H. J. et al.
The Fate of Autogenous Whole Joints Transplanted by
Microvascular Anastamoses.
Plast. & Reconst. Surg. No. 39, 4 April, 1967.
10. Buring K. & Urist, M.
Effects of Ionizing Radiation on the Bone Induction
Principle in the Matrix of Bone Implants. Clin. Ortho., No. 53, 1967.
11. Burkle de la Camp. Deutsch Ztschr. Chir. 217:109, 1929
12. Burwell, R. G.,
Biological Mechanisms in Foreign Bone Transplantation in
Modern Trends in Orthopaedics 4, 1964.

13. Burwell, R. G., The Scientific Basis of Bone Homotransplantation. Med. Ann. Review 1968.
14. Burwell, R. G. Studies in the Transplantation of Bone J.B.J.S. 48B, No. 3, 1966.
15. Campbell, C., Ishida, H., Takahashui, M. & Kelly, F., The Transplantation of Articular Cartilage J.B.J.S., Vol. 45A, No. 8, Dec. 1963.
16. Chalmers, J., Transplantation Immunity in Bone Grafting J.B.J.S. 41B, 160, 1959.
17. Chesterman, P. J., Smith, A. The Homotransplantation of Articular Cartilage and Isolated Chondrocytes J.B.J.S. 50B, No. 1, Feb. 1968.
18. Chrisman, O. D., Zemonsky, Charles, Bensch, K. G., Cathepsin in Articular Cartilage in "The Healing of Osseous Tissue" National Academy of Sciences, N.R.C. 1967, p. 169-192.
19. Craigmyle, M. M., Antigenicity and Survival of Cartilage Homografts Nature. Vol. 182, Nov. 1968.
20. Craigmyle, M. M. Studies of Cartilage Autografts & Homografts in the Rabbit. Brit. J. Plast. Surg. 8: 93-100, 1955.
21. Craigmyle, M. M., Regional Lymph Node Changes Induced by Cartilage Homografts & Heterografts in the Rabbits J. Anat. Hand, 92. p 74, 1958.
22. Cruess, R. and Mitchell N.. Effect of Synovectomy on Articular Cartilage Vol. 49A, J.B.J.S., 1967.
23. Curran, R. & Gibson, J. The Uptake of Labelled Sulphate by Human Cartilage Cells and It's Use as a Test for Viability. Proc. Ray. Soc. London 144B, 572-576, 1956.
24. Depalma, A. Tsaltas, T. & Mauler, G. Viability of Osteochondral Grafts as Determined by Uptake of S^{35} . J.B.J.S. 45A, 1565, 1963.

25. Di Ferante, N. & Paulin, G. M., Antibodies & Protein Polysaccharide from Nasal Cartilage
J. Lab. & Chir. Med. 63:945, 1964.
26. Dziewiakowski, D. D., Effect of Age on Some Aspects of Sulphate Metabolism in the Rat.
J. Exp. Med. 99:283-298, 1954.
27. Ekholm, R., Nutrition of Articular Cartilage
Acta. Anat. 24, 329-338, 1955.
28. Entin, M. A., Alger, J. R. & Baird, R. M.
Experimental & Clinical Transplantation of Autogenous Whole Joints.
J.B.J.S. 44A, 518-1536, 1962.
29. Entin, M., Daniel, G. & Kahn, D.,
Transplantation of 1/2 Joints
Arch. Surg. Vol. 98, Mar. 1968.
30. Erdelyi, R. Experimental Auto Transplantation of Small Joints
Plast. & Reconst. Surg. 31, 129-139, 1963.
31. Gibson, T. & Davies, W. B. The Fate of Preserved Bovine Cartilages in Man.
Brit. J. Plastic Surg. 6:4-25, 1953
32. Goodman, L. & Gillman, A. The Pharmacological Basis of Therapeutics.
Third Edition, 1968.
33. Hagarty, R. F. et al.
Surg. Gyn. & Obs. 110, 229, 1960.
34. Ibid. Surg. Gyn. & Obs. 110, 433, 1960.
35. Heiple, K. G., Kendrick, R., Herndon, Charles, Chase, S.
A Critical Evaluation of Processed Calf Bone
J.B.J.S. Vol. 49A, No. 6, Sept. 1967.
36. Herndon, C. & Chase, S. W. Experimental Studies in the Transplantation of Whole Joints
J.B.J.S. 34A 564, 1952.

37. Herple, K. et al. A Comparative Study of the Healing Processes Following Different Types of Bone Transplantation
J.B.J.S. 45A No. 8. Dec, 1963.
38. Hoagland, F. Experimental Hemiarthrosis
J.B.J.S. 49A, No. 2, Mar. 1967.
39. Hodge, J. McKibbin, B. The Nutrition of Mature & Immature Cartilage in Rabbits
J.B.J.S. Vol.51B, No. 1, Feb. 69.
40. Jaros, M., Ondrouch, A., Bone Transplantation, Part 2, Transplantation of Large Bone & Joint Parts
Acta Chirurgical Orthopiedicae et Traumatologica Cechoslavaca, Vol 32, No. 5, 1965.
41. Judet, H. Essai sur la Greffe des Tissus Anticulaires
Comp. Rend. Acad. D. Sci. Par 166;193, 1903
42. Khvorostukkin, I. Antigenic Properties of Articular Cartilage
Bull. Exper. Biol. & Med., 45: 90-93, 1958.
43. Kingma, M. J., Hampf, J. F. The Behaviour of Blood Vessels after Experimental Transplantation of Bone.
J.B.J.S. 46B, No. 1, Feb. 1964.
44. Kopriwa, B. M. & Leblond Improvements in the Coating Techniques of Radio Autography
J. of Histochemistry, Vol.10, p. 269, 1962.
45. Kosinka, E., Janecek, M. Bone Transplantation, Part 1, Transfers of Large Homoplastic Bone Grafts in Acta Chirugiae Orthopaedicae et Traumatologiae Cechoslavacae, Vol. 32, No. 5, 1965.
45. Lack, C. H., Ali, S. Y. The degradation of Cartilage by Enzymes in Cartilage Degradation & Repair
National Academy of Sciences, N .R.C., 1967. p. 61-71.
46. Lexer, L. S. G. & O. 40: 782, 1925.
47. Loewi, G. & Muir, M. The Antigenicity of Chondromuco-protein
Immunology 9:119, 1963.

48. Mankin, H. The Metabolism of Matrix of Articular Cart. Pittsburgh, 1967.
49. Mankin, H. Personal Communication, 1969
50. Mankin, H. The Structure, Chemistry & Metabolism of Articular Cartilage
Bulletin on the Rheumatic Diseases Vol 17 No. 7, Mar. 1967.
51. Maroudas, A. Bullough, P., Freeman, M. A. R., Swanson, S. A. V.
The Permeability of Articular Cartilage
J.B.J.S., Vol. 50B No. 1, Feb. 1968.
52. Maroudas, A. Physiochemical Properties of Cartilage in the Light of Ion Exchange Theory.
53. May, H. The Regeneration of Joint Transplants and Intra Capsular Fragments
Ann. Surg. 116: 297-310, 1942.
54. McLean, F. & Urist, M. Bone: Fundamentals of the Physiology of Skeletal Tissue, 3rd. Edition, 1968, p. 45-46
University Press Chicago.
55. Meachim, G. Sulphur Metabolism of Articular Cartilage After Surgical Interference with the Knee Joint.
Amer. Rheum. Dis. 1964, 23 12.
56. Meachim, G. The Histology and Ultrastructure of Cartilage pp 3-16. In Cart. Degradation & Repair
National Academy of Sciences & National Research Council, 1967, Wash.
57. Mitchell, N. & Cruess, R., Synovial Regeneration after Synovectomy
J.B.J.S. Vol. 47, 1965.
58. Ottolenghi, C. Massive Osteoarticular Bone Grafts
J.B.J.S. 4813 No. 4, Nov. 1966.
59. Ouchterlony, O. Progr. Allergy 5,1, 1958.
60. Pap, K. & Krompecher, G., Arthroplasty of the Knee
Experimental and Clinical Experiences
J.B.J.S. 43A, 523-537, June, 1961.

61. Parrish, F., Treatment of Bone Tumors by Massive Autogenous and Homogenous Grafts
J.B.J.S. 48A, 1966.
62. Peacock. Antigenicity of Cartilage
Ann. New York Acad. of Sci. 1957.
63. Pearse, A. G., Histochemistry, Theoretical & Applied,
p. 180-213, Third Edition, Vol. 1, 1968.
64. Pelc, S. R. & Gluchsman, S.,
Exp. Cell Res. 8:336, 1955.
65. Russell, P. & Monaco, A. Tissue Transplantation
N.E.J.M., Vol 271, Sept. 1964.
66. Sandson, J., Rosenberg L. & White, D. The Antigenic
Determinants of Protein Polysaccharide of Cartilage.
Exp. Med. 123, 817, 1966.
67. Sandson, J. Personal Communication, 1969.
68. Sandson, J., Hamerman, D., Jassis, R. & Rojkind, M.
Immunologic & Clinical Similarities Between the
Streptococcus and Human Connective Tissue - Transactions
of the Association of American Physicians, Vol. 81,
1968, p. 249-257.
69. Schallen, W. E. et al. Biological Survival and Growth of
Cartilage Cells
Plastic & Reconst. Surg. 22:11-28, 1958.
70. Seif, S. Comments of the Antigenicity of Cartilage
Immunochemistry of Connective & Skeletal Tissues.
71. Slone, D. & Reeves, B. Experimental Homotransplantation
of the Knee Joint
Lancet, July 23, 1966.
72. Spicer, S., Ham, R & Leppi, T. Histochemistry of Connective
Tissue Mucopolysaccharide. The Connective Tissue International
Academy of Pathology Monograph 7.
Williams & Wilkins, 1967.

73. Tietz, A. Chir-Konge Verhandl 1:77, 1902.
74. Tuffier, M. Resection Chonchoplastique de la tete
numerals pain fracture communicative.
Burl et Mein Eoc. Chir 27, 492-493, 1901.
75. Urist, M., Silverman, B., Buring, K., Dubuc, F., Rosenberg, J.
The Bone Induction Principle
Clin. Ortho. No. 53, 1967.
76. Urist, M. & Adams, J. Cartilage or Bone Induction by
Articular Cartilage
J. B. J. S. No. 1, 50B, Feb. 1968.
77. Vavrda, J. Homoplastic Transfer of Joint Surfaces
Bone Transplantation Part III, Homoplastic Transfer
of Joint Surfaces. Acta Chirurgiae Orthopaedicae et
Traumatologiae Cechoslavacae, Vol. 32 No. 5, 1965.
78. Weiss, C., Rosenberg, L. & Helfet, A.
An Ultrastructural Study of Normal Young Adult Human
Articular Cartilage.
J. B. J. S. 50A, No. 4 June, 1968.