

**INTRA-ARTICULAR RADIOACTIVE CHROMIC PHOSPHATE
IN AN ANIMAL MODEL OF RHEUMATOID ARTHRITIS ©**

by

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INTRARTICULAR ^{32}P CHRONIC PHOSPHATE IN A MODEL OF RHEUMATOID ARTHRITIS

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ABSTRACT

Radioisotopes have been employed in the therapy of chronic arthritis, in particular, rheumatoid arthritis for many years. A variety of isotopes have been popularized, and in the last ten years, a colloidal solution of radioactive chromic phosphate has been in use. No controlled studies on this modality have been reported clinically and few animal studies were found. The efficacy of ^{32}P as a medical synovectomy, its effects on the articular cartilage on normal and arthritic knee joints in the rabbit was examined.

Forty knee joints in 20 adult rabbits were treated with 0.1 mCi ^{32}P , a dose comparable with regimens in man, and were examined over 26 weeks by histological and biochemical means. While fibrotic changes in the synovium suggested some previous insult there was no evidence that synovectomy had occurred; there was, however, clear suggestion of articular cartilage damage in all groups and evidence of cartilage degeneration at 26 weeks.

Forty arthritic knee joints in 20 rabbits treated on one side with ^{32}P , the opposite serving as control were sacrificed over 60 days. The synovium showed no evidence of radiation necrosis in treated joints. Cartilage of treated and control joints showed similar changes consistent with chronic arthritis with progressive chondrocyte degeneration, decreased matrix metachromasia and decreased hydroxyproline content. These changes moreover appeared less pronounced in treated joints suggesting a possible protective function of the isotope.

The popular ovalbumin antigen-induced arthritis model was reassessed prior to implementation in the forementioned study. Marked changes in the articular cartilage consistent with acute cartilage necrosis were found, making this model inappropriate as an model of rheumatoid arthritis. A useful modification of the model was described.

RESUME

Les radioisotopes sont employés dans le traitement de l'arthrite chronique; en particulier l'arthrite rhumatoïde, et ce depuis de nombreuses années. Une variété d'isotopes a été rendu populaire. Au cours des dix dernières années, une solution colloïdale de chrome phosphate a été utilisée. Aucune étude contrôle sur cette modalité n'a été rapporté de même que peu d'étude sur animaux. L'efficacité du ^{32}P comme synovectomie médicale a été étudiée chez le lapin, ainsi que son effet sur le cartilage articulaire de genoux normaux et arthritiques.

Quarante genoux chez 20 lapins adultes ont été traités avec 0.1 mCi ^{32}P , une dose comparable à celle utilisée chez l'homme. Ceux-ci ont ensuite été examinés biochimiquement et histologiquement sur une période de 26 semaines. Tandis que des changements fibreux dans la synoviale suggère une insulte précédente, il n'y avait aucune évidence qu'il y ait eu une synovectomie, il y avait cependant évidence de dommage du cartilage articulaire, dans tous les groupes et évidence de dégénération du cartilage à 26 semaines.

Vingt lapins (40 genoux arthritiques) traités d'un côté avec ^{32}P , le côté opposé servant de contrôle, ont été sacrifiés après 60 jours. La synoviale ne montrait aucune évidence de nécrose de radiation dans les articulations traitées. Le cartilage des articulations traitées, et de contrôle ont démontré les mêmes changements consistants de l'arthrite chronique avec dégénération progressive des chondrocytes, diminution de la métachromasie de la matrice et du contenu en hychoxyproline. Ces changements cependant, apparaissent moins marqués dans les articulations traitées suggérant qu'il y ait possiblement une fonction de protection de l'isotope.

Le populaire modèle d'induction d'arthrite "antigène ovalbumine" a été réévalué avant le début de cette étude. Des changements marqués dans le cartilage articulaire consistant avec une nécrose aiguë du cartilage ont été notés, faisant de ce modèle, un modèle inapproprié pour l'arthrite rhumatoïde. Une modification utile de ce modèle est décrite.

PREFACE

Radioisotopes have been employed in the intraarticular therapy of chronic arthritis in particular rheumatoid arthritis for many years. The aim of this form of therapy has been to produce an non-surgical synovectomy in a joint in order to delay or prevent joint pathology and joint symptomatology. A variety of elements has been employed; more recently ^{32}P has been popularized. To date, no controlled studies on this modality and few animal studies, have been reported. Chapter 1 of this thesis, defines the structure and histology of the joint tissues, defines rheumatoid arthritis, describes the current theory of pathogenesis of rheumatoid arthritis, gives a brief summary of radiation pathology, reviews the literature of surgical synovectomy in the management of the rheumatoid joint, and reviews the literature of medical synovectomy in the management of the rheumatoid joint. In Chapter 3 Part A of this paper, the efficacy of ^{32}P in producing a synovectomy and its effect on articular cartilage in the knee joint of normal adult rabbits is examined. In chapter 3, Part B the effect of ^{32}P on the knee joints of rabbits with arthritis is reported.

In the course of design and investigation of these experiments, an accepted model of rheumatoid arthritis was chosen. However, surprising and unexpected observations were made of the model making it inappropriate as a tool, and necessitating the repetition of much of the experiments of Chapter 3 Part B using a modification of the model. For this reason, Chapter 2 on studies in antigen-induced arthritis was placed first in this thesis and concerns itself with the model and the modification made of this model for use in the experiments on ^{32}P . It also contains a review of animal models of rheumatoid arthritis.

It is considered that the studies of the animal model of arthritis put into question many of the conclusions drawn from the model as previously employed and is of value in cautioning future workers who use this model in the interpretation of their results.

Secondly, to our knowledge the studies reported are the first detailed examination of the effect of ^{32}P in the experimental arthritic animal. The experiments detailed in Chapter 3 suggest that normal tissues exposed to ^{32}P are adversely effected by it while pathological tissues are, at worst, uneffected and may be favourably effected by it. It is considered that this thesis cautions those physicians employing ^{32}P in a clinical setting, of the still questionable validity of this therapeutic modality.

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The following abbreviations have been used:

acetosalicyclic acid	ASA
American Rheumatism Association	ARA
antigen induced arthritis	AIA
cetylpyridinium chloride	CPC
ethylenediaminetetraacetic acid	EDTA
Erbium	Erb
erythrocyte sedimentation rate	ESR
Gold	Au
hematoxylin and eosin	H&E
hydrogen peroxide	H ₂ O ₂
interphalangeal joint	IPJ
metacarpophalangeal joint	MCP
microcurie	μCi
micromilliliter	μml
micron	μ
millicurie	mCi
milligram	mg
milliliter	ml
millimeter	mm
millimole	mmol
Molar	M
molecular oxygen	O ₂
monocyte inhibitory factor	MIF
Normal	N
Phosphorous	P
proximal interphalangeal joint	PIPJ
Rhenium	Re
rheumatoid arthritis	R.A.
transmission electron microscopy	TEM
United States Nuclear Regulatory Commission	USNRC
Yttrium	Y
Radiation Biological Equivalent	RBE

CHAPTER 1
BACKGROUND

1.1

DEFINITIONS AND HISTOLOGY

Since rheumatoid arthritis is a systemic disease whose principle manifestations are in the synovial joints, it is appropriate to begin here.

1.1.1 Synovial joints: Synovial joints are articulations surrounded by and sealed by an outer capsule of fibrous tissue, reinforced by tendons and aponeuroses, and an inner lining of which is the synovium - a single cell layer arranged in folds or villi allowing unfolding and folding or crimping of the synovial layer with the demands of the articulating bones in movement and fluid requirements of the joint cavity. The articulating ends of the bones are covered by a cap of cartilage - hyaline cartilage, which is responsible for the biomechanical properties of the joint in absorbancy, load bearing, and reduction of surface friction. Synovial joints contain a fluid which has biomechanical, nutritional and circulatory properties and in many joints there is also a meniscus of fibrous tissue which serves a complementary and supportive role to the properties of the cartilage.

1.1.2 Synovium:

Of mesenchymal origin, the synovium is thought to have formed by differentiation of the area between the two abutting primary cartilaginous bony models. A cystic space forms and shortly thereafter, the space becomes lined with primitive mesenchymal cells which differentiate into synovial cells. The synovial cells are ovoid lying with their long axes parallel to the surface. By scanning EM, the membrane is furrowed like an accordion and pores are visible (118). By L.M., the synoviocytes appear as large ovoid cells with cytoplasmic processes with many vacuoles and evidence of lysosomal enzymes in the cytoplasm. By E.M. no basement membrane, adhesion plates or desmosomes can be detected. The cells may be recognized as two types: A cells, whose morphologic and functional activities resemble macrophages, and B cells

whose morphologic and functional activities resemble fibrocytes.

Type A cells, in the highest proportion, show many large vacuoles with cytoplasmic processes directed toward the cell surface, with branched fibrils lying parallel to the cell membrane, and with micropinocytotic vesicles, many mitochondria, and Golgi arranged near the apical part of the nucleus and cytoplasmic processes. A dense fibrillar and granular material has been identified coating the cell membrane.

Type B cells have the same cell body configuration but have well developed rough endoplasmic reticulum, only occasional and smaller mitochondria, prominent Golgi apparatus, longitudinal microfilaments, vesicles and secretory granules (infact fewer in number than type A cells). These secretory granules (8) are in intimate association with the Golgi which is involved in the granule formation which contain mucopolysaccharides (104,8). The intercellular matrix is of an amorphous substance devoid of fibrils.

Beneath the lining layer, the fibroareolar tissue or subsynovium is of variable depth, of variable density with collagen fibres, of variable organization and thickness. Elastic fibres are interspersed throughout, as are vessels. Randomly interspersed are fibrocytes and scattered histiocytes, mast cells; the blood vessels are ramifications of larger vessels supplying the fibrous capsule. With age, the veins often show sclerotic changes and hyaline thickening even as early as 20 years. The synovium may be supported by this fibrous subsynovium of variable density or may sit directly on areolar tissue which is strengthened by elastic and collagen fibres which extend out into the capsule (133).

1.1.3 Cartilage:

Cartilage is described as a "viscoelastic" material coating the articulating ends of bones. The "hyaline" nature, which describes its pearly appearance, is actually a surface with many humps and ridges caused by underlying and protruding chondrocytes.

TANGENTIAL ZONE

TRANSITIONAL ZONE

RADIAL ZONE

TIDEMARK

CALCIFIED CARTILAGE

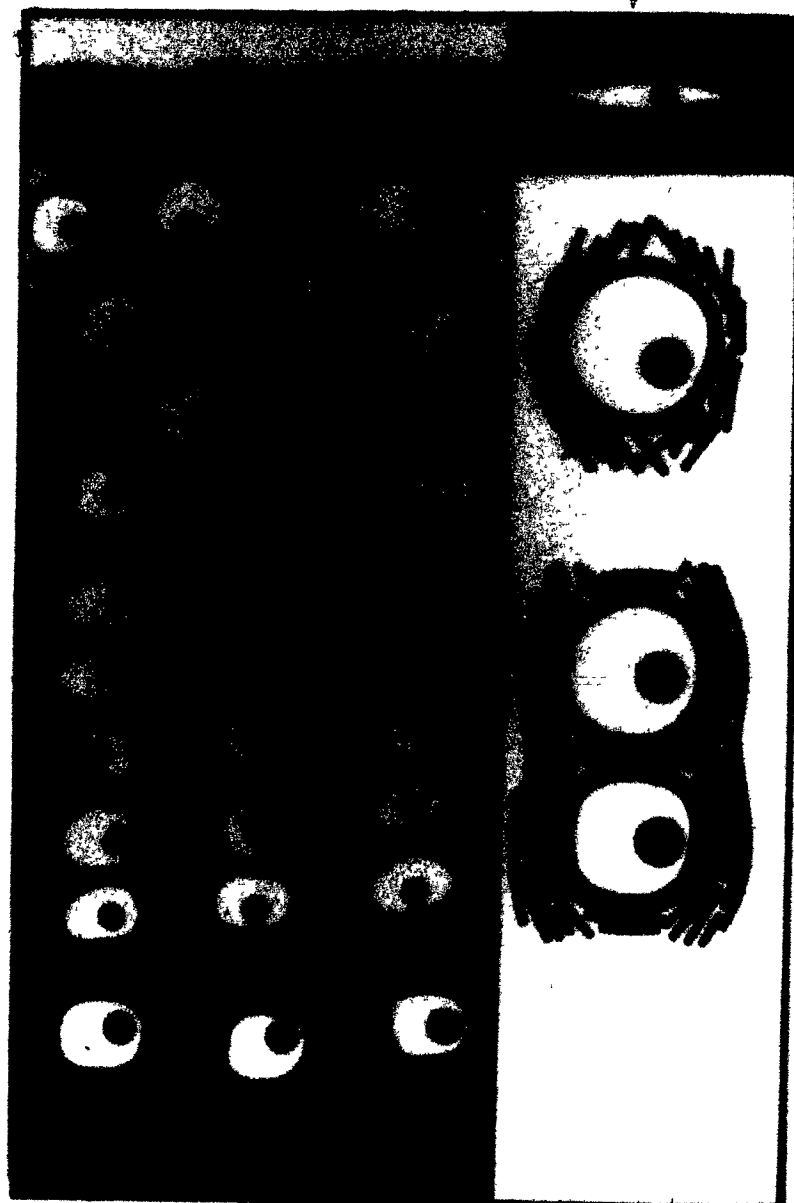


Fig 1. schematic representation of the microscope architecture of articular cartilage.

The ridges, which tend to run perpendicular to the axis of rotation of the joint are thought to be secondary to the mechanical properties of the joint as the corrugations of a dirt road are after tires have rolled over it innumerable times.

Cartilage is composed of a fluid matrix of water and proteoglycans strengthened by collagen fibrils into which are embedded chondrocytes.

Microscopically, one is able to identify zones or layers in the architecture of cartilage from the inner surface down to the bone interface (fig 1).

The most superficial layer is the **tangential** zone in which thin collagen fibres lie parallel to the surface in a mat. The most superficial layers do not stain with cationic dyes indicating a lack of proteoglycan. Cells here lie with their long axes parallel to the surface. They have little endoplasmic reticulum and have flattened inactive Golgi saccules. In the transitional zone beneath, collagen fibres are haphazardly arranged with proteoglycan aggregates and water as matrix stabilizing and strengthening it. The cells are round, small, and also haphazardly arranged with one or more prominent nucleoli as a prominent feature. Around the cells is a marked concentration of proteoglycan and collagen arranged in a circular lattice-work. As one descends through the zone the collagen fibrils become more perpendicularly arranged. In the **radial** zone in the lower half to one third of the tissue, the collagen and cells gradually appear to lie perpendicular to the surface. The cells are arranged in columns several cells high, have a notably well developed Golgi, rough endoplasmic reticulum and glycogen (for anerobic metabolism). Around the cells are halos of concentrated proteoglycan and an over all increased in the intercellular matrix. The fibrils are much thicker and more densely packed in this zone and the radial zone meets a basophilic wavy line called the **tidemark** whose significance is speculative but is thought to be the attachment point of the collagen network of fibrils acting to transmit and dissipate stresses imposed. Below this is the **calcified** zone

extending to the underlying bone. Here the cells are arranged in columns; more of them appearing with a darkly staining cytoplasm and more obviously degenerating. The collagen fibres are arranged perpendicular to the surface and though proteoglycans are known to be present, their tertiary structure has not been defined. The area is fully calcified and below this lies bone with its Volkman's canals and cells resembling osteoclasts.

On a biochemical level, the matrix is composed of collagen proteoglycan aggregates and 65 to 80% water. The proteoglycan is of three species: hyaluronic acid, a proteoglycan subunit, and a link protein. The proteoglycan subunit is composed of a protein core to which are attached the glycosaminoglycans, keratin sulfate and chondroitin sulfate, negatively charged molecules which repel each other and therefore arrange themselves about the protein core in a rigid fixed orientation. Link protein a small protein of approximately 40,000 M.W., links the proteoglycan subunits to the hyaluronic backbone to form an aggregate. The proteoglycans may associate the hyaluronic acid molecule in aggregates as small as 15,000 Å or as large as 100,000 Å in developing cartilage. The proteoglycan subunits with their rigid glycosaminoglycans are thus hooked to hyaluronic acid in a molecule which then attains a quaternary formation as a globule. These interact with the collagen fibres, crosslinking them; water molecules become trapped in this meshwork due to the hydrophilic nature of the globules. Linkages of collagen fibrils with adjacent ones are accomplished with bonds with hyaluronic acid and also with link protein.

The collagen triple helix structure accounts for the tensile strength of the cartilage but has no compressive properties. The proteoglycans are structured with hydrophilic tendencies and maintain hydration and keep the collagen in random but fixed position. As a strain is applied to cartilage, the water escapes from the matrix to the surface adjacent to the load, and the matrix tends to compress under the load to a point where it is resisted only by the collagen and proteoglycan bulk (97).

Biomechanically, this arrangement of protein with trapped water molecules and a collagen latticework lends an amazing strength to this thin layer of cartilage which in humans is less than 7 millimeters thick. Normal synovial joints sustain a load of five times body weight, have a coefficient of friction of .002 (for 1000 pounds of load on a joint, only two pounds would be required to slide the joint), and a sliding speed of less than 65 meters per second (97).

1.1.4 Rheumatoid Arthritis:

Rheumatoid arthritis is a systemic disease with wide spectrum of manifestations, the most prominent of which is chronic inflammatory polyarthritis whose natural history is total joint destruction. The joint changes in rheumatoid arthritis are characterized in stages by a congested edematous synovium infiltrated with lymphocytes, hypervascularity, with superficial patchy necrosis covered by fibrinoid material (mainly fibrin, immunoglobulins and complement). This evolves to a lining principally of plasma cell infiltration with lymphoid foci and multinucleated giant cells. Perivascular infiltration prevails and the fibroblastic and capillary proliferation called granulation tissue causes gross villous thickening from the periphery of the joint lining. The synoviocytes become hyperplastic and hypertrophy of the membrane occurs, sometimes extending 10-20 cells thick. Granulation tissue spreads as "pannus" creating a physical barrier between the nutritive synovial fluid and the underlying cartilage which is wholly dependent on the fluid for circulation. This pannus secretes proteinases which, once activated, erode away the cartilage. As joint destruction proceeds, the pannus becomes quiescent, shrinks and becomes fibrotic, the capsule contracts and the additional stresses on the joint contribute to shifting of the articulation further creating unnatural stresses on the cartilage. Later, calcification of the fibrotic capsule may cause bony bridges to form with an end stage of denuded bone interface which ankyloses (2).

Changes observed in early arthritis where only synovitis is grossly apparent have shown polymorphonuclear cells adherent to, and apparently invading, the superficial layer of the cartilage (60). As well, pannus cells resembling rheumatoid synovial cells with "long cytoplasmic processes which penetrated for a variable distance into the superficial layers of the cartilage have been identified and under the electron microscope, chondrocytes could be seen to be surrounded by a foreign cytoplasm possessing the features of the pannus cells lying above" (90).

Early changes also include erosion of the superficial layer of cartilage. Even earlier changes suggested by Mitchell and Shepard are the increased production of a fibrillar material around the cells of the mid and deeper zones as a protective device against the destructive enzymes being secreted and diffusing through the cartilage. Cells which did not respond in this way often appeared with large lipid accumulations in the cytoplasm and no moat of material. As well, proteoglycan could be seen deficient in the matrix surrounding the cells and precipitated proteoglycan lying on the surface of collagen fibrils and being phagocytosed by chondrocytes (93).

As more cells die, proteoglycan can not be produced in amounts enough to compensate for the amount being degraded. The loss of compressive resilience soon leads to fibrillation of the cartilage surface and the now exposed matrix, rapidly degrades. The whole joint surface becomes denuded and bone rubs against bone.

1.2

Pathogenesis of Rheumatoid Arthritis:

Though the etiology of rheumatoid arthritis remains an enigma, the many theories on pathogenesis are beginning to become consolidated into a more cohesive picture. Poole et al (113) summarized the current thinking of the inflammatory arthritides of which rheumatoid arthritis is the prototype.

The pathogenesis "involves the destruction of connective tissues"... and may be ... "directly related to the development of autoimmunity to some of the macromolecules from which these tissues are assembled." These "immune processes stimulate excessive secretion of proteinases from synovial cells and fibroblasts. Further destruction of connective tissue results in the presentation of more autoantigens and enhanced stimulation of proteinase secretion. A chronic cycle of immunopathology is established in which the connective tissue provides the seeds for its own destruction." (113).

Thus, connective tissue damage, for example, caused by trauma, by inflammation, or by infection allows presentation of the antigenic material to B cells and T cells by macrophages and polymorphonuclear cells, setting up first, the humeral antibody response forming immune complexes with the antigen. Vascular permeability increases and monocyte and polymorphonuclear cells infiltrate. The complexes, through intermediates cause the increased permeability and vasodilation through degranulation of mast cells, and cause the polymorphonuclear cells to release proteinases causing degradation of collagen and proteoglycan, the principle components in joint tissues thus exposing more of the previously "privileged" antigenic sites on, and degradation products of collagen and proteoglycan, which stimulate and amplify the acute immune response. At the same time, the chronic system is set up through the T cell-B cell lymphocyte - monocyte system releasing monocyte collagenase factor (MCF) or catabolin which controls and stimulates the synovial cells to produce proteinases for further destruction, the fibroblasts and the chondrocytes for repair, and

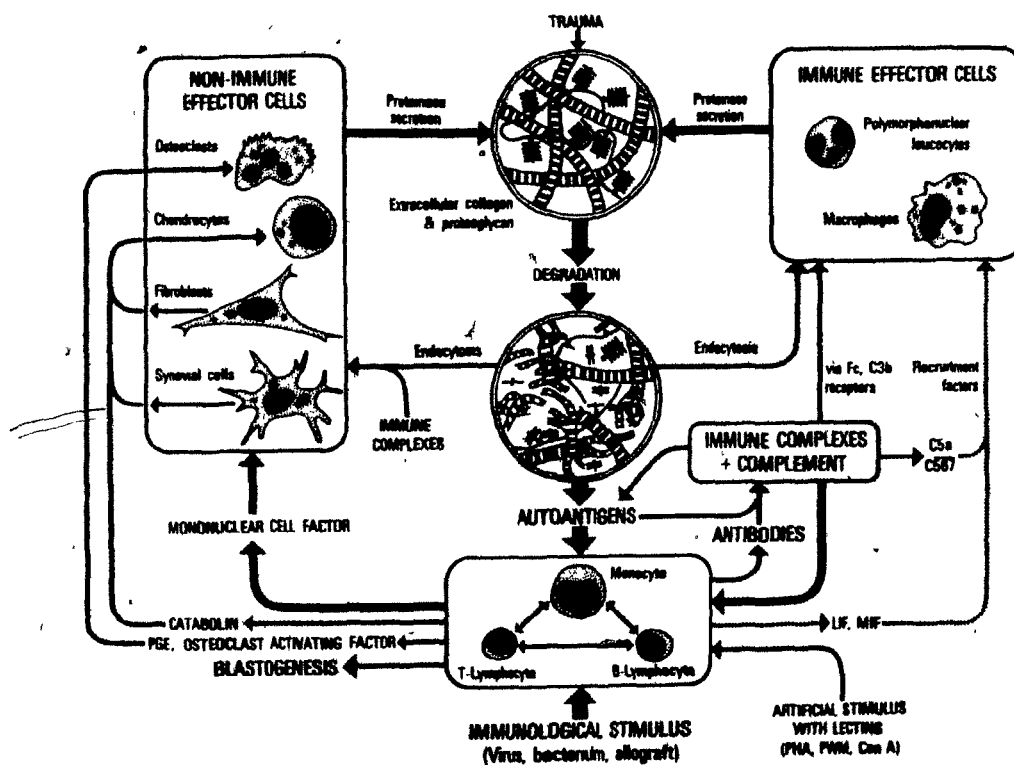


fig 2 Schematic representation of the pathogenesis of inflammatory arthritis
Diagram kindly supplied by Dr. A.R. Poole. Reproduced with permission from
Adult Orthopaedic Surgery. ed. Cruess RL and Rennie W.

the osteoclasts for dissolution of bone. The more the destruction, the more the system is amplified.

The systemic manifestations of the disease are believed by some (personal communication Dr. A.R. Poole) to be an expression of cross-reactants in other tissues. For example, reactants on corneal and other collagens and proteoglycans in the eye may result in uveitis and iridocyclitis; reactants on nerve sheath may result in central nervous system manifestations.

1.3

RADIATION PATHOLOGY:

The effects of radiation on tissue are determined by the type, dose, duration of exposure and type of tissue and its environment.

Elementary particles with their accompanying energy take several forms -the beta particle and gamma particle being those in medical use. The basic action of these particles is penetration of mass, removing electrons from the mass and in its course expending energy and allowing the particle to come to rest. The mass which has lost electrons is thus "ionized". In biological systems a number of things may happen: on a macromolecular level, water molecules are disrupted "resulting in the formation of hydrogen atoms, hydrated electrons and hydroxyl radicals. In the presence of oxygen, both oxygen and subsequently H_2O_2 are formed. This extracellular O_2 flux may result in the formation of oxygen dependent chemotactic factor resulting in the emigration of granulocytes into the irradiated region and possibly the activation of these cells by other products of irradiation" (28).

The kind of radiation, dose rate, and ionization density will influence the tissue's ability to recover from insults before a demonstrable cumulative effect on the function of the tissue as a whole, occurs. The sensitivity (the mitotic and G2 stages being most vulnerable), and ability of DNA to repair, (and therefore the tissue's inherent turnover rate), the age of the tissue exposed, the species differences, the fitness of the metabolic system surrounding (eg. O_2 tension) are other variables which effect the end result.

A variety of things may occur in biological systems, but the most critical are the effects on the genetic macromolecules. The extent of damage depends on among other things, the stage of cell cycle. "In vitro radiation of DNA solution results in breaking of hydrogen bonds, formation of cross-links with adjacent strands or opposed regions of the same strand, base damage, disruption of the sugar-phosphate backbone of the molecule,

impairment of the ability of 'DNA to act as template for the synthesis of a new DNA strand' (2) - all of which effects the ability of a cell to translate or transcribe a replicate. The effect on RNA in the cytosol of a cell is less for reasons that are unclear. In the cytosol radicals formed direct ionization or indirect reaction may react with solutes and block biochemical pathways.

Histologically one can observe the nucleus swelling, the chromatin becoming clumped and if the dose is high enough the nucleus becomes pyknotic, the chromatin fragments and there is a focal loss of nuclear membrane; the cytoplasm at the same time swells, demonstrates vacuolization and the membranes partially disintegrate.

On a morphological level, the various tissues will react according to their susceptibility base on their inherent turnover rate and metabolic activity: Capillaries show dilation and increased permeability. The endothelial cells swell, the lumen narrows and the walls sclerose further narrowing the lumen. Thrombosis then forms and completes the obliteration. The arterioles show the same sequence but initially the endothelium proliferates, followed by hyaline material deposition on the subendothelial layer. The wall becomes sclerotic, and the muscle cells degenerate. The arteries demonstrate changes only later with endothelial cell damage, the internal elastic lamella later fragments, smooth muscle cells degenerate, the adventitia fibrosis and a hyaline-like material accumulated in the media. These vascular changes secondarily cause necrosis of the surrounding tissue and loss of parenchyma. Fibrotic replacement occurs and the tissue demonstrates poor wound healing and susceptibility to infection.

Little is written in human pathology texts with specific reference to synovial cartilage and bone changes with radiation. But synovium of mesenchymal origin would be expected to react similarly to that described about vessels. It is thought that bone and cartilage changes are usually secondary to surrounding soft tissue and vascular insults in the adult and relatively resistant to radiation. In the developmental stages, however, when rapid cellular turnover and activity occur, bone and cartilage should be more susceptible to ionizing rays.

REVIEW OF SURGICAL LITERATURE OF SYNOVECTOMY IN THE MANAGEMENT OF THE RHEUMATOID JOINT:

The management of the joint pathology is the focus of attention in rheumatoid arthritic patients. Medical management with antiinflammatories analgesics, physiotherapy, and a variety of other regimens, for example, gold, penicillamine, is of primary and paramount importance in attaining, and maintaining active functional joints for independent activity. But what of the chronic, intractably painful, stiff joint? And what about the chronically swollen, but architecturally intact joint, at one end of the spectrum, to those of which may euphemistically termed "joints" but are complete ankylosis of a once functional joint?

Surgical goals have been to restore joint mobility, restore stability, relieve pain, and retard degeneration of the joint.

Synovectomy has been one of the earliest procedures aimed at these goals. Schuller (42) first reported relief of pain and stiffness in four patients after he removed synovium from rheumatoid knees in 1887. Swett in 1923 (143,144) reported the first series of 15 synovectomies for "chronic infectious" arthritis with good results at two years in eleven of twelve patients. Several of the cases presented, when reviewed in the light of the experiences of the 1980's were in all probability not infectious, but belonged to the collagen group or rheumatoid group of arthritides. The enthusiasm with this as a mode of therapy quickly gained momentum.

Does it work? Obvious questions one must ask in evaluating this procedure are (1) does it relieve pain long term? (2) does it change the degree of independence of the patient? (3) does it increase the mobility and functional level of the joint? (4) does it affect the natural history of the disease in that joint, and how? (5) which joints are candidates? (6) what is the association with systemic disease? (7) how much of the subjective changes are placebo?

1.4.1 Clinical Studies

Downie et al (32) gives a comprehensive bibliography of reported series between 1923 and 1970 and then reports his own series of 145 synovectomies in 145 patients. In his Glasgow series with follow-up of 6 to 63 months with an average of 36 months using parameters of pain, stiffness, range of motion and clinical signs of joint inflammation, he found a successful outcome of 82% in terms of functional grade of the patient, subjective and objective assessments of the joint. No breakdown as to the stage of the disease in the joint preoperatively with respect to outcome was made nor objective radiological assessment reported. And of course, it is impossible to address questions of natural history and placebo effect without a double blind study - obviously unethical proposition. His results closely parallel those he reviewed to 1970 in his paper

Goldie (45) assessed radiological changes in 27 knees pre and post synovectomy in the first three years, in 2 knees at four years, and in one knee at seven years. He graded them on the basis of permanent signs of joint destruction, narrowing of joint space, periosteal elevation, rarefaction, erosions, subchondral changes. Only two of 25 knees lost further joint space (one because of septic arthritis and one due to marked progression of systemic disease); periosteal changes and bone rarefaction remained stable; no new erosions appeared in any of the knees, though none healed, and preexisting ones either increased "slightly" or remained unchanged. Subchondral changes increased in one of fifteen.

Ranawat has examined 40 knees one to eight years post synovectomy and found decreased joint space in all, but improvement in subchondral bone, and healing of cysts radiologically. Subjectively and objectively, 60% improved, but the patients tended to appear late with varis and valgus deformities. The severity of the disease did not correlate with the result. The picture is confused, somewhat, however, because both

synovectomy alone and synovectomy with patellectomy patients were included (117).

In a review of 175 knees with a follow-up of four years Marmor found 50% overall had improved subjectively, most had increased joint space narrowing. Sixty percent had improved their functional status. Those who failed usually showed preexisting, and therefore, continued joint destruction. Sixty-nine of 175 lost an average of 29 degrees flexion and 69 patients gained a small amount. Seventy percent (26/36) of patients with early changes (grade I by A.R.A. grading) did well at least one year post synovectomy (80).

Graham (49) reviewed 122 knees at five to nine years and though his results are difficult to integrate he indicates approximately 50% improvement and implied that milder forms of disease produced better even results.

The most comprehensive review to date is reported by Laurin et al (71). In 66 knees with follow-up of five to seventeen years he confirmed what those with small numbers and incomplete data had suspected. The stage of the joint disease is related to the end result. The A.R.A stages 1,2 and 3 have 100, 75 and 64 percent success respectively. Sixty-six percent overall did well; synovectomy did not accelerate the disease but did in fact appear to delay degenerative changes in the cartilage and underlying bone despite exacerbation of the systemic disease.

Regenerating synovium has been examined by several authors and found microscopically to have the same features as the original synovitis; by others to appear normal (4,41,46,79,140,123); and by others to be quiescent, thin, with synovial cell layer of two to three cells deep, with slight plasma cell and lymphocytic infiltration of the synovium and hypervascularity of the same degree as preoperatively (46). Ranawat noted a thick fibrous subsynovial layer in addition in his five quiescent but demonstrably rheumatoid post-synovectomy biopsies examined at one to six years (116).

1.4.2 Experimental Studies:

If then, despite return of synovitis, clinically there is improvement, what is the pathophysiology? Why does the synovectomized joint, with a regenerated synovium that still looks rheumatoid, not deteriorate as would be expected. Why does the patient obtain lasting (years) relief? Why do some get worse?

There are perhaps no answers to the latter questions but insight into the latter has been gleaned from the observations of Key (70) and Mitchell (87,88,89,91).

Key, in 1925 reported on the results of 24 knees of normal rabbits after partial synovectomy with partial medial meniscectomy. He observed them at 2,3,4,6,15,86 and 104 days and saw a pattern of, first, the joint space filling with a fibrinous exudate and blood, cells appearing like fibroblasts in active division and a capsule becoming infiltrated with polymorphonuclear cells and monocytes. New capillaries were already forming at two days, the fibrin exudate gradually cleared and the fibrin adherent to the capsule was hyaline-like. The underlying fibroblasts proliferated and spread into the fibrin layer. Inflammatory cells were still prominent at four days. By six days a layer of fibroblast lined the surface with many extending into the fibrin layer. By eight days, the cells were oriented parallel to the surface. By sixteen days, new collagen was being formed in the subsynovial tissue. The surface cells were one to four cells thick and closely packed. By four weeks an areolar layer separated the cellular and fibrinous layer. Over the ensuing month to two months, the synovium took on a normal pattern with villi, and organized fibrous subsynovium. Key's work consolidated the commitment that regenerating synovium comes from mesenchymal elements.

Wolcott (87) working independently confirmed in his report the findings of Key. It was not until 1967 that much attention was paid to synovium post synovectomy. Mitchell and Cruess (87) demonstrated with thymidine labelling that post synovectomy cells of "mesenchymal" appearance, that is, undifferentiated fibroblasts, proliferated and that the relatively small increase in proliferation of the remnants of synovium

in the joint did not meet the areas of new synovium and could not be active enough to account for the new membrane formed. They also examined the cartilage (88) and observed that during the regeneration of synovium, at no time did the chondrocytes show any ill effects as measured by their ability to take up sulfur for proteoglycan synthesis. There was, however, a significant decrease during the first 45 days of metachromasia suggesting a loss of proteoglycan matrix material. Since the chondrocytes appeared normal it was postulated that degradative enzymes (cathepsins, plasmin and fibrinolysins) released with the cell injury, diffused from the joint into the cartilage, degraded the proteoglycan, once exhausted, the cartilage was allowed to recover. No structural changes were observed. This work was supplemented with electron microscopy studies (89) which demonstrated convincingly the similarities of type A (secretory) synovial cells to the progressively differentiating humoral macrophages and type B (protein producing) synovial cells to differentiating fibroblasts in the joints of rabbits examined after subtotal synovectomy.

Ranawat (116) in 1971 described the features of rheumatoid joints one to 15 years after synovectomy in attempt to correlate clinical state with histological findings. Interestingly, of 12 knees biopsied, three had clinical disease and histologically one could see the same pattern as prior to synovectomy, with multiple layers of synoviocytes, marked infiltration with plasma cells, polymorphonuclear cells and lymphocytes with villous hypertrophy and pseudolymphoid follicles. The other none had quiescent joints, all with preoperative evidence of joint damage. Five had quiescent joints with thickened synovial membranes, no villi and some hypervascularity and marked subsynovial fibrosis. The other four had proliferative synovitis with infiltrates but less severe than preoperatively. The question then arose whether the fibrosis of capsule and subsynovial tissues has anything to do with protection of the joint and/or remission of the disease.

Mitchell (91) examined ten rheumatoid patients by transmission electron microscopy at three weeks to five years, and compared them with the synovial and cartilage tissue preoperatively. All of these patients had had quiescent joints post synovectomy. The synovial pattern was a familiar one: gradual differentiation of fibroblastic and macrophage components over a thick collagenous layer with appearance at three and a half months of healthy type A and type B cells without the predominance of A type cells with tremendous vacuolization and lysosomal bodies that was seen preoperatively. The cartilage, on the other hand, showed an improvement in morphology only after the synovium had regenerated; the chondrocytes of the stripped lamina splendens had reappeared, and those of the middle zones lost their cytoplasmic processes, contained less fat and much more endoplasmic reticulum.

1.5 REVIEW OF THE LITERATURE ON MEDICAL SYNOVECTOMY (SYNOVIORTHESIS) IN RHEUMATOID JOINTS:

Medical synovectomy, or synoviorthesis, to use a word coined by Delbarre is the ablation of the synovial lining of a joint by exposing it to a substance with selective toxicity, usually by injection. The advantages of such a regimen are obvious: the cost and hospitalization time of major surgery is far greater than synoviortheses; the patients who is a candidate for synovectomy is, by nature often debilitated, and thus not a good operative risk. The rehabilitation time after arthrotomy is often three to six months while after synoviorthesis, it should be far shorter. Synoviorthesis came to attention after the beneficial effect of removing the synovium of inflamed joints had been recognized.

"In many old and inveterate cases of joint diseases it is the pain from the joint which is the most troublesome symptom for the patient...It is well known that these pains originate in the synovial membrane and that, often, it is possible to eliminate them for a brief period though anaesthetization of the inner surface of the synovial membrane... total synovectomy, which, (sic) in extensive American research, has given very good results... our object has been to eliminate the nerve endings in the synovial membrane in a simpler manner. By injecting into the joint a substance that coagulates the entire inner surface it should be possible to obtain total synovectomy without opening the cavity." (von Reis, 154).

This is a simple concept, and, one would think an easy problem to solve. Even the required characteristics of the substance as defined by Von Reis and Swensson are simple and, have not changed.

"It must not be toxic. It should act on the surface. It must not cause too violent irritation. It must not cause delayed degenerative or proliferous (sic) injuries in the joint."

It is the first and the last rules that have caused most consternation and burying of therapeutic regimens over the years.

Chemicals:

Von Reis' solution was osmium tetroxide (Os_3O_4) which after animal experiments, he injected into 35 patients' knees and obtained symptomatic relief in at least 66% for

at least six months and found no joint deterioration by Xray at one year. Initial histological studies of animals showed complete coagulation necrosis of the surface with regeneration but no evidence of cartilaginous destruction. As this becomes relevant later it is not certain how long after injection the tissues were necropsied.

Enthusiasm rose and osmium along with several other substances were tried over the next twenty years (1,81,128).

Mötonen et al (96) documented in antigen induced arthritis in rabbits, coagulation necroses of the superficial layer of the synovium with polymorphonuclear cell infiltration, followed by plasma cell lymphocytic infiltrate and collagen formation in the subsynovium, in the area surrounding the osmium particles. This suggested that the chemical killed the surface layer and then induced a reactive inflammatory fibrotic change deeper down as the chemical's effect penetrated and/or the particles themselves penetrated down into the deeper layers carried by macrophages from the surface.

Thiotepa, thorium, and nitrogen mustard were other agents reported as successful measured by clinical improvement and lack of radiological or clinical evidence of deterioration over and above that of the natural history.

Then Menkes et al (83) hinted at, and, working independently, Mitchell (92) demonstrated, by transmission electron microscopy that osmium and nitrogen mustard caused early and extensive chondrocyte necrosis which was not apparent by light microscopy initially. One might think that this would have ended the use of osmium but other reports still followed, varying the dose of osmium employed or using it in combination with steroids (86).

Scattered reports on other substances have trickled in: Caruso (12) has tried Rifampin in 35 patients, followed them for six to thirty-six months and reported good clinical improvement in 32. Even 5-fluoro-uracil, a systemic chemotherapeutic agent has been reported used intra-articularly.

1.5.2 Radioisotopes:

In 1962 a new concept in the search for an agent for synovectomy -that of radioisotopes, was introduced (see Appendix I). Radioisotopes produce calculable radiation effect on the surface, calculable penetration or effect, known duration of effect (half-life),~~and~~ are easy to inject, could be prepared in solutions which are inert or nontoxic to tissues except for the radiation effect and thus should fill all of Von Reis' original requirements.

With some bravado, Ansell (5,6) reported on the results of injecting 50 μ Ci of colloidal ^{198}Au into the knee joints of four patients, surveyed the lymph nodes, blood, and the joints over 24 hours for leakage of isotope out of the joint space and then followed this with the injection of 30 knees of twenty-four patients with the estimated dose of 600 to 800 roentgens. At one year follow-up, Ansell reported that 23 of 30 knees had improved and 7 had not changed. There were 14 patients in this initial series who had bilateral involvement - one side only was treated providing a mini-controlled series. Eleven had improved with treatment as against six controls and three had remained unchanged as against eight of the controls.

Virkkunen et al (153) were one of the earliest groups to become involved in radiation synovectomy. In 1967, they published the clinical results showing 67 of 85 knees doing well but introduced a discussion in the problem of leakage to regional lymph nodes which, in one patient, was in excess of 15,000 rads and in which 27 of 67 demonstrated significant (4,000 to 15,000 rads) uptake over the lymph nodes.

Following Ansell's suggestion, Delbarre began using ^{90}Y , pure beta emitter, with a greater penetrance or depth, a higher energy and the same half-life (see Appendix II).

By the early 1970's radioisotopic synovectomy had become accepted mode of therapy in some large centres - namely France, England, Sweden and Finland. Yttrium and ^{32}P were used over gold and newer isotopes such as Erbium and Rhenium were

introduced for use in smaller joints due to their characteristic penetrance, energy and half-life. Almost any joint became a candidate after initial success with knee: interphalangeal and metacarpophalangeals joints, elbows, wrists, ankles and hips.

1.5.3 Clinical Aspects of Radio-Synoviorthesis:

Patients evaluated for synovectomy, historically, were those with intractable painful swollen joints, unresponsive to analgesic and anti-inflammatory medicines. They might or might not be rheumatoids; any intractable knee effusion (147) (defined as lasting more than six months with medical treatment) might be considered for this procedure - villous synovitis, osteoarthritis, ankylosing spondylitis, to name a few. Other factors such as advance age, significant multisystem disease, and poor surgical risks, would make synoviorthesis an excellent second line of therapy. The patients were graded on their range of motion, joint enlargement, pain and redness and heat. Serum studies of Rheumatoid factor, ESR, blood count and radiographs estimated the stage and activity of the systemic disease. While exacerbation of the systemic disease did not preclude treatment, expectation of success was greater if treated earlier. This expectation was based on the generally better results obtained by surgical synovectomy when joints treated were in earlier stages of destruction (by radiological criteria) (103). Patients understandably, were on a variety of antiinflammatories; all had had a full course of A.S.A., and most had had gold treatment and a course or courses of corticosteroids at some point. Children and premenopausal women were not treated.

In the early days, the size of the joint space was estimated to determine the isotopes dose needed for the projected exposure to the synovium. These estimates were a function of the surface area, the concentration of isotope in the synovial fluid, the thickness of the synovium and amount escaping the joint. There was (and still is) no standard of estimation; however, the joint in question was usually drained of fluid and the

volume measured at one visit. The surface area of that volume, assuming a sphere, would be calculated and the dose derived according to any of several formulas (see Appendix IV).

The volume of an average knee is around 50-100 mm³ and that of a metacarpophalangeal joint, 1.0 mm³. Doses delivered in review of the literature have varied widely, partly because of different methods of calculation and estimation. Some estimated by surface area (S.A.) of a sphere, some by volume of a liquid within a sphere, and many just by interpretation of publications by previous authors. Ansell delivered 600-800 rads in her first series and later used 500-1000 rads per joint (translation into mCi was not specified) (5,6). Makin kept his in order of 2000 rads (78). However, Jaffe (67) estimated that ¹⁹⁸Au delivered 76 rads/gm/ μ Ci and ³²P delivered 885 rads/gm/ μ Ci. These figures paralleled the radiation doses estimated by Winston et al (161) who stated that 1.0 mCi ³²P delivered 10300 rads/25cm². Most regimens call for a total of 10,000 rads (161,151,51,100,67,157,122).

When ¹⁹⁸Au gold was used with its ten percent gamma radiation, elaborate protection with lead shielding over syringes, lead shielding for the operator and expensive decontamination procedures for clothing and instruments were required. The synovial fluid was withdrawn and the solution injected by syringes and needle - often with a three-way connector so that a small amount of saline could be flushed through the syringe afterward. This helped to prevent radiation necrosis of the soft tissues as the needle was withdrawn. The pure beta emitters, which have replaced ¹⁹⁸Au, require only plastic insulation. In some centres which use the beta emitters, because of their short half-life, instruments and clothing are stored, the activity allowed to decay, and then are washed and returned to general use.

These procedures were sometimes done as outpatients, however most were hospitalized on bed rest for three to four days afterward (160).

Early post injection mild fever, malaise, and anecdotal skin eruptions have been reported by several authors (86), radiation necrosis of the skin and bone of small joints (105), flare-ups of the synovitis in up to 50% of patients (101) and pseudo-gout. Two cases of acute joint rupture were reported (22). There have been no reports of iatrogenic septic arthritis.

Radiation effects to other organs has generated a great deal of investigation (23,24,34,48,50,52,102,103,115,121). Stevenson (141) first reported chromosomal aberrations in humoral lymphocytes of patients treated with intraarticular gold. The number of aberrations could not be explained by exposure to the cells in the joint alone and had to have been exposed to radiation which had leaked to the regional lymph nodes. The number of aberrations correlated with the amount of isotope found in the regional nodes and was decreased by bed rest (23). The translation of this into risk of neoplasm was estimated as one in one thousand patients treated with 10 mCi of ^{198}Au or 5 mCi of $^{90}\text{Yttrium}$ (141). This, apparently is the same estimated risk for a 30 year old man living in Britain to develop naturally occurring cancer. Tetragenic effects have not been reported; the calculated dosage to the ovaries is within the safe range but dosage to the testes could possibly be high enough to damage gametes in spermatogenesis in the presence of high leakages as with gold (140) (see Appendix III).

1.5.4 Clinical Studies:

A review of 24 authors in 29 publications between 1963 and 1982 was made and attempted to separate out results according to anatomical site and isotope used. It was difficult to make any generalizations because of the variable methods of analyses and overlap of authors' early and late series; however, all success rate of radioactive synovectomies at one year was generally around 80% (Table 1). The effectiveness of the different isotopes was about the same when the dose was equivalent. Whether the

TABLE I

Author	Joints	Isotope used	Dose	Estimated exposure	Results		Follow-up
					Improved	Poor	
Ansell 1963(5,6)	30 knees	^{198}Au	?	480-1000 rads	23	7	1 year
Makin 1968(78)	41 patients*	^{198}Au	3-10 mCi	2000 rads	34	7	at least 1 yr for 25 cases
Gynning 1965(56)	21 knees	^{198}Au	5 mCi		18	3	8 mo - 4 yrs
Makin 1966(77)	33 knees	^{198}Au	10 mCi	2000 rads	27	6	? - 9 yrs
Delbarre & Roucaryl 1969(26)	(79 joints) 36 knees	^{198}Au & ^{90}Y	6-7 mCi		more than 50%	?	more than 6 mo
Virkkunen 1967(153)	85 knees	^{198}Au	10 mCi		67	18	4 mo - 2 yrs
Cayla 1967(13)	14 knees	^{198}Au	6 mCi		12	2	7 mo
Fine 1967(39)	11 knees	^{198}Au	10 mCi		8	3	2 yrs
Topp 1970(147)	18 knees	^{198}Au	4-8 mCi		15	3	1 yr
Fearn 1979(37)	40 knees	^{198}Au	5-8 mCi		28	12	? +
Bridgman 1973(10)	23	^{90}Y	3 mCi	10000 rads 3000 rads	13	10	1 yr
	21	Saline			0	?	
Menkes & Tubiana 1973(84)	122 wrists	^{169}Er	?		78	44	
	96 MCP				67	29	
	72 PIP				55	17	1 - 2 yrs****
Jalava 1973(68)	63 knees	^{90}Y *	6 mCi		47	16	7 - 19 mo
Delbarre & Menkes 1974(27)	106 knees	^{90}Y &*	0.5-6.0 mCi		94	12	
	20 MCP	^{169}Er &			13	7	2 yrs
	114 others	^{186}Re			87	27	

Author	Joints	Isotope used	Dose	Estimated exposure	Results improved	No effect or poor	Follow-up
Oka 1975 (100,101,103)	48	90Y*	3-6mCi	5000-6000 rads	35	13	1 yr
	46				26	20	2 yrs
	42				20	22	3 yrs
	30				5	25	4 yrs
Gumpel & Roles 1975(54)	10 knees	90Y surgery	5 mCi		7	3	2 yrs
	10 knees				8	2	
Menkes & LeGo 1977(85)	85 MCP	169Erb steroid	0.5-1.0 mCi		55-58%***		1 yr
	36 PIP						
	80						
Ott 1977(106)	62 IPJ & MCP	169Erb*	.25-0.50		42	20	6 mo
		169Erb*	0.50-1.0		37	25	2 yrs****
Szanto 1977(145)	19 knees	90Y	3-4 mCi		10	9	3 yrs
	20				11	9	2 yrs
	22				13	9	1 yr
	19	steroid			5	14	3 yrs
	20				5	15	2 yrs
	22				3	19	1 yr
Gumpel & Matthews 1979(55)	18 wrists	169Erb	2 mCi		44	38	1 yr
	55 MCP		1 mCi				
	9 PIP		0.5 mCi				
	64 joints	steroid	10-40 mg		32	32	
Menkes 1979(86)	156 knees	90Y*	3-6 mCi		100	56	1 - 2 yr
	99 shoulders				50	49	1 yr
	97 elbows				60	37	1 yr
	70 ankles				36	34	1 yr
	212 wrists				101	111	1 yr
	212 MCP				115	97	2 yr
	96 PIP				57	39	2 yr
Ruotsi & Hypen 1979(127)	70 MCP&PIP	169Erb steroid	0.25-0.50	6-9000 rads	64	6	1 yr
	51				45	6	1 yr

Author	Joints	Isotope used	Dose	Estimated exposure	Results improved	No effect or poor	Follow-up
Deckart 1979(24)	54 knees**	90Y	1240 rads/mCi		42	12	1 yr
	101 knees**	198Au	1500 rads/mCi		72	29	1 yr
Doherty 1981(30)	15 knees 15 knees	90Y steroid	5 mCi		14	1	6 mo
Onetti 1982(105)	3 hips	32p*	4 mCi	10300 rads/mCi			
	112 knees		6				
	10 ankles		2.5				
	9 elbows		2.5		183(90%)	20(10%)	1 - 10 years
	72 wrists		2.5				
	6 MCP		0.3				
	3 PIP		0.3				

* combination with steroids

** some were non-rheumatoid inflammatory arthritis

*** no absolute numbers given in paper for examination

**** author notes that good results deteriorated after 2 years

+ anecdotal report

treatment gave only an apparent improvement, to present later a greater degree of deterioration than would be expected for its natural course, authors had few parameters to measure. Radiological assessment at treatment and at follow-up using the Steibrocker's staging was empirical but none could find any increased rate of deterioration (149). In other words the disease went inevitably forward. However patients in earliest stages of disease gained symptomatic relief for a time, and did so more than patients in the later stages of disease (101).

Delbarre and Menkes (27) reviewed their 7000 patients who had synoviorthesis using any of several isotopes $^{90}\text{Yttrium}$, $^{186}\text{Rhenium}$ and $^{169}\text{Erbium}$. Three thousand two hundred and seventy two were treated with beta emitters; 346 had follow-up at two years; 240 could be traced. Initial failures tended not to come in. If they did well at six months they did well generally at two years. However if they did badly at six months they might still improve and do well at two years. Of 106 knees, 94 did well, 12 failed or relapsed. Evaluation of results was difficult however, because cases were treated with combinations of steroid and isotope.

Oka (149) reviewed their 48 cases of $^{90}\text{Yttrium}$ synovectomies, 30 knees of which had been followed for 4 years and found no correlation of success with stage of disease as assessed radiologically. The author had found a clinical improvement in 35/48 of these at one year while the number still in clinical remission at 4 years was only 17% (5/30).

Bridgman (10) in a double blind study with saline controls, found improvement in 13 of 23 knees. He tried to assess radiological change, but did not use the standard rheumatoid arthritis staging nor had histological documentation. He found almost insignificant leakage and chromosomal change but used only 3 mCi $^{90}\text{Yttrium}$ estimating absorbed dose of 10,000 rads at 1 mm.

Menkes and Lego conducted a double blind study of Erbium and steroid verses saline

and steroid in small joints of the hand, documenting duration of disease, radiological stage, ESR and Rhese-Waaler test. In overall clinical assessment the isotope treated group showed better results. Patients in the earlier radiological stages of disease fared better in both groups. Progression of radiological changes over the 2 years was not discussed (85). R  tsi however conducted a similar study using steroids verses Erbium and found at twelve months no significant difference in success rate between the two groups; but he did secure 90% improvement rate for both groups (127). In a similar study, Gumpel (55) could find only 50% improvement but again no difference between steroid and radioisotope treated joints. Ott (106) using Erbium and steroids in finger joints noted 68% improvement at 6 months and by 2 years 60% remained quiescent. Oka and Hypen (101) in their series noted also a correlation with stage of disease radiologically and progression of systemic disease. At 12 months, 60% of treated knees in patients who had flare-ups of their disease were quiescent; 90% of treated knees were quiescent in patients where the disease was stable and the figures approached 100% (8 cases) in those who were in remission. Later reports of the same series at 4 years showed only 5/30 knees was still in remission. Twenty-five were rated as poor (103).

Only one clinical study using ^{32}P has been reported since that mentioned in 1967 by Johnson (69) and the report in 1973 by Winston (161). Onetti (105) injected 0.3 to 6 mCi ^{32}P into various joints from the proximal interphalangeal joints of the hand to the hip and found in 217 joints, evaluated at least 1 year later that improvement in the joint clinically had occurred in 90% while 10% showed no response. However his results are complicated by the fact that steroids were also administered with the isotope. It is of note that with 0.3 mCi, in the finger joints, the author encountered significant radiation skin necrosis and osteonecrosis; he subsequently stopped injecting small joints.

In European centres, enthusiasm with this procedure was, and is to date, sustained

with support from clinical results and histological examinations. Attention, moreover, has centered on the concern for long term sequelae - the effect of radiation to the extra-articular organs, the long term effects on the cartilage and bone. Surprisingly, however, little investigation has actually been carried out on bone and cartilage.

5.5 Pathological and Experimental Studies:

Yates (163) arthroscoped and biopsied the synovium of a patient treated with 5 mCi $^{90}\text{Yttrium}$ four weeks earlier and noted an increase in thickness of the synovial lining cells and more fibrin. The subsynovial tissue had the same degree of monocytic infiltration. These findings might be explained they said, as a state of "temporary rective synovitis"; Webb, Low and Bluestone used an animal model of rheumatoid arthritis and demonstrated uptake of $^{90}\text{Yttrium}$ into the surface synoviocytes and also into the occasional macrophage with the subsynovium at 24 hours using between 100 μCi and 1 mCi of colloid, which by their estimation gave 27000 to 81000 rads (157). This suggests that the cells were still alive when the isotope macromolecule entered the cell and had not destroyed the cells by radiation at 24 hours.

Oka views the histological effects of isotopes as similar to those produced by the fixative osmic acid which reacts with proteins forming gels and oxidizes unsaturated fats to form reduced black osmium compounds. Coagulation necrosis of the synovium occurs followed by an intense inflammatory response as the necrotic tissue is cleared organizing fibrosis follows along with regeneration of a synovial cell layer. There was no cell death in the subsynovial tissues which were still active; the cartilage, survived and appeared normal; in immature animals, growth retardation of bone was observed. One expected he deduced, a similar response in isotope treated or chemically treated tissue with limited penetration powers. In fact, osmium has now been condemned as late cartilage degeneration has been identified (92).

Delbarre and Menkes compared synovial biopsies in 44 patients before and after one to two months, and observed "a fibrinous necrosis which disappeared after one to two months, being replaced by new synovial tissue that was usually sclerotic, but sometimes normal. The number of inflammatory cells diminished and the supporting tissue was the site of a hyaline sclerosis." (27) (The only histological parameters that seemed consistent however were a decrease in the number of villi and number of inflammatory cells). He also compared his centre's results of synoviorthesis verses three authors' surgical synovectomy series. Without breakdown, 114 of 367 cases (31%) treated surgically had relapsed or had bad results at 2 years (The surgical papers quoted by Delbarre were not reviewed in this thesis).

Isomaki (65) examined synovial biopsies of rheumatoid patients three days and seven days after treatment with $^{90}\text{Yttrium}$ and the fluid at 10 minutes, 30 minutes, and 24 hours. In 10 minutes the particles were found in the cytoplasm of macrophages and some loose synovial cells, which, by transmission electron microscopy, were severely damaged or degenerated. If they survived 24 hours, the particles were found in the cytosomes and there was evidence of membrane damage (decreased flow and decreased pinocytotic vesicles). The lining cell ultrastructure, membrane function, mitochondria, rough endoplasmic reticulum was also severely damaged. The type A cells seemed to survive better than the type B cells, more evident at 3 days. By then the particles could be seen throughout the intercellular space lying free in apparently empty vacuoles. At one week no activity could be seen by radioautography. This pattern then that the particles killed cells in the fluid; some cells survived, some cells in synovial lining survived long enough to pinocytose the particles and either die releasing their contents into the subsynovium with cell debris or migrate into the deeper layers of the subsynovium acting as a minigrenade deeper down. The particles could then make their way into the lymphatic system to the

nodes.

Oka and Hyphen (101) examined one failed case of ^{90}Y trium injection and found an ulcerated synovium with fibrinoid layering, subsynovial fibrosis, infiltration, and hypervascularity.

Muirden and Mills looked at 42 human synovectomies and observed that the greater the thickness of the synovium the greater the lymphocytic infiltration and the less the damage appeared to be (98).

Animal studies, though not always comparable have given us clues as to the pathology and pathogenesis of the synovium and the effects of isotopes. Webb (157) observed ^{90}Y trium on the synovium of arthritic rabbits and found at 24 hours that the particles had entered the cytoplasm of synovial cells and in a few deeper macrophages. One must wonder, then, if the cells were still alive, and if so why?

Pavelka (109) could not demonstrate a true destruction of the synovium with 0.4 mCi ^{90}Y trium in arthritic rabbits; but he did remark on the thrombotic occlusion of the capillaries in the villi with some recanalization at 2 weeks. At four weeks he demonstrated a marked decrease in cellular infiltration and marked fibrosis. More importantly, he found the cartilage at four weeks was normal except for foci of degenerative cells in the tangential layer near the cruciate ligaments. In other animals there were new and old degenerative foci with histocytic reaction.

In immature and mature rats treated intra-articularly with 10 to 100 times the therapeutic dose of ^{198}Au , growth retardation was observed (126). After a 30 day period of bone marrow suppression and growth retardation was noted in the weanling rats. The bone marrow recovered, moreover, and no degeneration of the cartilage or of increased metabolic functions was seen up to one year in those treated with the lower dosage. In adult rats no effect was noted at any dose given. These results though induced by ablative

amounts of radiation, none-the-less discouraged use of isotopes in children and premenopausal women and provided evidence to encourage use in adults with impunity.

More recently, Onetti (105) examined several biopsies of rheumatoid joints before and after treatment with intraarticular ^{32}P chromic phosphate. The changes in the synovium were consistent with a less active synovitis with reduction in synoviocyte layers, decreased infiltrates and decrease fibrinoid deposit. Findings consistent with radiation effect were the structural changes of the synviocytes: vacuolization, chromatin changes, freeing of the cells from the cell to cell association. They did not find any evidence of radiation vasculitis. No cartilage studies were reported.

Ubios (148) put 0.25 mCi of ^{32}P into the knee joints of immature rats. If a rat knee is about 1/70th the size of a human knee, this compares to approximately four times the clinical dose in man. At 56 days, the articular cartilage was markedly disorganized, a finding consistent with Rubin's studies with massive doses of ^{198}Au (126). Whether this is relevent to the clinical situation is the subject, in part, of this thesis.

CHAPTER 2

STUDIES ON AN INDUCED ARTHRITIS MODELS OF RHEUMATOID ARTHRITIS IN THE RABBIT.

2.1

INTRODUCTION:

Progress in the field of rheumatoid arthritis research has often been discouraged by the lack of a counterpart in the experimental animal. For many years an auto-immune pathogenesis was hinted at and it was upon this that Dumonde and Glynn (33) developed the first animal model of chronic (rheumatoid-like) arthritis in the rabbit. This was based on the fact that the chronic synovial and extraarticular lesions demonstrated plasma cells and lymphoid follicles, suggesting immune reaction to some self or foreign antigen. In their study animals were sensitized to fibrin and a 20-30 mg solution was later injected intra-articularly. When examined in groups up to 8 weeks later, the animals injected with autologous fibrin demonstrated a chronic synovitis but no cartilage destruction. Those injected with human fibrin developed a gross arthritis, with articular cartilage destruction by 8 weeks.

The fibrin model gained popularity (157) and many modifications of this model have been proposed and used on different animals. Rats develop a polyarthritis when their foot pads are injected with Freund's adjuvant alone (131,43) or with other antigens such as collagen (142,95) collagen type II (17) and Mycobacterium cell, without any intra-articular challenge. This, moreover, is restricted to rats and certain strains of rats.

Intravenous injections of antigens have been employed (111,112,15) to create a rheumatoid-like state to study the immune complexes in synovial joints and fluid and although the method has the advantage of making the joint a privileged site, the results were variable in degree, and unpredictable in numbers.

Polysaccharides (74,139) polyene antibiotics (99,158) have been used following Dumonde and Glynn's methodology, the substances chosen on the premise that since they have some direct lysosomal action, they might mimic the lysosomal action of rheumatoid synovial cells.

Bacterial extracts (138) have been used similarly on the premise of an expected

immune complex disease.

Breakdown products of immunoglobulins and enzymes found in arthritic joints have been introduced as well but produce a state which resolves too fast, destroys the underlying articular cartilage too fast, or has widely variable results (3,44,38,94,136). Each, however, has been of value in demonstrating some aspect of the pathogenesis of the rheumatoid-like state.

Mycobacterium species produces a granulomatous type lesion which is often smoldering and thus has been tried as a method of inducing a chronic arthritis. An added benefit was the fact that neither the *Mycobacterium* itself nor any remnant of it could be detected in the joint after the first four weeks despite an arthritis which remained active for up to a year (156). The problem is that the articular cartilage is all but destroyed by four weeks.

In 1971, Consden, Doble, Glynn and Nind reported using ovalbumin as the antigenic stimulus in producing a rheumatoid-like arthritic state (14). This was an obvious offshoot of using bovine gammaglobulin with its multitude of cross-reactants. Interestingly while establishing a minimal dose for production of a chronic arthritis, they demonstrated with radioisotopic tagging, that the antigen need not be present in the joint for the perpetuation of the arthritis. Animals were sensitized and then challenged with ovalbumin in doses from 10 mg to 1 μ g and in the 14 animals where 0.1 mg or more was used all developed gross arthritis at 8 weeks. Substantiated by histological examination of synovium and cartilage, the lowest doses produced no significant arthritis. Since then this animal model has become more popular for a variety of studies on rheumatoid arthritis (132,57,59,61,58). Stastny used it with a challenge dose of 215 mg for an MIF study of 6 weeks duration (135); Fox and Glynn used their maximal doses of 10-12.5 mg as challenge dosage and followed the rabbits for up to 8 weeks noting a "widespread arthritis" (40,41). Goldlust varied the challenge

schedule and frequency using a single 1 mg injection, he found a chronic synovitis at 10 weeks with no gross articular damage; with weekly 1 mg injections he produced an erosive arthritis (47). Lowther and Sandy et al have used this model with 1 mg challenge dose in their studies since 1976 and found a consistent dependable reaction of "marked joint changes" in rabbits (128,75). Lowther et al (75) used 2.5 mg human serum albumin challenge after sensitization to the same. Fifty percent developed a "chronic joint swelling in the 28-57 day period". Of 26 rabbits, 13 showed signs of acute inflammation but were "recovering" and had no joint swelling, normal appearing joint fluid, normal appearing cartilage microscopically and minimal synovial membrane changes. The acute synovitis was characterized by thickening, infiltration with polymorphonuclear cells and small round cells and perivascular infiltration. Thirteen showed gross joint swelling, cloudy fluid, erosions and pitting of the cartilage and synovitis characterized by thickening, villous proliferation, perivascular infiltration, and lymphocyte clusters. They found no change in the degree of cellularity. Biochemical parameters indicated no significant change in collagen or cellularity but significant drop in proteoglycan content and synthesis in cartilage, with time. Sulfur uptake studies and quantitative hexuronic acid studies of animals sacrificed in the first week showed approximately 80% inhibition of synthesis in weight bearing areas especially in the superficial layers and to a lesser extent in other areas. They further explored the very early changes in this animal model and found the proteoglycan decrease is due to both decreased chondrocyte synthesis and secretion (not accounted for by the decreased numbers of living chondrocytes) (128). Thus by 2 months their model produced a significant cartilage insult and evidence of degeneration. This was thought to be an appropriate model for rheumatoid arthritis.

Hunneyball supported the model, reporting that this animal model produced an arthritis that was comparable right to left but that there was a "high degree of

variation between animals in the severity of the arthritis" (63). He used 10 mg in 11 animals and graded the synovial reaction by histology. No articular cartilage studies were reported.

Van Beuskom (150) compared two different intra-articular doses of ovalbumin, 0.5 mg and 2.5 mg, after sensitization, and followed the disappearance from the joint with ^{131}I labelled ovalbumin and scanned the joints with ^{99}Tc . After 1 week the scanning as a measure of inflammation was the same in the knees with both dose regimens.

The antigen-induced arthritis animal model using ovalbumin was reexamined in respect to its appropriateness as a chronic symmetrical model of rheumatoid arthritis.

2.2

Part A

THE MODEL

In the belief that the sensitization of the rabbit and a subsequent intra-articular challenge with an antigen were the important steps in inducing a chronic arthritis and that the dose was relatively unimportant a middle dose range of 7.5 mg ovalbumin was chosen to challenge our group of rabbits in the hope of reproducing an appropriate model of rheumatoid arthritis. The animals were then sacrificed at intervals and examined with alarming results.

2.2.1. MATERIALS AND METHODS:

99 adult New Zealand white rabbits were used and 84 rabbits harvested by the end of the experiment. Sex was mixed, age was a minimum of six months (range six to nine months). A one milligram solution of ovalbumin (Sigma A-5503 grade V crystallized and lyophilized) in sterile water emulsified in an equal volume of Freund's complete adjuvant (Difco Labs 063859) to give a final concentration of one (mg/ml) emulsion was prepared and all animals sensitized intradermally in five sites each on the back which had been shaven and prepared with alcohol. On day fourteen, a second sensitization was made with 0.5 mg ovalbumin in 0.5 ml emulsion in 3 sites. On day 24, the animals were skin tested with 20 micrograms ovalbumin in 0.1 ml sterile water in 2 sites with controls of sterile water in 2 sites. All animals had positive skin tests. The animals were then challenged on day 30 with 7.5 mg ovalbumin in sterile saline injected into each knee joint through the intrapatellar tendon towards the intercondylar notch.

Early losses of animals usually occurred from one to seven days post challenge. Afterwards the animals were divided into groups and sacrificed at 1, 2, 5, 7, and 14 days, 19, 26, 33, 50, 90, 160, 230d (33 wks) post challenge. Anaesthesia was induced with Nembutal 25 mg/kg and supported with ether. The joints were opened under sterile



Sensitization and intra-articular injection method, for induction of antigen-induced arthritis.

conditions and the joint fluid cultured.

Each group was handled similarly. The knees were harvested for synovium in the area of the infrapatellar fat pad and fixed in Bouin's solution for 24 hours. The solution was changed to 70% alcohol and then processed for paraffin sectioning.

The femoral condyles were immediately fixed in 10% buffered formalin (Fisher S0-F-100) containing 0.5% cetylpyridinium chloride (C.P.C.) for two weeks and then decalcified using 10% EDTA (pH 7.4)

Synovium was harvested, incubated for 60 minutes at 37°C in a Dubnoff metabolic shaking incubator under a controlled atmosphere (95% O₂ and 5% CO₂) in a proline free incubation media (Minimal essential media Flow Laboratories 12-102) containing 50 µCi methyl 1,2-³H-thymidine (NEN NET-512) (S.A. 101 Ci/mmol). The specimens were then rinsed four times with a total of 200 ml iced saline, agitated, then fixed in Bouin's solution prior to paraffin embedding and preparation for radioautography (73).

Whole joints were harvested 24 hours after intra-articular injection of 250 µCi ³⁵sulfate (NEN NEX-041 sodium sulfate) and immediately fixed in 10% buffered formalin with 0.5% C.P.C. for 2 weeks, decalcified and embedded in paraffin for radioautography.

Cartilage was sliced, from the superior aspect of the patellar groove of the femoral condyle, incubated in 10 or 20 µCi L² 3'- ³H-proline (NEN NET-323, S.A. 29.5 Ci/mmol) in Krebs Ringer bicarbonate solution under the same conditions of incubation for 4 hours. The tissue was then cold chased 15 minutes and transferred to 2% glutaraldehyde 0.1 M sodium phosphate for 3 hours. All tissue was then decalcified overnight in 4.13% EDTA (pH 7.4) (155).

Slices of cartilage were also immediately fixed in 2% glutaraldehyde for 3 hours and then decalcified in 4.13% EDTA pH 7.4 prior to fixation in osmium and prepared routinely for transmission electron microscopy (TEM).

Two weeks after intra-articular injection of 250 μCi L(2'³ - ³H proline (NEN NET-323 S.A. 29.5 Ci/mmol) and 24 hours after 250 μCi ³⁵Sulfate injection, animals were harvested of all cartilage of the femoral condyles, patellar groove and tibial plateaus, care being taken to avoid areas of fibrocartilage. The shavings were blotted, wet weights obtained and frozen for analyses of hexuronic acid and hydroxyproline.

Light microscopy:

Paraffin embedded synovial specimens were sectioned at 3 microns and stained with acid hematoxylin and eosin. Cartilage was sectioned at 6 microns and prepared and stained with toluidine blue and iron hematoxylin-fast green-safranin-O (73). Paraffin sections prepared for radioautography were coated with Ilford-4 (Ilford Ltd., Ciba-Geigy, Essex, U.K.), exposed, developed and stained with standard hematoxylin and eosin.

Transmission electron microscopy:

Articular cartilage was cut prior to fixation so as to permit full thickness sectioning following embedding. Samples for transmission electron microscopy were fixed in 2% glutaraldehyde/0.1 M Na phosphate (pH 7.4) containing 1% toluidine blue a proteoglycan stabilizing agent (130) for two to three hours; rinsed in 0.1 M Na phosphate buffer containing 0.2 M sucrose and 0.025% toluidine blue 0 for 30 minutes - postfixed in 2% OsO₄ in 0.1 M Na phosphate plus 0.025% toluidine blue 0 for 2 hours - ethanol dehydrated (30%, 50%, 75%, 95%, 100%) - 10 min. each for 3 changes). Blocks were then transferred to a Spurr/absolute ethanol (1:1) infiltration mixture (134) for 2 hours -then into Spurr alone and placed in a dessicator and held under vacuum overnight. Blocks were flat embedded in Spurr to permit full thickness sectioning and polymerized at 70°C for 18 hours. One micron plastic sections were cut with glass knives and placed on subbed glass slides (108) and the ultrathin (600-800Å⁰) sections were cut with diamond knives and placed on 200 mesh filmless copper grids. A Reichert Ultracut was

used for sectioning. The one micron sections required no further staining as they were stained in situ with the toluidine blue during the fixation process (130). The ultrathin sections were stained for 30 minutes with 2% uranyl acetate (in 50% alcohol) and for 10 minutes with 0.2% Pb citrate (152). A Philips 400 transmission electron microscope was used in the study.

Radioautography:

Tissue was fixed and processed as above without the addition of toluidine blue 0 and decalcified. Toluidine blue, if used, reacts with the photographic emulsion resulting in artifact (129). One micron thick sections were placed on subbed slides and dipped in total darkness in Ilford L4 nuclear track emulsion (1:2 dilution) (11) air dried; stored in sealed black plastic boxes containing a drying agent (silica-gel) with a blank slide between each slide containing sections and stored at 4°C for 2-3 weeks. Following exposure the slides were brought to room temperature and developed in D 170 (6 min), rinsed in tap water, fixed in 24% sodium thiosulphate (10 minutes), rinsed for an additional 10 min in running tap water, and air dried. To permit heating and prevent swelling of the slides, they were hardened in 10% formalin at room temperature for 10 minutes, rinsed, and again air dried (120). They were then heated on a hot plate stained with 1% toluidine blue in 0.5% sodium borate. All slides were cover slipped using unpolymerized Spurr and placed on a hotplate overnight to polymerize the plastic.

Biochemistry:

Cartilage shavings from each joint were analyzed separately. The tissue was powderized using a freeze miller, and then suspended in 0.5 M acetic acid. A pepsin solution of 1 mg/ml/50 mg tissue (Sigma P-7012) was added and the tissue allowed to digest at 4°C with constant stirring for 7 to 9 days. The digest was neutralized to pH 8.6 with NaOH to inactivate the pepsin and then dialyzed in cellulose tubing against 0.5

M acetic acid till the dialyzate was free of counts. The solution was made up to constant volume and divided into aliquots for hexuronic acid, hydroxyproline and DNA analyses.

Hexuronic acid analysis was made according to the method of Bitter and Muir (9) using sodium tetraborate in sulfuric acid and carbazole in ethanol with a standard of glucuronic acid. An aliquot was analyzed for counts of ^{35}S and ^3H using a Beckman scintillation counter with a two channel simultaneous readout and automatic quench correction.

Hydroxyproline analysis was made according to the method of Stegemann (137). The solution was lyophilized in a centrifuge dryer overnight and resuspended in 1 ml of 6 N HCl, and hydrolyzed for 18 hours, dried, and resuspended to constant volume with 6 N HCl. A 100 μml aliquot was then transferred to heavy chromatography paper (Whatman 3 MM). Using a butanol, glacial acetic acid water solution (4:1:2) the sample was separated over 16-18 hours, dried, the proline and hydroxyproline bands cut out and eluted in water (18). Hydroxyproline assay using chloramine T and perchloric acid was carried out and scintillation counting of the proline and hydroxyproline eluants were made.

Deoxyribonucleic acid analyses were made according to the method of Erwin Stoschecki and Florini (36).

2.2.2 RESULTS:

Marked reaction did, indeed, occur in the joints of animals sensitized and challenged intra-articularly with ovalbumin. Unchallenged joints did not react.

Synovium:

Twenty-four hours after challenge, the joints were grossly swollen, and hot; a clear or bloody effusion was present with a markedly hemorrhagic synovium (Fig 3a). Tissue incubated in vitro with thymidine showed no surface cell turnover - indeed no surface at times was visible. At 5 and 7 days the hemorrhagic synovium was less profound, synoviocyte hyperplasia and mixed polymorphonuclear and monocytic infiltration are noted (fig 3b). By 14 days a thick, red, villous synovium with predominantly perivascular monocyte infiltration prevailed (fig 3c). Thymidine incubated tissue showed more cells with uptake than normal but proportionate to the increases in number of synoviocytes present in the section. At 13 weeks, the synovitis was still active with a purely monocytic population and evidence of new subsynovial fibrosis (fig 3d). By 22 weeks half of the animals showed a fibrotic synovium; the others had regenerated normally (fig 3e).

Cartilage:

Grossly all joints showed a classic enlargement with effusion, some with rice bodies and cloudy fluid by 26 days. The cartilage however appeared intact. By 33-50 days early osteophyte formation was evident, the articular surface appeared dull with occasional spotty erosions (fig 4a). These changes were obviously progressive: by 13 weeks post challenge, severe degeneration of all joints was evident with articular cartilage eroded, eburnated bone evident, large joint margin osteophytes, pannus invasion and degenerative ligamentous structures (Fig 4b).

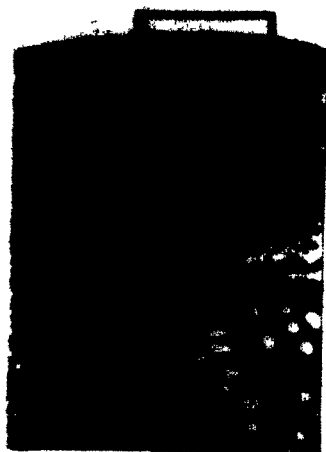
In joints necropsied in the first 14 days, morphology and proteoglycan staining of



fig 3(a) Synovium 24 hours after intra-articular challenge with 7.5 mg ovalbumin. Note subsynovial spaces filled with blood. 3 μ paraffin H+E section.



3(b) Synovium 7 days after challenge 7.5 mg ovalbumin. Note polymorphonuclear cell infiltrate and synoviocyte hyperplasia. 3 μ paraffin H+E section.



3(c) Synovium at 14 days after challenge 7.5 ovalbumin. Note mixed infiltrate extending deep into a synovium and presence of reparative fibroblasts. 3 μ paraffin H+E section.



3(d) Synovium 13 weeks after challenge with 7.5 mg ovalbumin. Note hypervascularity, monocytic infiltrates and accumulations; villi. 3 μ paraffin H+E section.



3(e) Synovium 22 weeks after challenge 7.5 ovalbumin. Note fibrosis, hypervascularity.

cartilage was not significantly different from adult controls. Radioautographs of ^{35}S incubated tissue showed uptake around the cells of the non weight-bearing areas and areas protected by meniscus. By 33 days, weight-bearing areas of femur and tibia had clefts into the radial zones, pannus creeping from the periphery and encircling the peritendinous areas. There was significant reduction of Safranin-O staining throughout, and uptake of ^{35}S sulphate was present only in the deeper zones of the non-weight bearing areas. By 33 weeks there was complete fibrillation, disorganization or even erosion of the articular cartilage (Fig 4c).

The early severity of the antigen-induced arthritis was not appreciated, moreover, until one micron sections of plastic embedded cartilage were examined.

Sections of cartilage fixed in vivo with toluidine blue and sections incubated in vitro with tritiated proline precursor as a measure of chondrocyte metabolic activity were examined.

At twenty four hours, serious effects on chondrocyte activity were seen throughout the depth of the cartilage (Fig 5b). Decreased uptake by the chondrocyte, an abnormal number of necrotic cells and decreased staining are noted. At seven days, these changes persisted and at 14 days, little uptake was noted over the chondrocytes (fig 5c). Furthermore significant fatty degeneration was noted in these cells which in our experience has correlated very closely with degenerating chondrocytes. At 33 days, little sign of recovery is noted; indeed degenerating and dead cells were more evident. The pattern of cell death was not a surface phenomenon or even a phenomenon restricted to one morphological zone, but seemed to be sporadic and diffuse (Fig 5d&e). At 33 weeks, sections demonstrated spotty survival and activity of chondrocytes. The matrix was coarse and irregular and staining decreased (fig 5e). T.E.M. studies of these cells substantiated our light microscopic findings of severe cell compromise and degeneration. Figures 6a to e demonstrate signs of surface collapse, degenerating and



fig 4(a) Rabbit knee joint at necropsy at 7 1/2 weeks (50 days) on right; a normal knee joint pictured on left for comparison. Note active synovitis, osteophyte, and focal erosion of articular cartilage. Below: a 6 μ paraffin toluidine blue O section non weight-bearing area, posterior aspect of femoral condyle. Good preservation of cartilage architecture with cloning of chondrocytes.





4(b) Rabbit knee joint necropsy at 13 weeks on right; a normal knee joint pictures on left for comparison. Note fibrinous exudate, rice bodies, osteophyte formation, articular cartilage erosion and synovitis. Below, a 6 μ paraffin toluidine blue-O section in non weight-bearing area, posterior aspect of femoral condyle. Cartilage acellular, eroded almost to osteochondral junction.





4(c) Cartilage of 33 week AIA with 7.5 ovalbumin. Note complete disorganization of articular surface. 6 μ paraffin section, toluidine blue.



fig 5(a) One micron sections of normal adult cartilage. On left radioautograph ^3H proline incubated tissue. On right, insitu toluidine blue stained tissue.



fig 5(b) On right cartilage at 24 hours, after challenge 7.5 mg ovalbumin. One micron Spurr embedded section, toluidine blue. On the left at 48 hours, ^3H proline invitro incubated tissue showing metabolic activity, decreased staining, and necrotic cells.

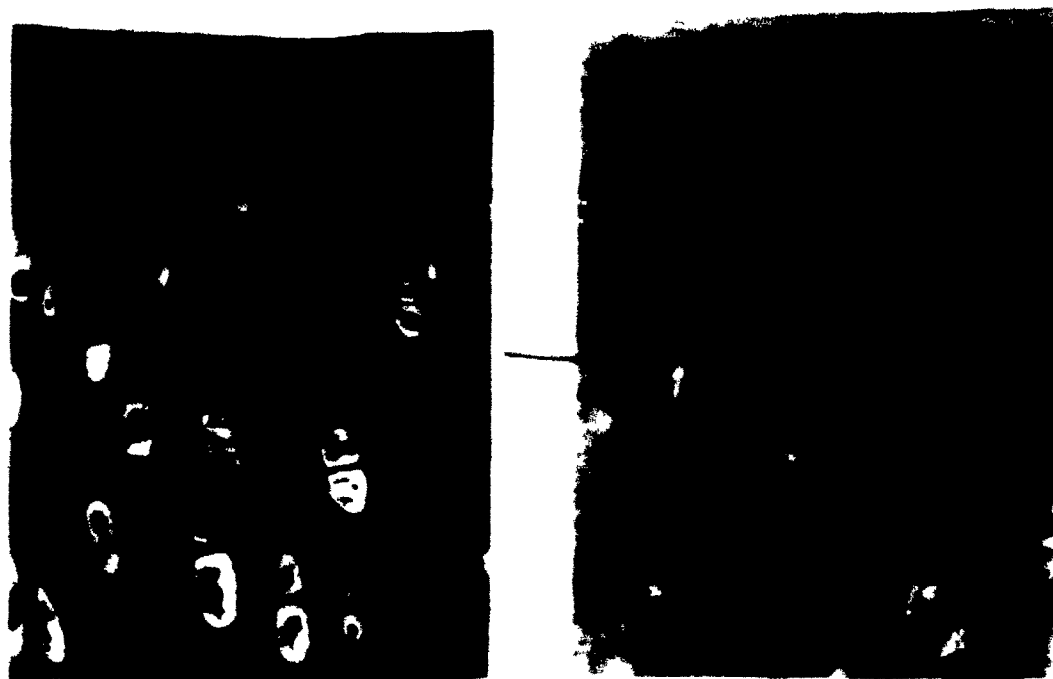


fig 5(c) One micron radioautographs of ^3H proline incubated cartilage, 14 days after challenge 7.5 mg ovalbumin. Note decreased uptake over chondrocytes and fatty accumulations in cytoplasm.



fig 5(d) One micron radioautograph of ^3H proline incubated cartilage, AIA with 7.5 mg ovalbumin at 33 days. Only 2 cells in whole field show active uptake. Empty lacunar spaces and retraction of cells from matrix typify chondrocyte degeneration.

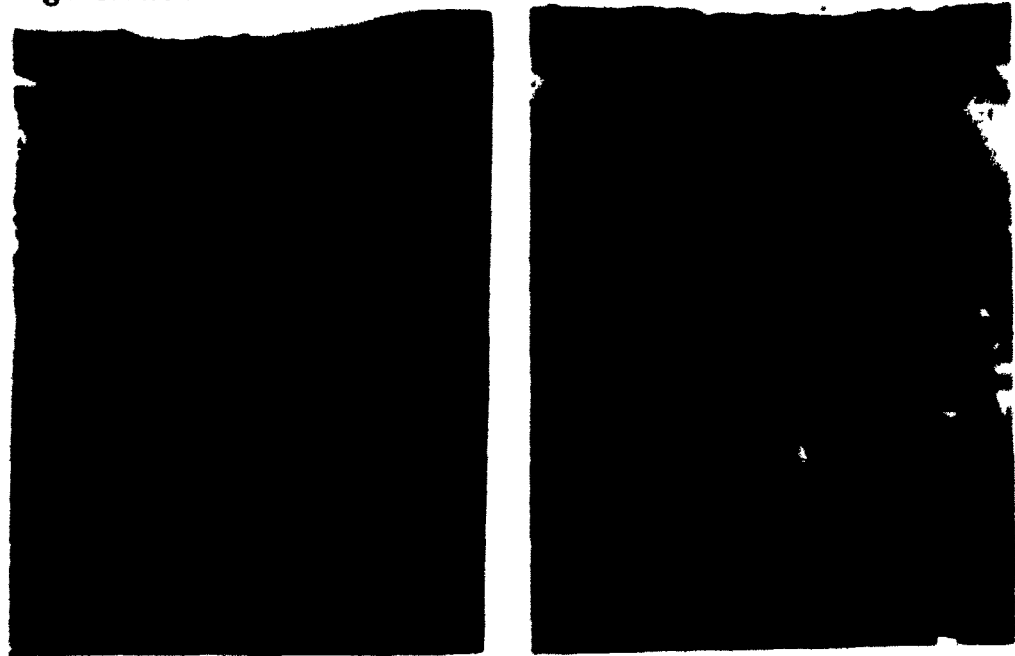


fig 5(e) One micron radioautograph of ^3H proline incubated cartilage AIA 7.5 mg ovalbumin at 33 weeks. Cell population markedly degenerate with loss of transitional cell layer and decreased toluidine blue staining of matrix. In the section on the right, some cells show intense activity in middle zone with loss in upper and lower radial zones.

necrotic cells at surface, radial and tidemark areas. Viable cells contained accumulations of fine fibrillar material associated by some authors with the ageing of the cartilage. The wavy arrangement or kinked appearance of the matrix collagen is an abnormal feature which our laboratories have observed repeatedly in both human and animal arthritic cartilage. At the level of the tidemark, normal chondrocytes became necrotic but did not have the appearance of those in fig. 6e (contrast fig. 12f).



fig 6(a) Transmission electron photomicrograph. Antigen-induced arthritis, 8 weeks after challenge with 7.5 mg ovalbumin. Note surface collapse and necrotic tangential cells.



fig 6(b) Ageing, decompensating chondrocyte near the tidemark. Note lipid (L), fibrillar material (F), retraction of cell membrane from matrix.

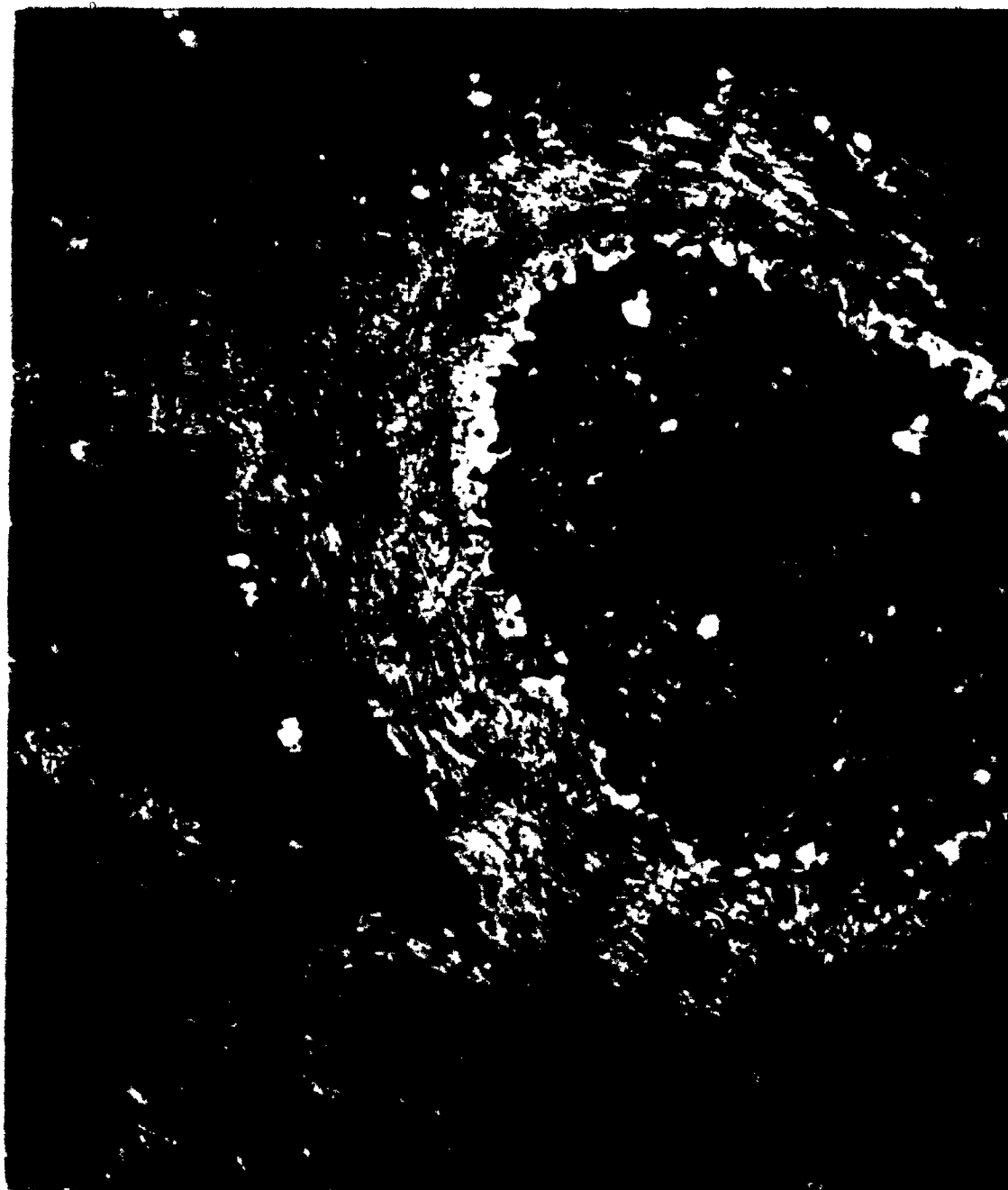


fig 6(c) Degenerate radial zone chondrocytes. Wavy arrangement of matrix collagen noted, termed "kinking" or "matrix streaks".



fig 6(d) In this radial zone group of seven, only one cell is viable.



fig 6(e) Deep in cartilage, the tidemark (T) is shown. A group of atypical flattened necrotic cells are noted above.

Biochemistry:

Hexuronic acid content of the tissue measured in $\mu\text{g}/\text{mg}$ dry weight of cartilage as a measure of proteoglycan content dropped after 14 days and remained depressed (7b). Similarly the hydroxyproline content dropped and remained depressed; these changes were consistent with the gradual breakdown and loss of cartilage matrix (Fig 7a).

Specific activities of ^3H proline and ^{34}S ulphate in-vivo incubated cartilage, were counted in attempt to assess the absolute and relative metabolic activity of the tissue. However, due to errors in design and delay in counting, the radioactivity of the digests decayed to the point where correction factors were not useful and no reliable pattern of different counts in animal groups could be discerned. The results of this portion of the investigation have been omitted.

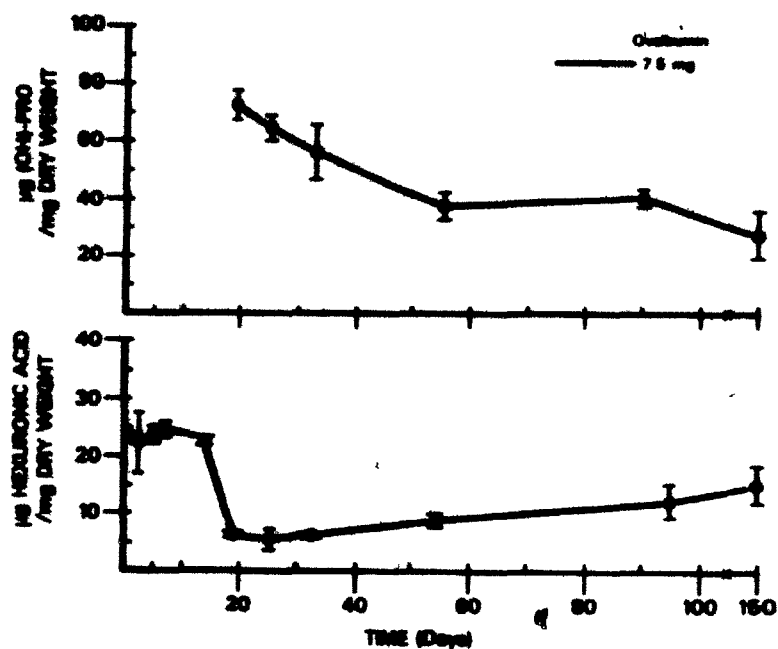


fig 7(a) Upper: Hydroxyproline in $\mu\text{g}/\text{mg}$ dry weight of cartilage. Antigen induced arthritis with 7.5 mg ovalbumin intra-articular challenge dose.

fig 7(b) Lower: Hexuronic acid is $\mu\text{g}/\text{mg}$ dry weight of cartilage. AIA 7.5 ovalbumin intra-articular challenge dose.

2.1.4 DISCUSSION:

The ovalbumin antigen induced arthritis of Consden et al (14) produced in rabbits, is early and severe. The histological changes were more compatible with acute cartilage necrosis as was demonstrated by light microscopy supported by radioautographs of precursor uptake studies, and verified by transmission electron microscopy. Routine histological preparations and biochemical assays detected changes only to a lesser degree and far later in the course of the disease.

With these rather dramatic and obvious effects, the reasons for the model not having been questioned before, may be entertained.

This model has been employed for a number of immunological and biochemical studies into the etiology and pathogenesis of rheumatoid arthritis. Most controls and assessments of the model were in terms of the degrees of gross joint enlargement and histological appearance of the synovium. Few studies have included specific attention to the cartilage. Furthermore, no studies have included the more sensitive techniques of plastic embedded light microscopy with precursor uptake studies, and TEM.

Even more germane, moreover, is the probable interpretation of the original paper by Consden et al (14). They examined rabbits sensitized and then challenged with variable doses of ovalbumin (1 microgram to 10 milligrams) and noted that 0.1 mg was the minimal dose to produce a chronic synovitis, lasting at least 8 weeks and then directed the remainder of their paper to the presence of the antigen in the joint and the possible pathophysiology involved. The unstated axiom seemed to be that any dose between 0.1 mg and 10 mg produced consistent joint inflammation lasting at least 8 weeks and as long as 1 year. The dose then, seeming relatively unimportant.

Even more recently indirect evidence that the dose was relatively unimportant was reported by van Beusekom et al (150) who scanned with ⁹⁹Techneium

pertechnetate the joints of ovalbumin induced arthritis using two different challenge doses 0.5 and 2.5 mg and found at one week that the uptake was the same suggesting an equivalent amount of inflammation.

Having witnessed the profound chondrocyte necrosis by 7.5 mg on our animals this study indicates that the model using the antigen challenge in the upper ranges quoted by Consden is inappropriate as a model of rheumatoid arthritis. It is obvious, then that all immunochemical conclusions previously drawn using this model should be seriously questioned.

2.3

Part B

MODIFICATION OF THE MODEL

Because of the startling results found, using the model described above, we then investigated a modification of the protocol for an antigen induced arthritis. Consden experimented with different challenge doses and schedules and found that 0.1 mg would produce arthritis but .01 mg would not (14). It was unclear if the arthritis became chronic. Others have used as little as 2.5 mg, apparently with success (128,75,149). A challenge dose of 0.1 mg ovalbumin was then selected and the effect observed in synovial reaction and articular cartilage.

2.3.1 METHODS AND MATERIALS:

An identical protocol was used, with the exception that 0.1 mg ovalbumin challenge dose was implemented. 44 joints were examined between 24 hours and 65 days, histologically and biochemically.

2.3.2 RESULTS:

Using the "minimal dose" intraarticular challenge of 0.1 mg suggested by Consden grossly, a synovitis persisted at least 66 days, the articular cartilage having a normal appearance, with some increase in joint fluid (fig 8a, b).

The synovitis lacked the gross hemorrhagic stage of the first days seen with the high dose challenge but persisted as an active synovitis at 14 days (Fig 9a). At 40 and 66 days, the surface had returned to a single cell layer, but subsurface monocyte infiltrates persisted (Fig 9b&c).

Paraffin sections of cartilage at 66 days appeared not unlike normal controls (fig 10a). ³⁵Sulphate in-vivo incubated tissue generally showed good uptake around the



fig 8(a) Rabbit knee joint necropsy at 66 days. Synovitis evident in intrapatellar fat pad. Articular surface is normal.

fig 8(b) Below: a 6μ paraffin toluidine blue photomicrograph in non weight bearing area, posterior aspect of femoral condyle. Normal architecture and metachromatic staining.



fig 9(a) Synovium 14 days after intra-articular challenge with 0.1 mg ovalbumin. Note monocytic infiltrates, synoviocyte hyperplasia and hypervascularity. 3 μ H+E section.



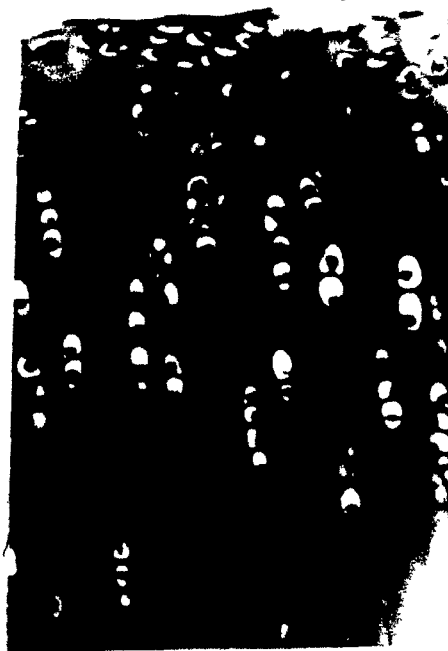
fig 9(b) Synovium 40 days after challenge with 0.1 ovalbumin. Note chronic synovitis with hypervascularity and infiltrates on left. Reparative fibroblasts noted on right. 3 μ paraffin H+E section.



fig 9(c) Synovium 66 days after challenge with 0.1 mg ovalbumin. Note chronic synovitis with subsurface fibrosis, and round cell infiltrate. 3 μ paraffin H+E section.



fig 10(a) 6 μ paraffin toluidine blue stained photomicrographs. 40 day AIA; non weight bearing area of posterior aspect of femoral condyle. Normal architecture and metachromatic staining. (b) At 66 days architecture and metachromatic staining is unchanged from the 40 day group.



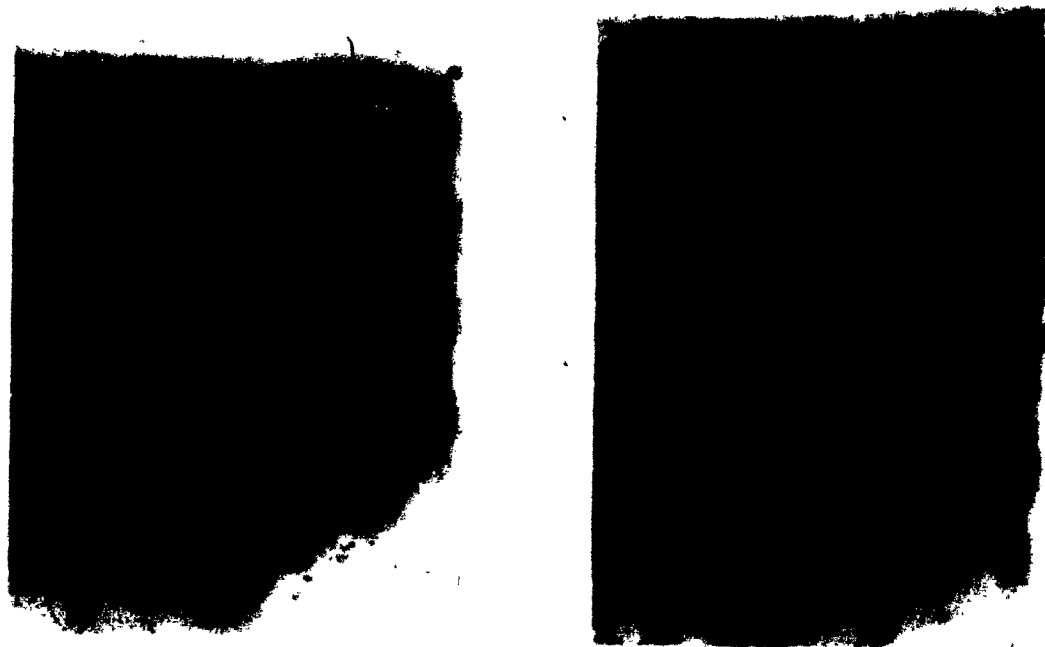


fig 11(a) One micron radioautographs of ^3H proline incubated cartilage, AIA 0.1 mg ovalbumin at 14 days. Note uptake over chondrocytes is present in most cells. Significant glycogen lipid accumulation is evident in cells. Architecture and staining are normal.

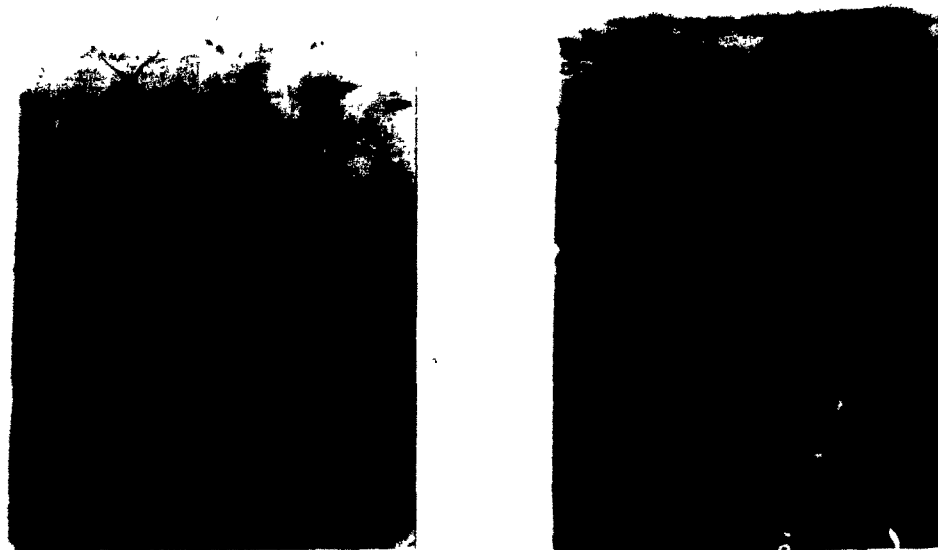
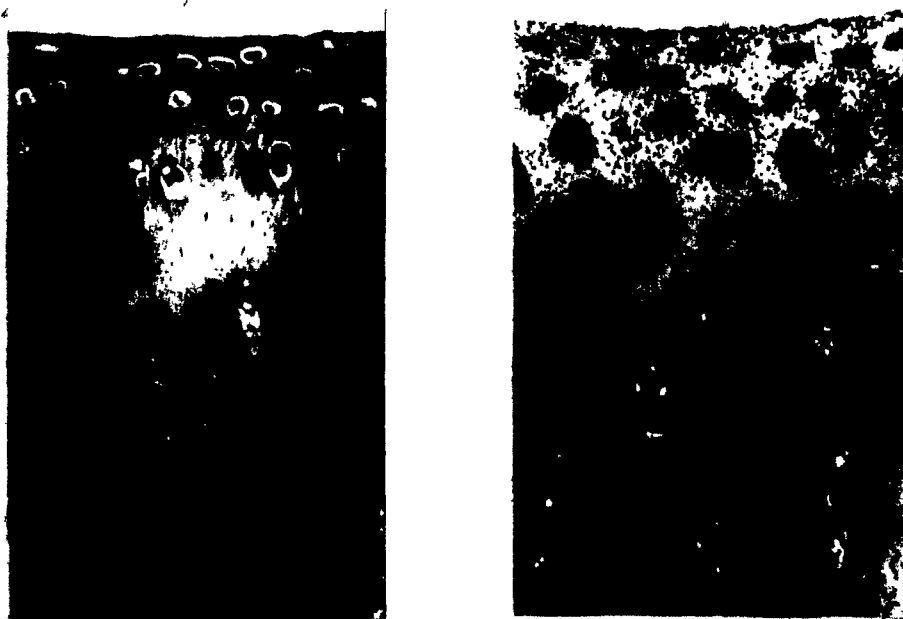


fig 11(b) One micron sections of cartilage AIA at 40 days, 0.1 mg challenge dose. Note in upper photomicrographs depletion of staining in upper zone matrix and intense rims of stain around cells. Other cells have pale halos. Fatty accumulation is significant in chondrocytes. A double tidemark is present. Lower photomicrographs of ^3H proline incubated cartilage demonstrate 2 patterns. On the left: upper zones absent of uptake; on the right: all cells are active. Deeper cells have acquired large amounts of lipid.



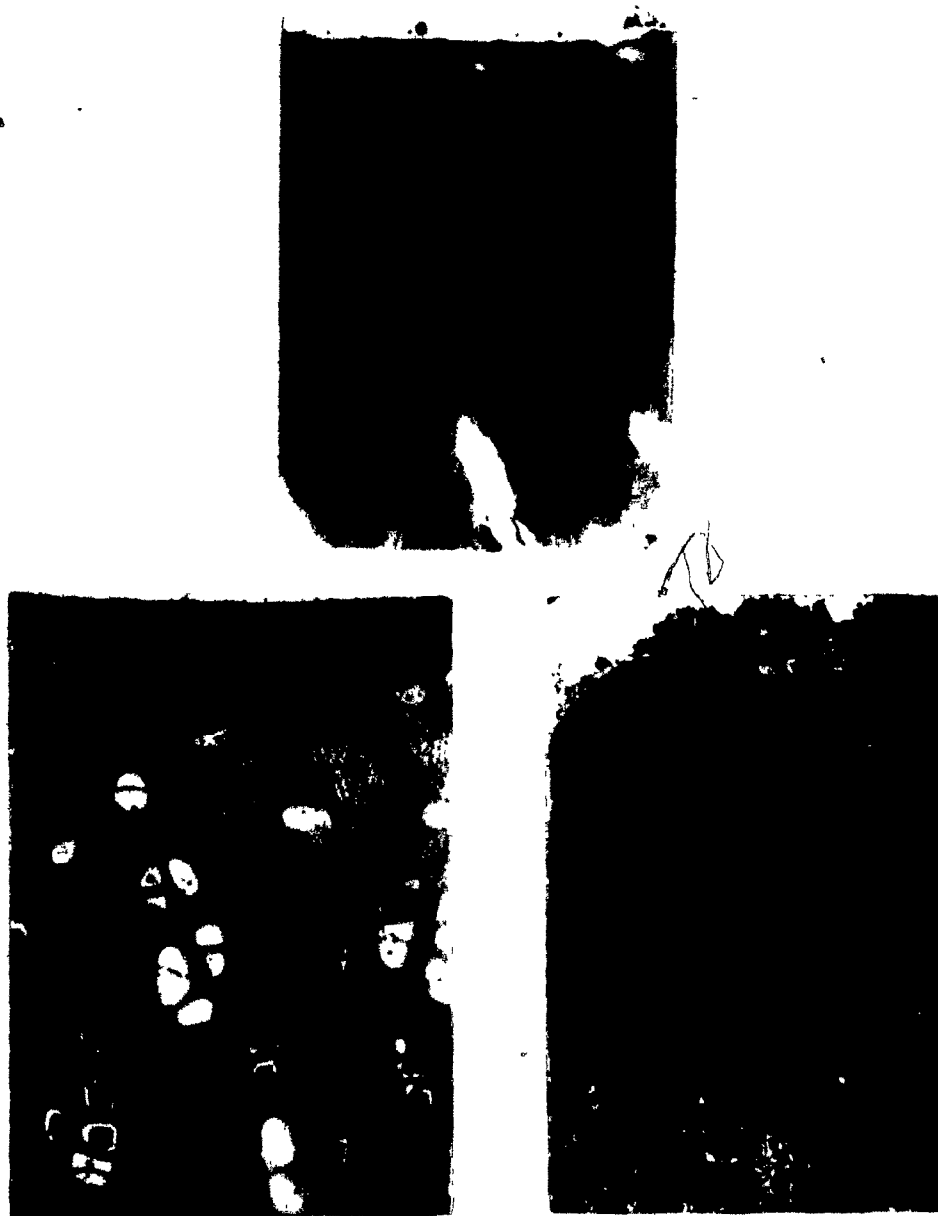


fig 11(c) Upper: one micron section of cartilage AIA at 66 days, 0.1 mg challenge dose. There is prominent fatty accumulation but the cells are generally alive. Lower right and left radioautographs demonstrate two different patterns. One where surface cell layers are preserved while lower zones are degenerate, the second where the surface is eroded to the radial zone with deeper chondrocytes still metabolically active.

chondrocytes in the deeper weight-bearing zones and through the whole depth in non-weight bearing zones. These findings were similar to controls. One micron sections incubated in vitro with tritiated proline showed active though decreased uptake at 14 days (Fig 11a). At 40 days significant fatty degeneration and sporadic cell death was noted (Fig 11b). Matrix changes with decreased staining, double tidemark and intense staining around chondrocytes was again demonstrated. These changes were more notable in the 66 day animals (Fig 11c).

Transmission electron microscopy of tissue at 40 and 66 days demonstrated characteristics of arthritic cartilage similar to those demonstrated with the high dose (7.5 mg ovalbumin) challenge. The surface showed signs of progressive collapse and erosion (Fig 12a) and transitional chondrocytes appeared near the surface (Fig 12b). Both healthy and degenerating cells were noted in the radial zone but degenerating ones appeared less frequently (Fig 12c,d,e). The tidemark cells were more typical of normal degenerating chondrocytes (Fig 12f) in contradistinction to the bizarre ones seen in figures 6e. The fatty degenerating chondrocytes and a double tidemark noted by light microscopy were again noted (Fig 12 l+m).

Hexuronic acid assays supported proteoglycan depletion of a lesser degree as expected (Fig 13b). Hydroxyproline content was analyzed in only 2 groups of animals at 40 and 66 days and indicated a small decrease in collagen content.

2.3.3. Discussion:

The minimal challenge doses suggested by Consden et al provided a far more satisfactory arthritis lasting at least 66 days.

The synovitis was persistent at least to 66 days; the articular cartilage changes were consistent with gradual degeneration of chondrocytes and matrix but were definitely viable at 66 days. Transmission electron microscopy demonstrated even more subtle changes, but, unavoidably, sampling was selective. However, the location of



fig 12(a) Transmission electron photomicrograph. Antigen induced arthritis 0.1 mg challenge at 5 1/2 weeks. Surface zone collapsed. Transitional cell lying just below surface.



fig 12(b) Fatty degenerating, transitional cell near surface. Above, a cell ghost.

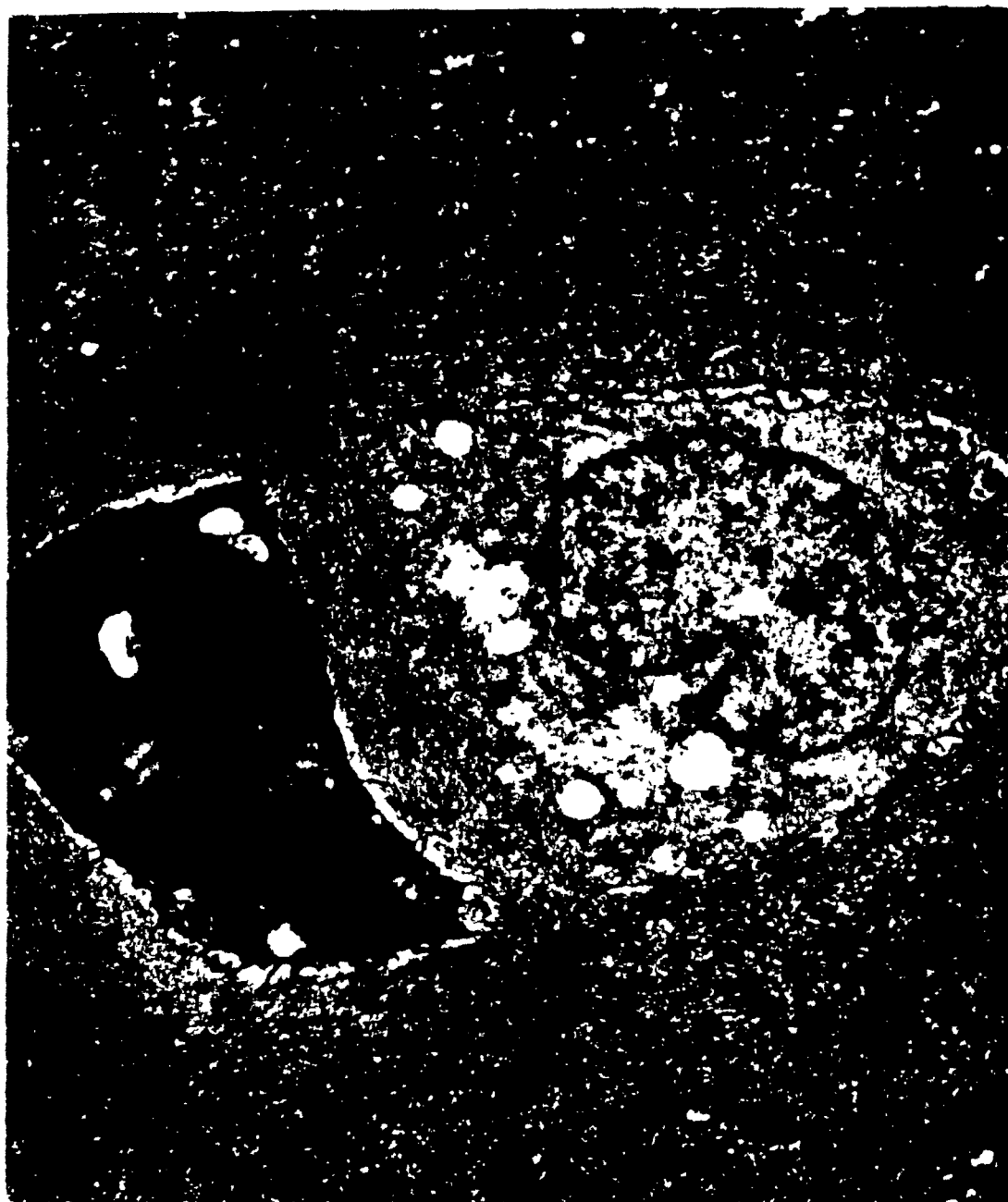


fig 12(c) Mid radial zone cell pair. On right a normal cell. The one on the left is degenerating.



fig 12(d) Near the tidemark, an ageing cell typical of those seen in normal cartilage.



fig 12(e) A cell similar to (d). Matrix kinking of collagen is noted in upper left.



fig 12(f) Normal appearing architecture of tidemark area. Vertically oriented collagen, round degenerating cells. Below, a calcified cartilage cell.



fig 12(g) Antigen-induced arthritis, 0.1 challenge dose at 9 1/2 weeks. Surface has eroded and round cells typical of deeper layers appear near surface.

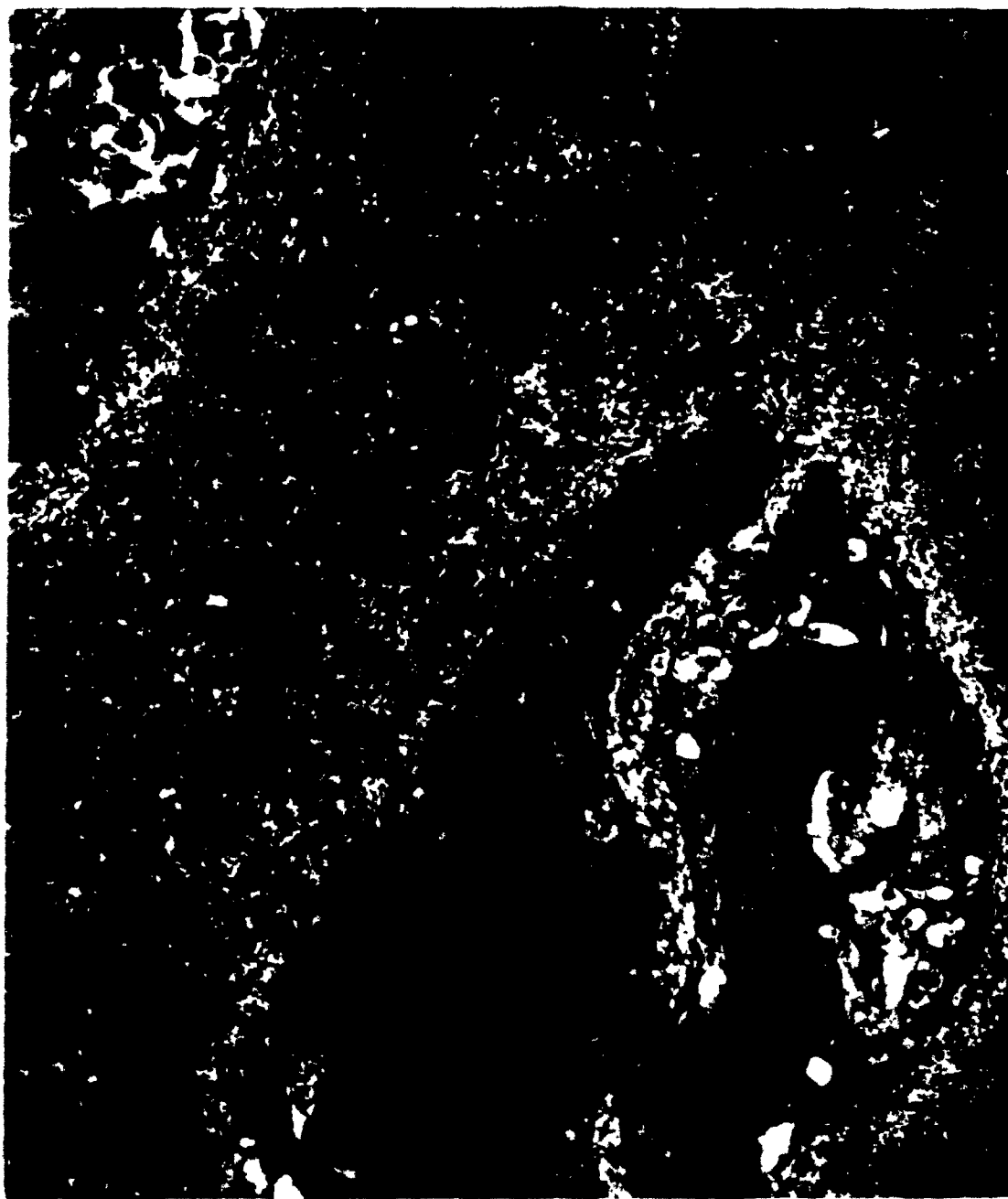


fig 12(h) Fatty degenerating chondrocytes near the surface.



fig 12(i) Healthy mid-radial zone chondrocyte.

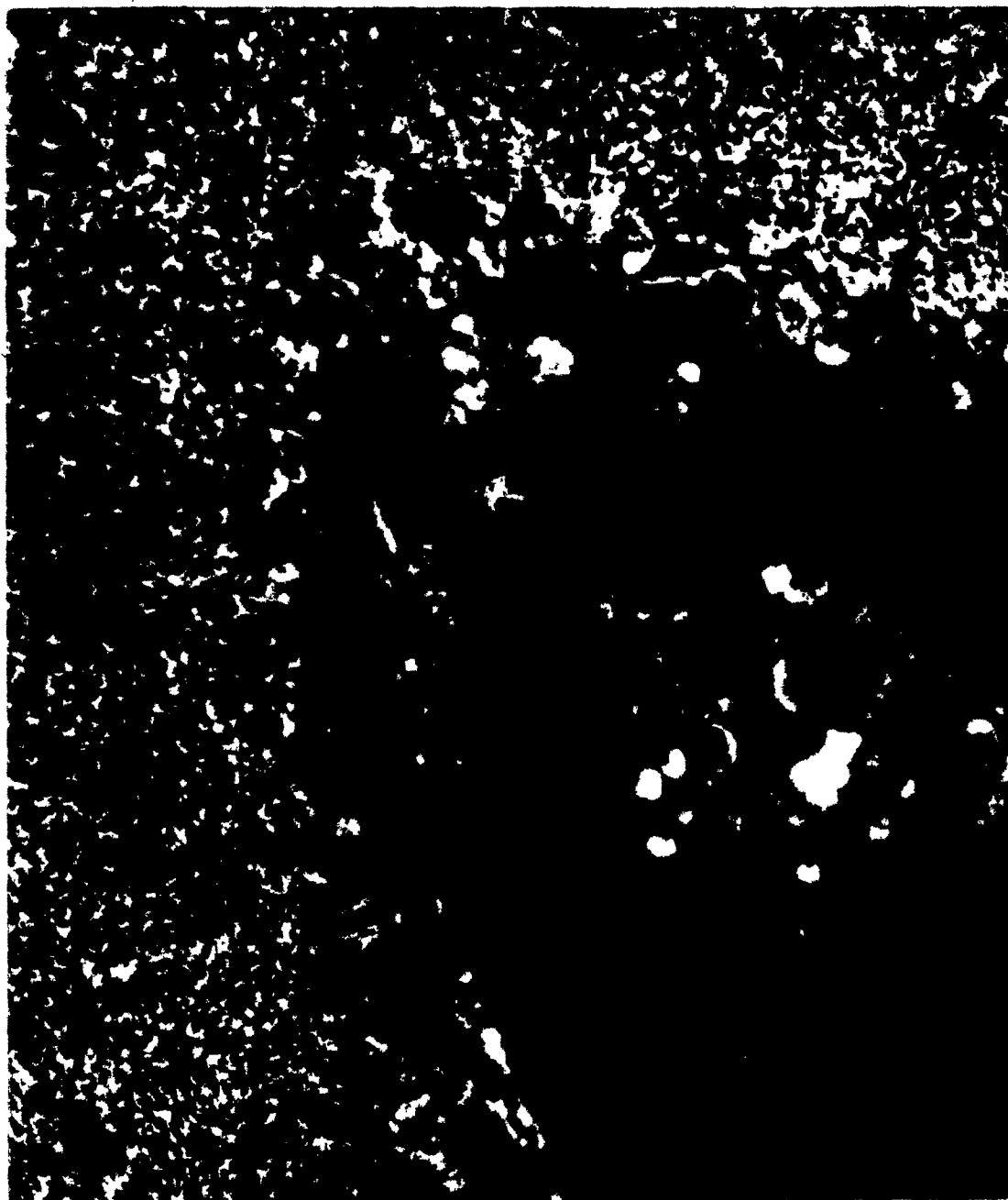


fig 12(j) Viable radial zone chondrocyte with dense material at extracellular margin and in cytoplasm, not membrane bound.



fig 12(k) A radial zone necrotic chondrocyte.



fig 12(l) A radial zone degenerating chondrocyte. Note lipid (L) and the space (G) believed to be an effect of the dehydration process on the intracellular stored glycogen.

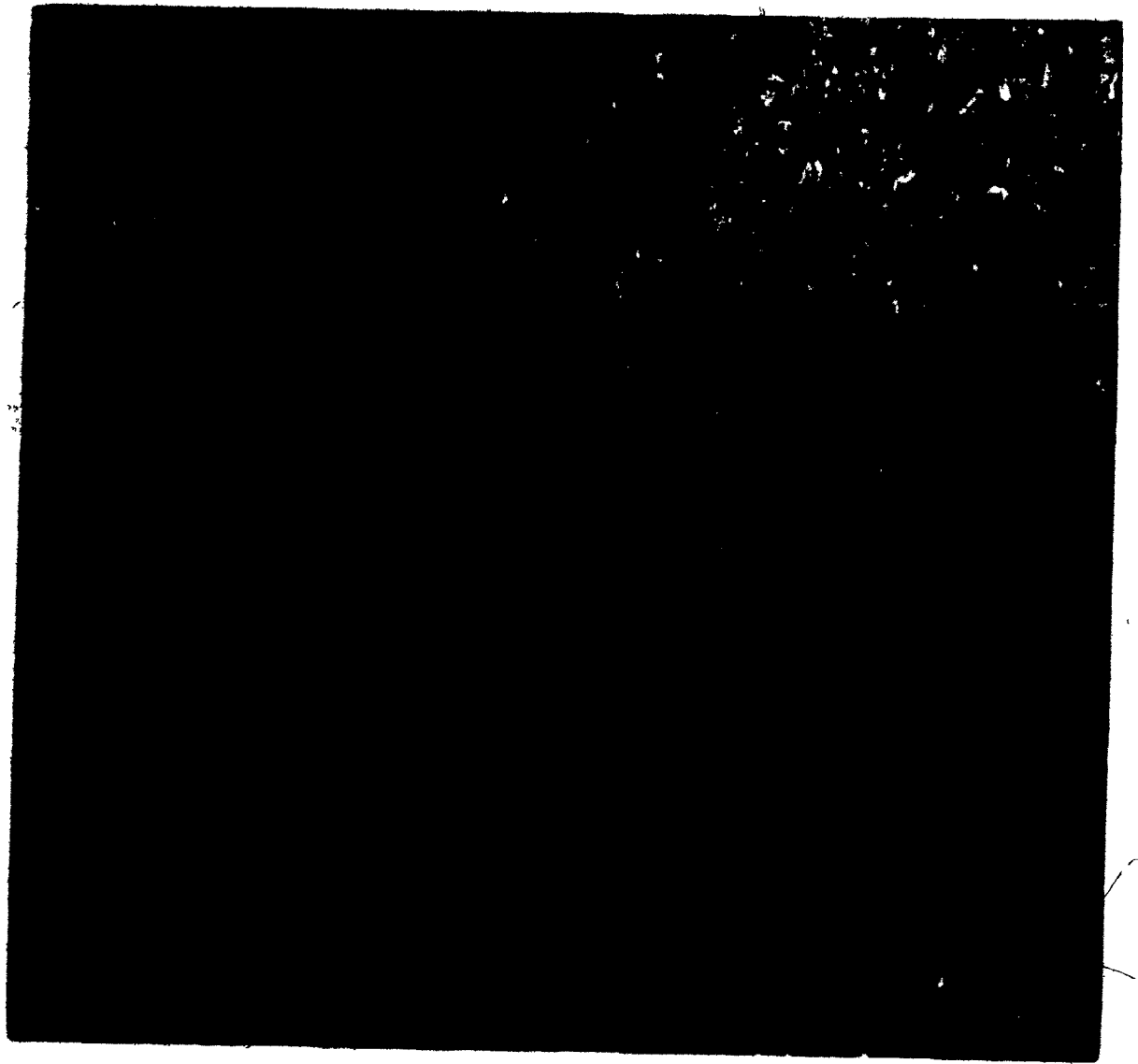


fig 12(m)A double tidemark. A feature seen only in pathologic material.

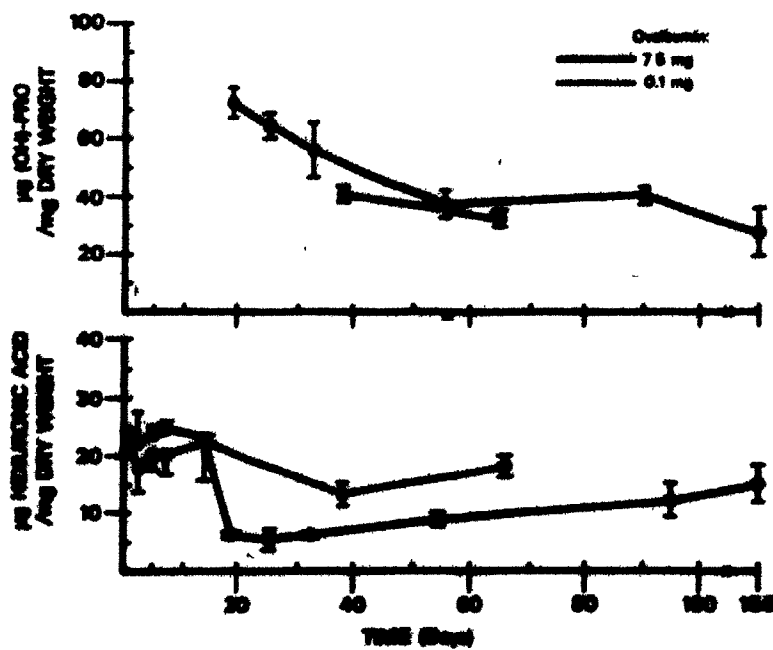


fig 13(a) Upper. Hydroxyproline in $\mu\text{g}/\text{mg}$ dry weight of cartilage. Antigen induced arthritis with 0.1 mg ovalbumin challenge shown with broken lines.

13(b) Lower. Hexuronic acid in $\mu\text{g}/\text{mg}$ dry weight of cartilage. Antigen induced arthritis with 0.1 mg ovalbumin challenge shown with broken lines.

sampling was consistently 1 cm proximal to the distal end of the patellar groove - an area which, we, anticipated, was non weight bearing. Thus, any changes seen here would be expected to represent the lower end of the spectrum of articular damage.

The biochemical studies were supportive evidence of ongoing articular damage. The magnitude of change in proteoglycan was significant but the decrease in collagen was minimal. Scintillation counting of digests and purified hydroxyproline to assess the specific uptake of precursors and conversion to collagen and proteoglycan were not reproducible.

The work done by Lowther et al is important in having demonstrated that even with 2.5 mg of challenge marked joint changes such as those produced with 7.5 mg, were found. We expect that if their material had been examined by TEM that even more severe chondrocyte degeneration would have been shown. One must conclude that if conditions similar to rheumatoid arthritis are expected the model must be used in the very early stages, that is, in the first two weeks even when the synovium is still "acutely inflamed", or must be developed using a much lower challenge dose such as the one reported. Histological techniques described are more sensitive and reliable assessments of joint pathology. The size of a joint and the appearance of the synovium are no longer adequate assessments of a model on which are carried out sophisticated immunobiochemical research in rheumatoid arthritis. A plea must be made to standardize the animal model of rheumatoid arthritis in the rabbit so that proper pre-clinical trials and basic science data may have a sound groundwork.

CHAPTER 3

STUDIES ON THE EFFECT OF ^{32}P -COLLOIDAL CHROMIC PHOSPHATE IN THE KNEE JOINT OF THE RABBIT

3.1

INTRODUCTION

As discussed earlier in this thesis, synovectomy has been one of the earliest surgical procedures aimed toward the management of joint pathology in rheumatoid arthritis. It appears to improve the clinical symptoms of the joint, pain, swelling and decreased mobility and appears to retard the degenerative changes in the articular cartilage (32,45,49,71,80,87,88,91,116,117). Synoviorthesis, a medical synovectomy, to use the word coined by Delbarre, is logical in the evolution of the management of rheumatoid arthritis as it is characterized by the ablation of the offending tissue synovium, by exposing it to a substance with selective toxicity, usually by injection (25). The advantages of such a regimen, are obvious; the patient, who is a candidate for synovectomy, by nature, is often debilitated and a poor operative risk. The rehabilitation time after arthrotomy is often 6-8 weeks while with synoviorthesis it is far shorter; and the cost and hospitalization time of major surgery is far greater than with synoviorthesis.

The use of radioisotopes of biological or biologically inert substances to induce selective tissue necrosis has been in use since the 1950's and in popular use only since the early 1970's. In Western literature, one of the earliest substances used was gold injected into pleural or peritoneal cavities to control malignant effusions (122). A mixed beta gamma emitter, it induced radionecrosis of the mesenchymal linings, and inhibited the progression of the effusion. However it had unpredictable total body effects and was very difficult to handle:

"It has been calculated that when 100 mCi are placed in a peritoneal cavity, there is emitted from the patient, 50 mr per hour at a distance of 5 feet. It is necessary, therefore, to keep such a patient at least 6 feet from other patients in order to stay within the maximum permissible daily radiation exposure; a nurse, for example, may be within 2 feet of the patient for no more than 20 minutes each day" (66).

Other emitters were sought that produced adequate penetration of tissue, low risk to the operator and patient, and would remain in the cavity being treated without diffusion

into the vascular and lymphatic compartments. Chromic phosphate in intracavitary use was first reported by Jacobs in 1950 (66) with several follow-up clinical reports in the following few years on its effect in malignant effusions (67,66,107,110,151) and on its metabolism (122) and distribution (29).

The principle of selective tissue destruction with radioisotopes (^{198}Au) was introduced in the human knee as a therapeutic modality in 1962 by Ansell (5). In an abstract in the Journal of Nuclear Medicine, Johnson in 1967 (69) is noted using ^{32}P for synovial effusions. In 1973, Winston and Bluestone (161), reported using chromic phosphate in lieu of ^{198}Au in nine patients with rheumatoid arthritis. They pointed out that ^{32}P had a similar energy level as gold, a longer half-life, a greater penetration of beta rays, no gamma rays, and could be found in the lymph nodes, liver and sacrum of only one of nine patients in whom activity had obviously escaped hematogenously. Furthermore, whatever activity was detected in urine or blood in these patients was cleared in two days. The highest value in urine obtained was $2.3 \mu\text{Ci/l}$ on the first day and in blood $0.15 \mu\text{Ci/l}$. No evaluation of short and long term results was made, save for the fact that there were no acute flare-ups post injection. Since that time, moreover, chromic phosphate has continued to appear in the literature as an accepted isotope for treatment of intractable synovitis (105,125). There have been no detailed studies of synovium and cartilage of normal rabbits treated with ^{32}P nor controlled studies in the arthritis animal model.

The experiment reported in Part A details the effect of ^{32}P -colloidal chromic phosphate on the rabbit knee joint, using 0.1 mCi, a dose calculated to be comparable to the 2-4 mCi employed clinically in the arthritic knee joints of patients with refractory joint symptomatology. In Part B the effect of the isotope on the antigen induced arthritic knee joint of the rabbit is reported.

3.2

Part A

EFFECT OF ^{32}P ON NORMAL KNEE JOINTS

3.2.1 METHODS AND MATERIALS:

Twenty new adult New Zealand white rabbits of both sexes of a minimum age of 6 months were used, all from the same breeding farm. ^{32}P Chromic phosphate (Phosphocol- ^{32}P Mallinkrodt Inc., Missouri. - S.A. 313 mCi/mg), 0.1 mCi in 0.2-0.5 ml volume was injected bilaterally into the knee joints of 20 rabbits through an infrapatellar tendon approach under general anaesthesia (Xylazine 5 mg/kg and ketamine 35 mg/kg). The knees were put through passive range of motion 25-30 times before returning them to their cages. The animals were divided into 4 groups of 5 animals and sacrificed at 4, 9, 16 and 26 weeks.

Each of the groups was handled similarly. All animals were harvested of synovium which was fixed in Bouin's solution for paraffin sectioning, staining and radioautographs to demonstrate ^{32}P . One animal was harvested live of synovium which was incubated for 60 minutes at 37°C in a Dubnoff metabolic oscillating incubator under 95% O_2 and 5% CO_2 controlled atmosphere in incubation media (Minimal essential media Flow Laboratories 12-102) containing 50 μCi methyl 1, 2- ^3H -thymidine (NEN NET-512) (S.A. 101 Ci/mmol). The specimens were then washed four times with a total of 200 ml iced saline, agitated, and the tissues fixed in Bouin's solution for paraffin embedding, and preparation for radioautography (73). Four joints were harvested of femoral condyles, immediately fixed in 10% buffered formalin (Fisher 50-F-100) containing 0.5% cetylpyridinium chloride (C.P.C.) for two weeks and then decalcified using 10% EDTA in 1 M NaOH at pH 7.4. Paraffin sections were stained with routine toluidine blue and Safranin-O, and radioautographs to demonstrate ^{32}P were prepared. Two joints in each group were injected intra-articularly 24 hours prior to sacrifice with 250 μCi ^{35}S (NEN-NEX-041 Sodium sulfate) and harvested of whole joints, immediately fixed in 10%

formalin and C.P.C., decalcified and paraffin embedded for radioautographs to demonstrate ^{35}S uptake. Prior to sacrifice, cartilage was hand-sliced from femoral condyles incubated in 10-20 μCi L2'3'-H proline (NEN-323 S.A. 29.5 Ci/mmol) in Krebs Ringer bicarbonate solution under the same conditions of incubation for 4 hours. The tissue was then cold chased for 15 minutes and transferred to 2% glutaraldehyde /0.1 M sodium phosphate for 3 hours. All tissue was then decalcified overnight in 4.13% EDTA (pH 7.4). Slices of cartilage were also immediately fixed in 2% glutaraldehyde for 3 hours and then decalcified in 4.13% EDTA pH 7.4, prior to fixation in osmium and prepared routinely for TEM (155).

Two weeks after intra-articular injection of 250 μCi L(2' 3')- ^3H proline (NEN-323 S.A. 29.5 Ci/mmol) and 24 hours after 250 μCi ^{35}S sulfate injection, 4 joints were harvested of all cartilage of the femoral condyles, patellar groove and tibial plateaux, care being taken to avoid areas of fibro-cartilage. The shavings were blotted, wet weights obtained and then frozen for individual analyses of hexuronic acid and hydroxyproline.

Light microscopy:

Paraffin embedded synovial specimens were sectioned at 3 microns and stained with acid hematoxylin and eosin. The cartilage was sectioned at 6 microns and prepared with stains 0.1% toluidine blue and Safranin-O (73). Paraffin sections prepared for radioautography were coated with Ilford-4 (Ilford Ltd., Essex, U.K.) and exposed, developed and stained with standard hematoxylin and eosin.

Transmission electron microscopy:

Articular cartilage was cut prior to fixation so as to permit full thickness sectioning following embedding. Samples for transmission electron microscopy were fixed in 2% glutaraldehyde/0.1 M sodium phosphate (pH 7.4) containing (0.1% toluidine blue) a proteoglycan stabilizing agent for two to three hours, rinsed in 0.1 M sodium

phosphate buffer containing 0.2 M sucrose and 0.025% toluidine blue for 30 minutes - postfixed in 2% OsO_4 in 0.1 M sodium phosphate plus 0.025% toluidine blue for 2 hours and ethanol dehydrated (30%, 50%, 75%, 95%, 100% - 10 min. each for 3 changes) (130). Blocks were then transferred to a Spurr/absolute ethanol (1:1) infiltration mixture for 2 hours then into Spurr alone, and then placed in a dessicator and held under vacuum overnight (134). Blocks were flat embedded in Spurr to permit full thickness sectioning and polymerized at 70°C for 18 hours. One micron plastic sections were cut with glass knives and placed on subbed glass slides; the ultrathin (600-800Å) sections were cut with diamond knives and placed on 200 mesh filmless copper grids (134). A Reichert Ultracut was used for sectioning. The 1 μ sections required no further staining as they were stained in situ with the toluidine blue O during the fixation process (130). The ultrathin sections were stained for 30 minutes with 2% uranyl acetate (in 50% alcohol) and for 10 minutes with 0.2% lead citrate (152). A Philips 400 transmission electron microscope was used in the study.

Radioautography:

Tissue was fixed and processed as above without the addition of toluidine blue and decalcified. Toluidine blue could not be used because of a chemical reaction that takes place between the toluidine blue and emulsion layer which results in artificial grains (129). One micron thick sections were placed on subbed slides and dipped in total darkness in Ilford L4 nuclear track emulsion (1:2 dilution), air dried, stored in sealed black plastic boxes containing a drying agent (silica-gel) with a blank slide between each slide containing sections, and stored at 4°C for 3-4 weeks (11). Following exposure, the slides were brought to room temperature and developed in D 170 (6 min), rinsed in tap water and fixed in 24% sodium thiosulphate (10 minutes) - rinsed for an additional 10 minutes in running tap water and air dried. To permit heating the slides, which is necessary to ensure adequate staining of plastic sections, the developed

emulsion required hardening to prevent swelling during heating. Hardening was done by placing the slides in 10% formalin at room temperature for 10 minutes, rinsing and again air drying (120). The slides were then stained on a hot plate with 1% toluidine blue in 0.5% sodium borate. All slides were coverslipped using unpolymerized Spurr and placed on a hotplate overnight to polymerize the plastic.

Biochemistry:

Cartilage shavings from each joint were analyzed separately. The tissue was powderized using a freeze miller, and then suspended in 5 M acetic acid. A pepsin solution of 1 mg/ml/50 mg tissue (Sigma P-7012) was added and the tissue allowed to digest at 4°C with constant stirring for 7 to 9 days. The digest was neutralized to pH 8.6 with NaOH to inactivate the pepsin and then dialyzed in cellulose tubing against 0.5 M acetic acid till the dialyzate was free of counts. The solution was divided into aliquots for hexuronic acid, hydroxyproline and DNA analyses.

Hexuronic acid analysis was made according to the method of Bitter and Muir using sodium tetraborate in sulfuric acid and carbazole in ethanol with a standard of glucuronic acid (9). An aliquot was analyzed for counts of ^{35}S and ^3H using a Beckman Scintillation counter with a two channel simultaneous readout and automatic quench correction.

Hydroxyproline analysis was made according to the method of (Stegemann) (137). The solution was first lyophilized in a centrifuge dryer overnight and resuspended in 1 ml 6N HCl and hydrolyzed for 18 hours dried and resuspended to constant volume with 6N HCl. A 100 μml aliquot was then transferred to heavy chromatography paper (Whatmann 3 MM). Using a butanol, glacial acetic acid water solution (4:1:2) the sample was separated over 16-18 hours, dried, the proline and hydroxyproline bands cut out and eluted in water (18). Hydroxyproline assay using chloramine T and perchloric acid was carried out and scintillation counting of the proline and hydroxyproline eluants were made.

Deoxyribonucleic acid analyses were made according to the method of Erwin Stoschecki and Florini (36).

3.2.2 RESULTS:

At sacrifice, the joints were generally unremarkable except for an annular area of fibrosis surrounding the point of insertion of the needle through the infrapatellar tendon and synovium. The remainder of synovium was not hypemic or villous; the cartilage was smooth and pearly white. In only the occasional joint was there any increase in joint fluid - one of which was slightly murky (fig 14).

The synovium of 4 week treated animals was generally reactive with new vessel formation and reactive subsynovial fibroblasts; a layer of disorganized new collagen seen beneath the surface of synoviocytes (fig 15b). At 9 weeks, the surface cells were either reactive with neovascularization, dead or showing signs of degeneration with loss of cell to cell contact and extrusion into the joint. The subsynovial layer was fibrotic (fig 15c). At 16 to 18 weeks, oddly, the surface cells were more degenerative, loss of cell borders, extrusion into the joint, and marked fibrosis (fig 15d). At 26 weeks, the surface appeared normal, but reparative fibroblasts, giant cells and new collagen indicated an ongoing reparative response to injury (fig 15e). Tritiated thymidine uptake by synovial cells incubated in vitro was proportional to the number of cells in the field and not different from controls.

Paraffin embedded sections stained with toluidine blue and safranin-O, were essentially normal with preservation of metachromatic staining in all areas save for the weight-bearing surface of the femoral condyle - a phenomenon considered normal in our laboratory (fig 16). ³⁵S incubated radiographs at 18 weeks showed uptake in all zones except superficial zones in weight bearing area. At 26 weeks, good uptake was noted in the 2 joints examined.

One micron Spurr embedded sections were more informative. At 4 weeks, the cartilage had good depth, preservation of the matrix and a generally metabolically



fig 14 Necropsy photograph, 26 weeks after intra-articular injection of ^{32}P 0.1 mCi. Note preservation of joint; injection site with annular fibrosis visible (arrow).



fig 15(a) ^3H thymidine in-vitro incubated synovium from a normal rabbit knee. Note the single cell layer on surface and fatty tissue below. 3μ H+E.



15(b) Synovium 4 weeks after intra-articular $0.1 \text{ mCi } ^{32}\text{P}$. Note the new vessel formation, focal fibrosis. Area in right photomicrograph is not fibrotic. 3μ H+E.

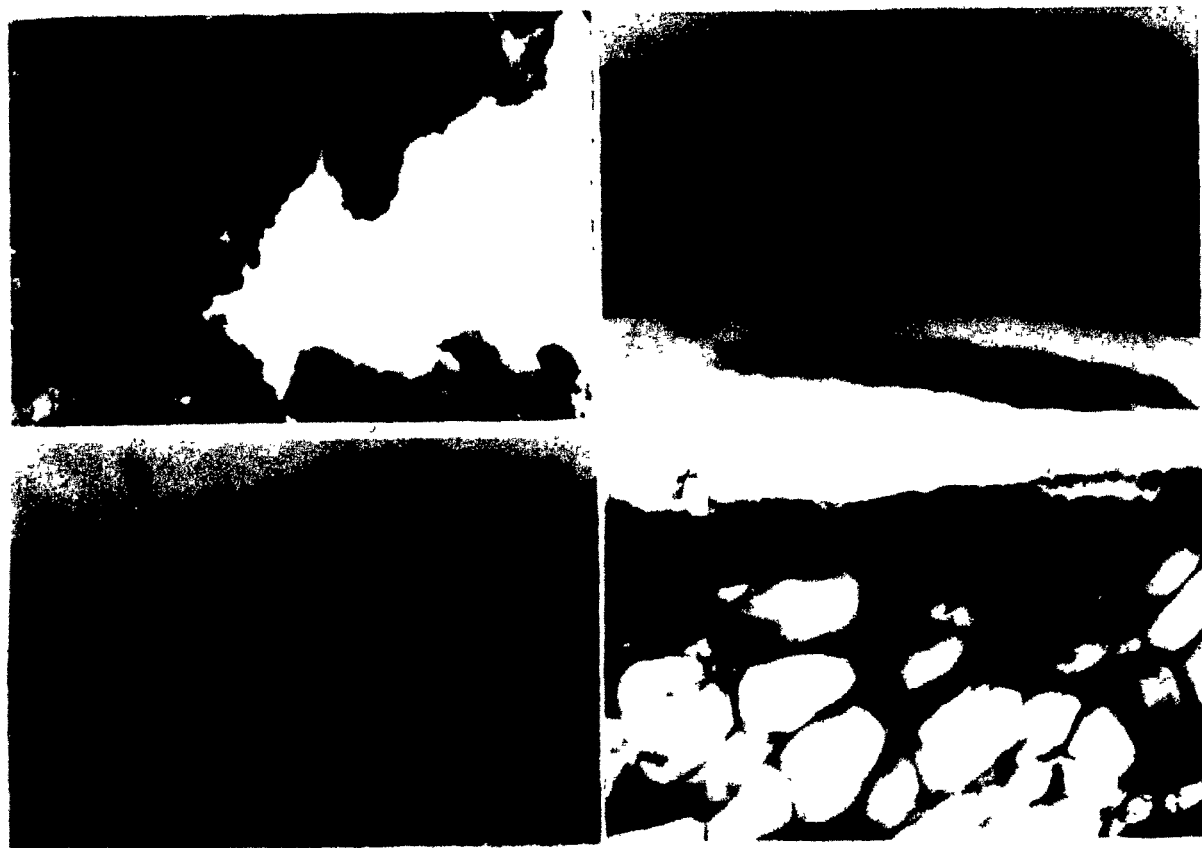


fig 15(c) Synovium 8 weeks after intra-articular injection 0.1 mCi ^{32}P . Note in some areas, thick acellular fibrosis (upper left); reactive synovium with new vessels and fibrosis (upper right); intact surface with loose new collagen (lower left); a surface with loss of normal cell-to-cell definition and subsurface fibrosis (lower right).



fig 15(d) Synovium 16-18 weeks after intra-articular injection 0.1 mCi ^{32}P . ³H Top, radioautograph of ^3H thymidine in vitro incubated tissue; two cells have uptake. Lower group: upper left extensive fibrosis, giant cell is visible; upper right, same knee as top section, demonstrating subsurface fibrosis. Lower left and right, demonstrate loss of normal cell to cell contact 3μ H+E.



fig 13(e) Synovium 26 weeks after intra-articular injection 0.1 mCi ^{32}P . Note intact and normal synovial surface, presence of reparative fibroblasts and a giant cell in fat. 3μ H+E.



fig 16 6 μ paraffin section Toluidine blue stained cartilage 26 weeks after intra-articular injection 0.1 mCi 32 P.

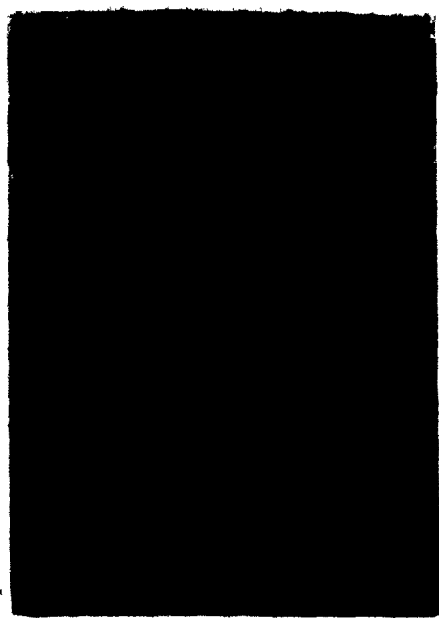


fig 17(a) One micron sections of normal adult cartilage. On left radioautograph, ^3H proline incubated tissue. On right insitu toluidine blue stained.



fig 17(b) One micron sections cartilage 4 weeks after 0.1 mCi ^{32}P intra-articular injection. Note marked fat and glycogen accumulations in cells. Surface is abnormal with loss of surface zones; empty lacunae and decreased staining. Toluidine blue. Radioautographs not completed at time of writing.

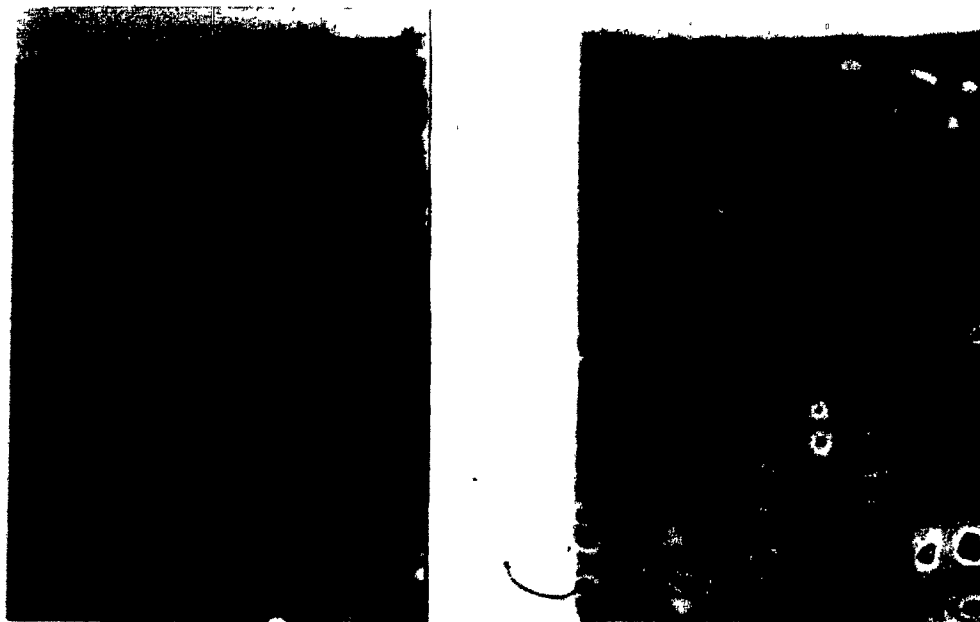


fig 17(c) One micron sections cartilage 8 weeks after 0.1 mCi ^{32}P intra-articular injection. Note loss of tangential layer, decreased stain, intensely staining halos around radial zone cells, fatty accumulation in cells.

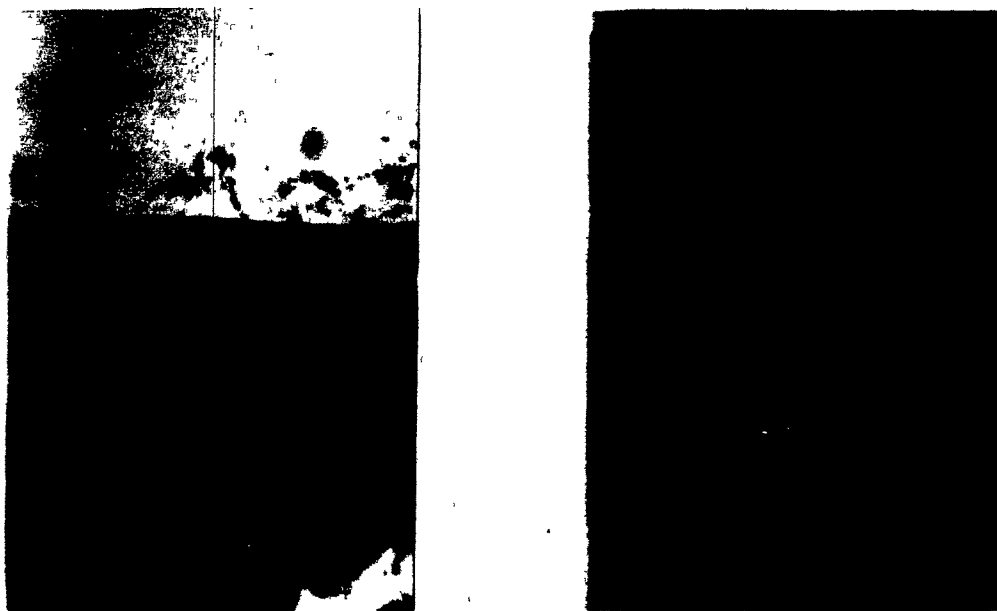


fig 17(d) One micron sections cartilage 18 weeks after 0.1 mCi ^{32}P intra-articular injection. On left toluidine blue section showing fatty and glycogen accumulation in cells and irregular superficial zone matrix. On right radiograph shows diminished uptake over most cells with necrotic chondrocytes in tangential zone.

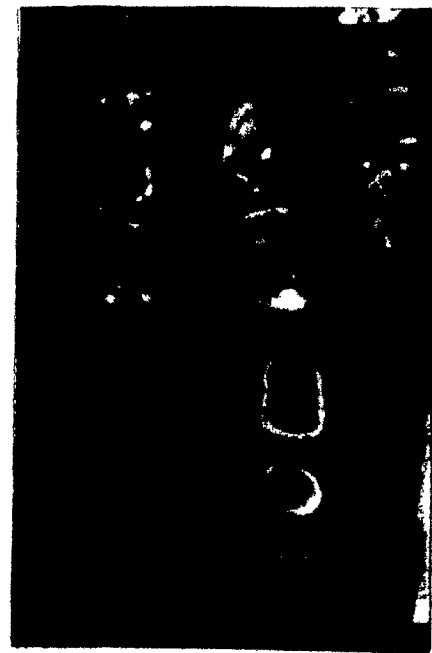
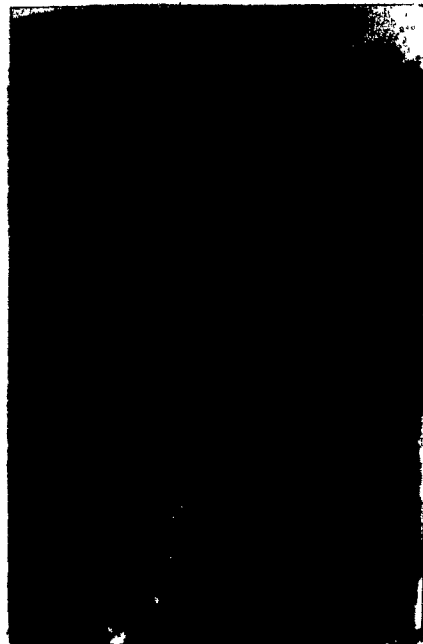


fig 17e One micron radiographs cartilage 26 weeks after 0.1 mCi ^{32}P intra-articular injection. Note complete absence of uptake of ^3H proline precursor by cells; abnormal cytoplasmic accumulations; necrotic cells; surface disorganization; deep zone matrix irregularity.

active chondrocyte population. Fatty accumulation, an early indicator of cell degeneration, in our opinion, was seen to an abnormal degree in all joints examined (fig 17b). At 8 weeks, the chondrocytes appeared to have had some insult; there was an intensely staining rim around some, a halo of depleted stain around others suggestive of failure to produce proteoglycan in an amount required or a depletion of proteoglycan (fig 17c). At 18 weeks the chondrocytes were still viable but metabolically less active than normal tissue. A curious pattern of decreased uptake of precursor was noted at all levels of the cartilage (fig 17d). At 26 weeks, the surface of the cartilage began to breakup, many chondrocytes were clearly necrotic. The remainder showed little or no uptake of the metabolic precursor, ^3H proline (fig 17e). The study of 26 week 1 micron sectioned cartilage was limited to 2 joints and may be anecdotal.

TEM studies of 4, and 26 week treated animals demonstrated strikingly abnormal findings. The cartilage depth was decreased (fig 18a); the matrix had an odd amorphous edematous appearance; many cells were genuinely necrotic and had a bizarre appearance (fig 18h-k); others had remarkable accumulation of glycogen material (fig 18c). The lacunar spaces contained small darkly stain spots, presumably proteoglycan (fig 18e). The areas immediately surrounding the cells were often devoid of stain suggesting depletion of proteoglycan (fig 18c). The matrix showed a curious irregularity, sometimes called "matrix streaks" in the literature and demonstrated earlier in our arthritic specimens. Whether this was a depletion of intercollagenous proteoglycan allowing kinking of the fibrils is still speculative (fig 18f).



fig 18(a) Transmission electron micrograph of articular cartilage, four weeks after intraarticular injection of 0.1 mCi ^{32}P . Surface cell and adjacent "ghosts".



fig 18(b) A chondrocyte scar. Lacunar space is filled with course disorganized collagen fibrils.



fig 18(c) A viable chondrocyte pair. Pericellular material with fine densities of proteoglycan and collagen fibers. Cell membranes have retracted from matrix to abnormal degree leaving a "halo". Lipid (L) and glycogen (G) and fibrillar (F) material in cytoplasm in abnormal amounts.



fig 18(d) Another chondrocyte pair similar to fig 18 c.



fig 18(e) Chondrocyte pair near surface. Note retraction of cell membrane from matrix and accumulation of proteoglycan in "space".



fig 18(f) Tidemark (T) and above, "matrix streaks" or waves of course collagen fibrils as previously seen in arthritic cartilage.

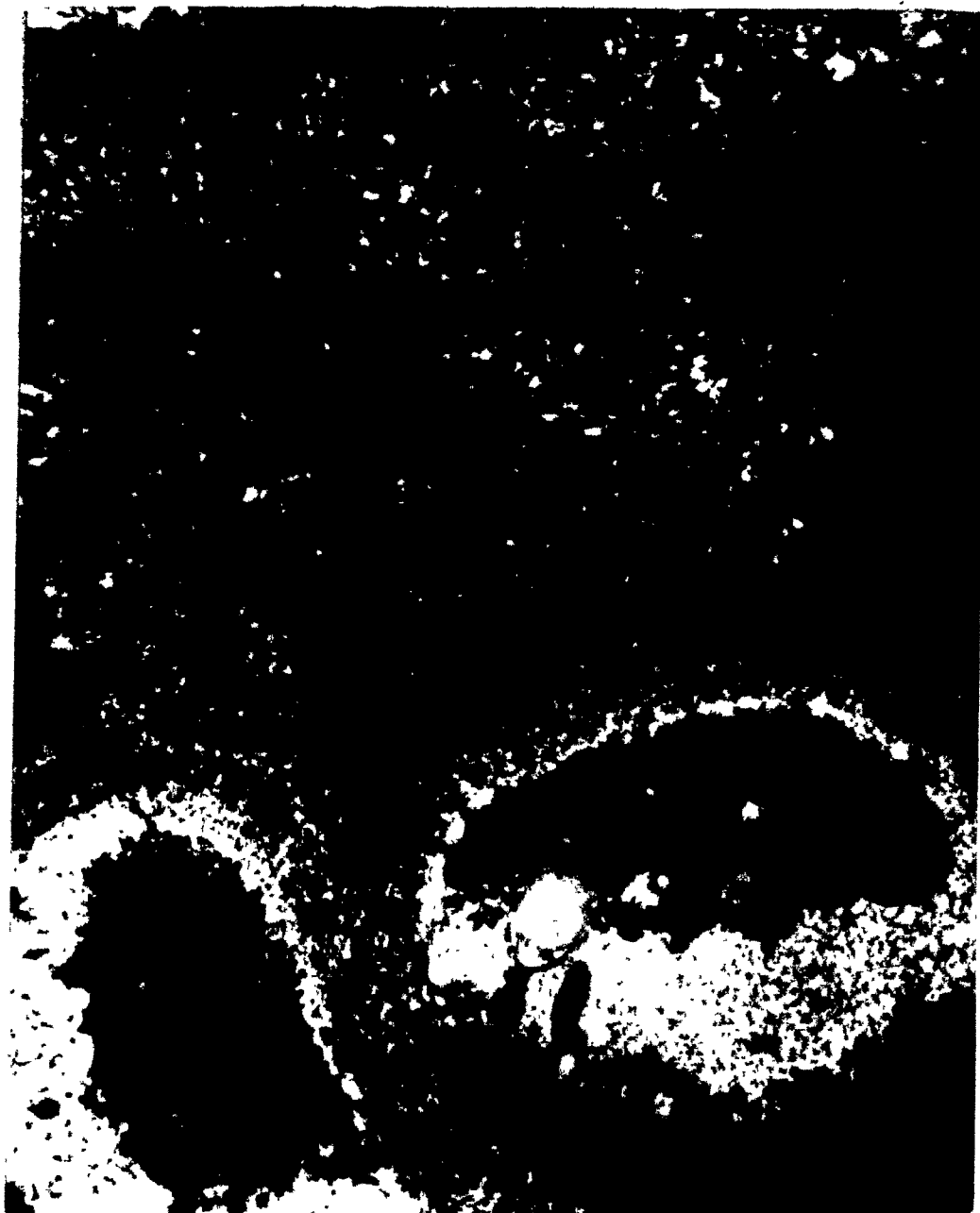


fig 18(g) Cartilage 26 weeks after 0.1 mCi ^{32}P injection. Surface (S) is nonhomogenous with necrotic tangential cells.



fig 18(h-k) Bizarre necrotic cells with huge accumulations of dense material (DM), self amputated cytoplasmic portions, and lacunar spaces of proteoglycan material (PG).



fig 18(i)

2

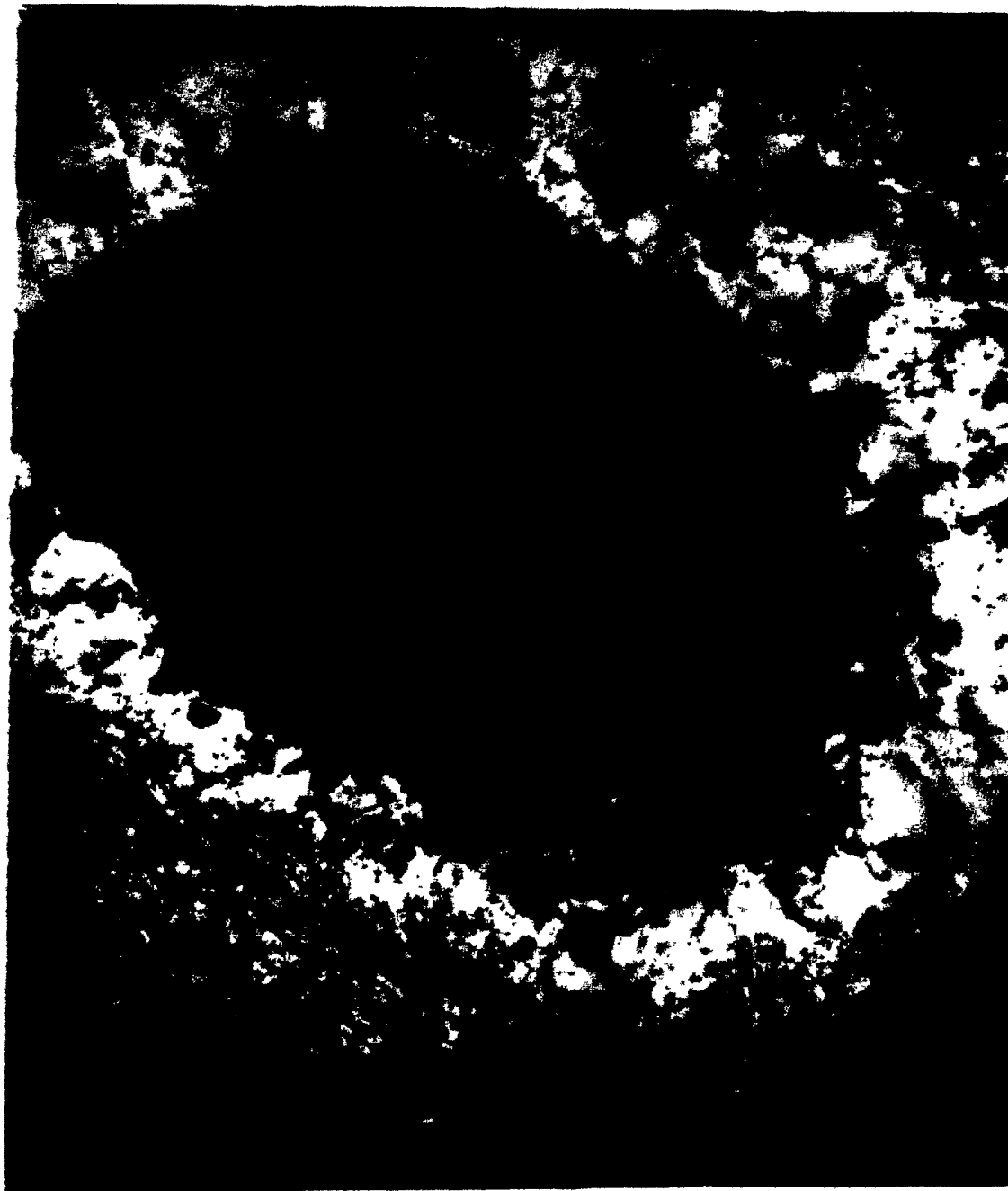


fig 13(j)



fig 18(k) High magnification of 18j.

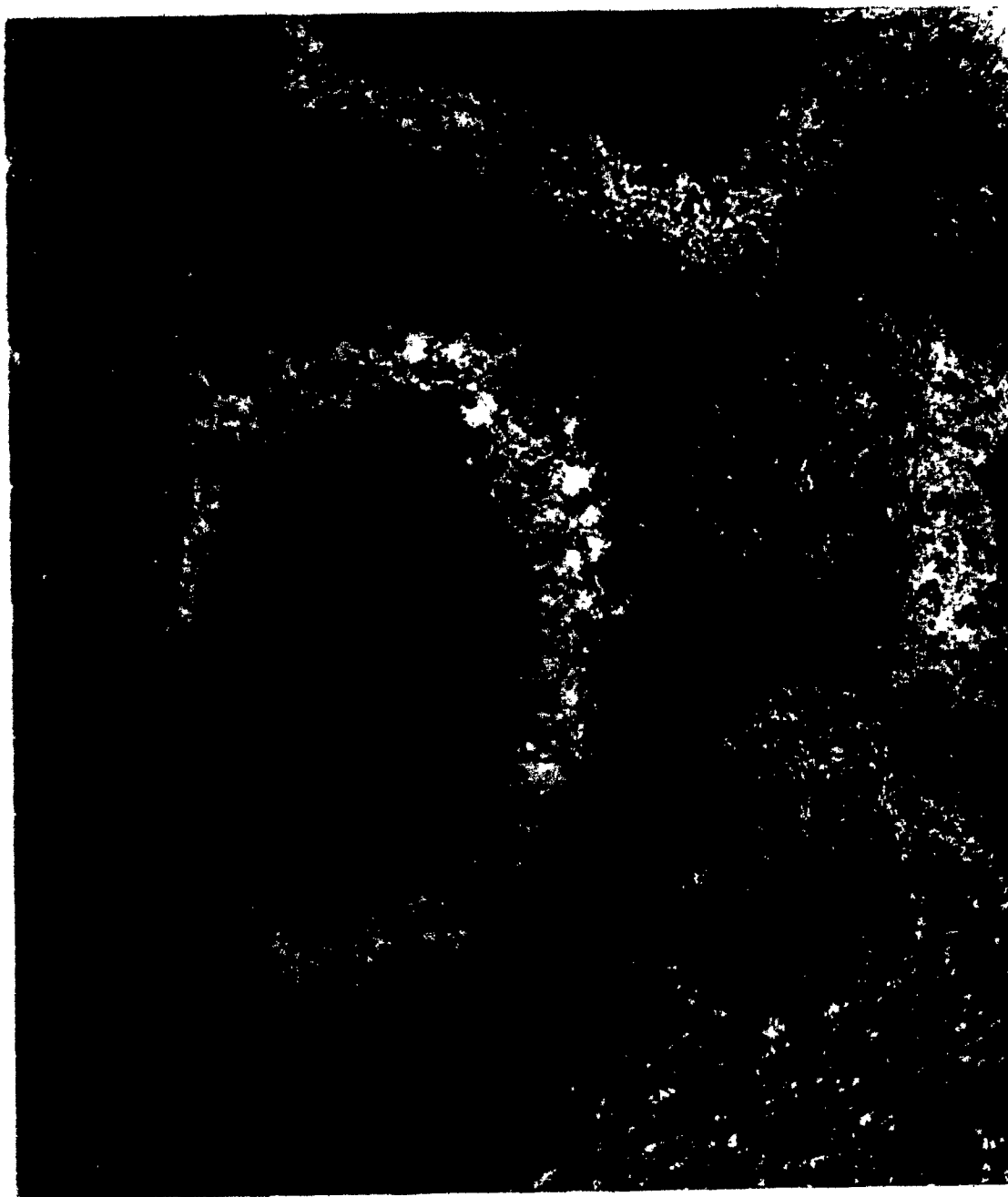


fig 18(1) A bizarre necrotic chondrocyte at the tidemark (T).

Biochemistry:

Hexuronic acid content measured in microgram per milligrams dry weight of cartilage as an indirect measure of proteoglycan content did not reflect the histological and staining evidence of depletion of proteoglycans (fig 19b). Hydroxyproline measured in micrograms per milligrams dry weight of cartilages and indirect measure of collagen content showed a significant decline in concentration with a recovery. The latter might be a spurious result or might reflect the loss of cartilage substance of cells and proteoglycan leaving a proportionately higher concentration of collagen despite less cartilage bulk and weight.

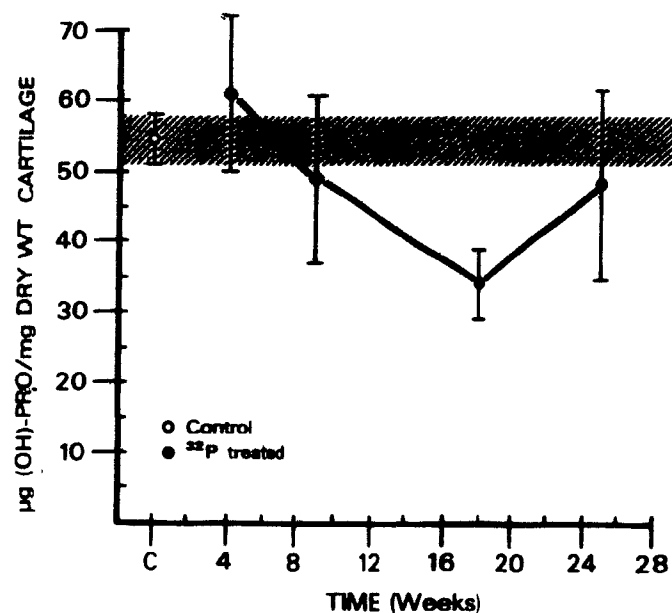
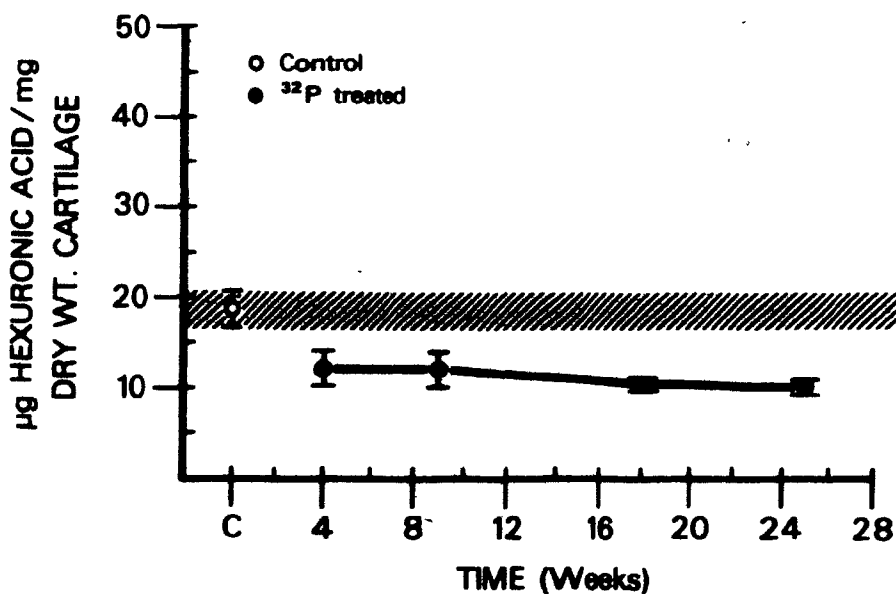


fig 19(a) Hydroxyproline content in $\mu\text{g/mg}$ dry weight of cartilage after intra-articular injection 0.1 mCi ^{32}P .



19(b) Hexuronic acid content in $\mu\text{g/mg}$ dry weight of cartilage after intra-articular injection of 0.1 mCi ^{32}P .

3.2.3 DISCUSSION:

The effects of ^{32}P on normal joints appears not only to be a real one, but also a negative one. The isotope at doses we believe to be "therapeutic" produced a spotty fibrosis in the synovium which as demonstrated, was alive and active, indeed, as early as 4 weeks after injection. This resembles little, the joint lining after surgical synovectomy described previously (87). The changes in the articular cartilage of 4, 9 and 18 week animals were suggestive of some insult; the 28 week plastic sections and TEM studies were impressive and alarming even though the latter are based on a study of 2 joints.

The study of ^{32}P in rat knee by Ubios (148) using rather high (0.25 mCi, four times therapeutic) doses reported severe cartilage disorganization with time. We wonder if his findings herald those of ours to come, in long-term studies presently underway.

3.3

PART B

**EFFECT OF ^{32}P ON KNEE JOINTS OF THE
RABBIT WITH ANTIGEN INDUCED ARTHRITIS**

3.3.1 METHODS AND MATERIALS

Twenty adult New Zealand rabbits of the same age and weight were used. One milligram solution of ovalbumin (Sigma A-5503 grade V crystallized and lyophilized) in sterile water emulsified in an equal volume of Freund's complete adjuvant (Difco Lab. 063859) to give a final concentration of 1 mg/ml emulsion was prepared and all animals sensitized intradermally in 5 sites each on the back. On day 14, a second sensitization was made with 0.5 mg ovalbumin in 0.5 ml emulsion in 3 sites. On day 24, the animals were skin-tested with 20 micrograms ovalbumin in 0.1 ml water in 2 sites with controls of sterile water. All animals had positive skin tests and were then challenged on day 30 with 0.1 mg ovalbumin in sterile solution injected into each knee as described earlier. Ten days after challenge 0.1 mCi chromic phosphate ^{32}P suspension (Phosphocol ^{32}P , Mallinckrodt Inc, Missouri) was injected under general anaesthesia into the right knee joint and 0.5 ml normal saline injected into the left joint. The knees were put through passive range of motion 25-30 times before returning them to the cages.

The animals were divided into groups of 10 and sacrificed at 4 and 8 weeks after treatment. The same protocol for analysis was used as described in Part A. 3 animals were harvested of femoral condyles; 3 animals were incubated in vivo with ^{35}S and whole joints fixed; 4 animals were harvested of cartilage for biochemical studies.

3.3.2 RESULTS:

Examination of the joints of control and treated animals up to 66 days revealed no gross abnormalities. The surface was smooth and pearly; the fluid was clear (fig. 20). One joint had early marginal osteophytes.

Synovium: At thirty days post treatment with 0.1 mCi ^{32}P -chromic phosphate, (40

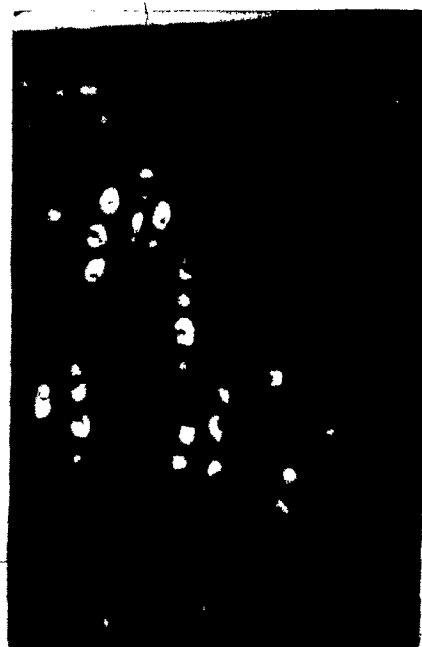
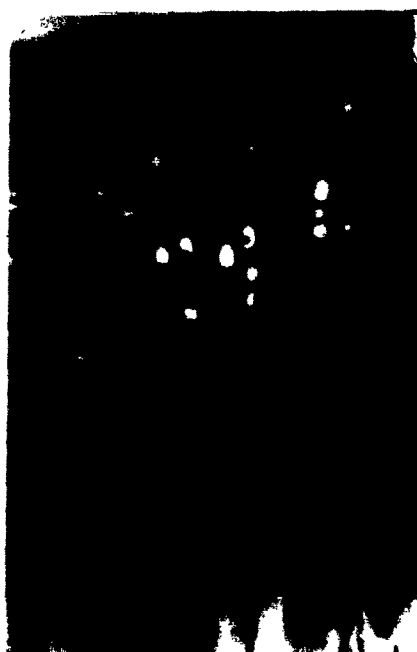


fig 20 Antigen-induced arthritic knee joints. Left, control. Right, 60 days after intra-articular injection of 0.1 mCi ^{32}P . Below 6 μ paraffin sections, toluidine blue stained at 60 days, control and treated. Note normal architecture.

days post induction of arthritis), tritiated thymidine incubated synovial samples demonstrated a monocellular surface layer with active uptake indicative of duplicating synoviocytes. Monocytic cellular infiltrates were present as evidence of ongoing synovitis. Most had subsurface fibrosis. The treated animals also demonstrated all the features of chronic arthritis but no changes specific for radiation damage. In particular, there was no swelling of synovial cytoplasm or nucleus, no chromatin clumping, no obliteration of the capillaries, or fibrosis out of proportion to the arthritis induced (fig 21b). At 60 days post treatment, a fibrotic subsurface remained; the synovial cells were again monolayer, and the subsurface still contained an infiltrate (fig. 21c).

Cartilage: Five micron sections of paraffin embedded tissue stained with safranin-O and toluidine blue demonstrated early clefts in the weight-bearing areas of tibial plateaus, marginal pannus and staining was decreased in weight-bearing areas of the femur. The tibia and non-weight-bearing areas of the femoral condyles stained normally - changes all compatible with early degenerative arthritis. These changes were not different in control and treated animals (fig. 20).

One micron sections of Spurr embedded cartilage stained in situ with toluidine blue demonstrated clearly the more subtle signs of chondrocyte degeneration. Fatty degeneration and retraction of the cytoplasm from the surrounding matrix creating a lacunar space was present. The changes appeared more noticeably in the upper radial zone in some sections but in others could be quite sporadic in distribution (fig. 22b). Subtle differences were noted with treated tissue demonstrating healthier cells.

Slices incubated in ^3H -proline, Spurr embedded and coated for radioautography were examined: uptake of ^3H -proline as evidenced by silver grains overlying the chondrocytes demonstrated the metabolic activity of the cells. It was evident that significant insult had occurred to the chondrocytes - again sporadically but often

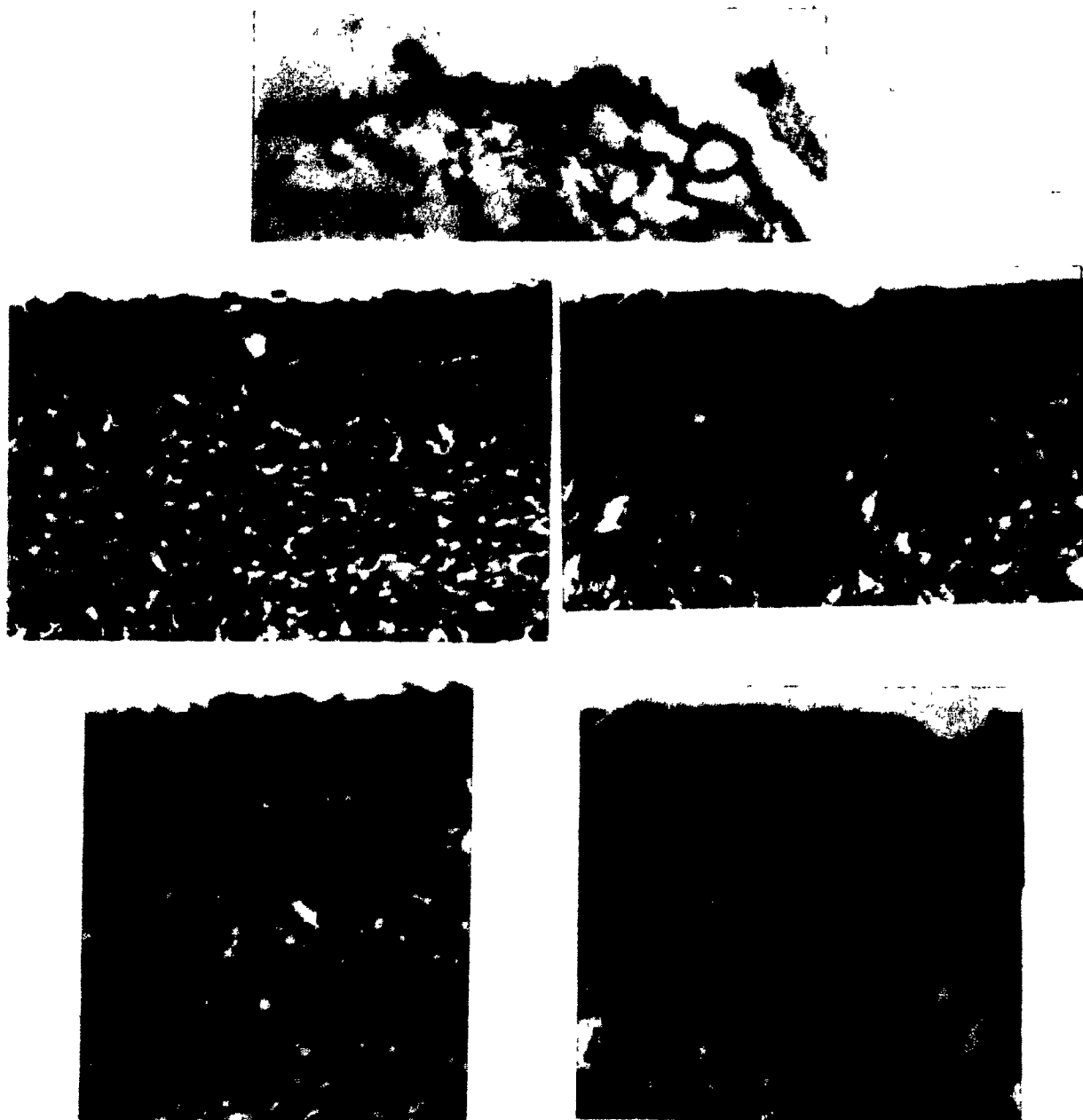
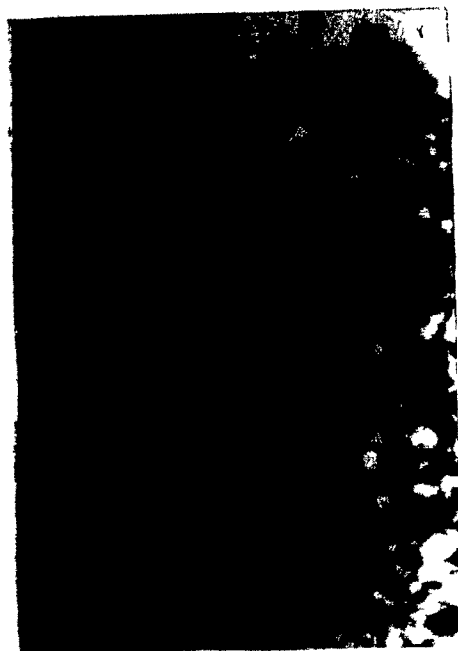


fig 21(a) Radioautograph ^3H thymidine incubated normal rabbit synovium. 3μ H+E.

(b) Synovium antigen induced arthritis control upper left, 3μ H+E and 30 days after $0.1 \text{ mCi } ^{32}\text{P}$ intra-articular injection upper right 3μ H+E. Lower sections, high magnification of control (left) and treated (right). Note hypervascularity and fibrosis in control and treated tissue.



CONTROL



TREATED

fig 21(c) Synovium antigen-induced arthritis control, upper left 3 μ H.E; and 60 days after 0.1 mCi ³²P, intra-articular injection, Upper right lower sections: high magnification of control and treated joints. Note persistence of infiltrates, fibrosis and vessels.



CONTROL



TREATED

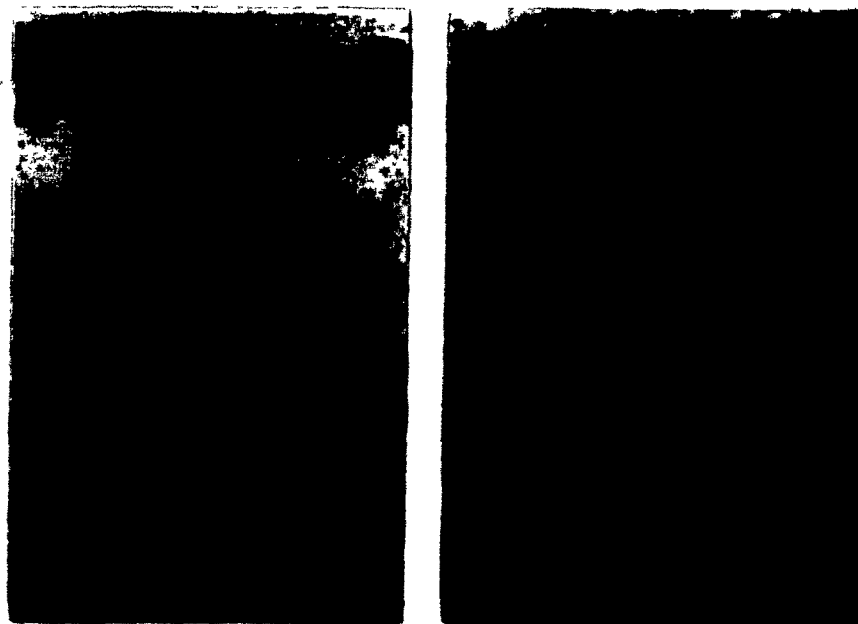
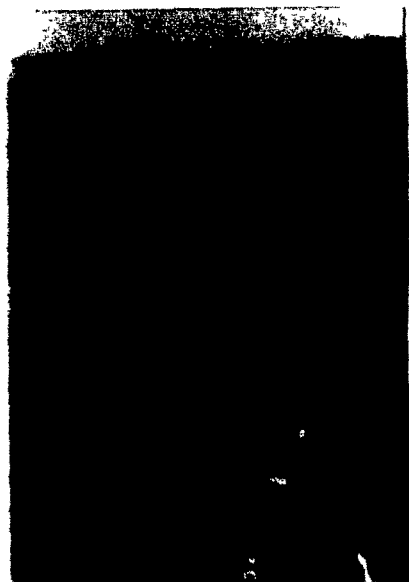
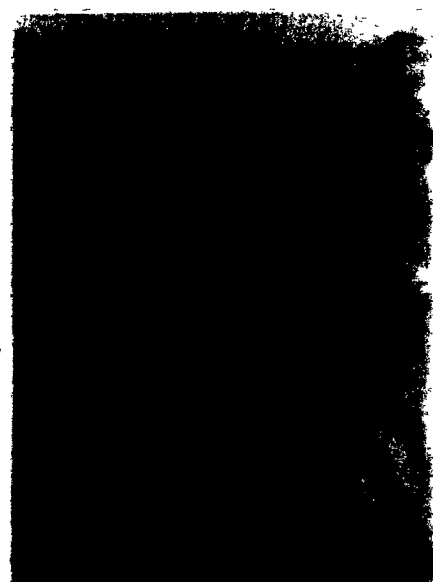


fig 22(a) One micron sections normal adult cartilage. Right: in situ toluidine blue stained. Left: radioautograph, ^3H proline incubated tissue.



CONTROL



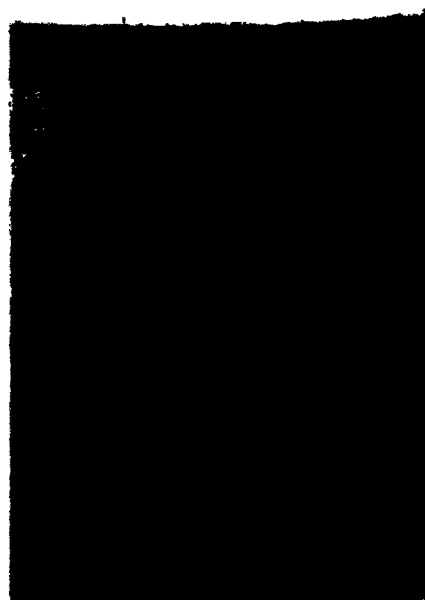
TREATED

fig 22(b) Above: one micron section insitu stained cartilage antigen-induced arthritis control on left, 30 days after 0.1 mCi ^{32}P intra-articular injection. Note fatty accumulations, lacunar spaces, particularly upper zones of both control and treated.

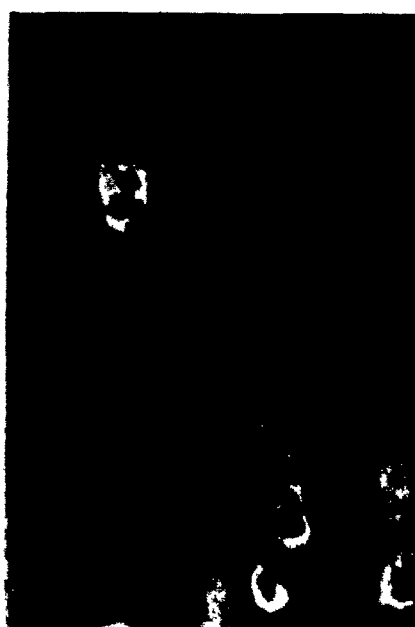
fig 22(c) Below: radioautographs of ^3H proline incubated cartilage. Sporadic decreased uptake noted. Treated joint demonstrated fewer metabolically inactive cells.



CONTROL



TREATED



CONTROL



TREATED

fig 22(d) Radioautographs, antigen-induced arthritis control, and ^{32}P treated at 30 days. High magnification demonstrates similar cell features. Note chondrocytes with uptake; chondrocytes with large fat globules, surrounded by halos.



fig 22(e) One micron insitu stained sections of arthritic cartilage. Left control. Right, 60 days after 0.1 mCi ^{32}P intra-articular injection. Note more loss of superficial cells, and fatty degeneration in control, verses treated. Significant decreased staining is noted about the chondrocytes in both groups.



fig 22(f) Radioautographs of ^3H proline incubated arthritic cartilage. Control on left. On right, 60 days after ^{32}P intra-articular injection. Note surface erosion, progressive chondrocyte degeneration, halos, fatty degeneration, and glycogen accumulations. Treated section changes are less profound.



CONTROL



TREATED

fig 22(g) Radiographs arthritic cartilage control and ^{32}P treated demonstrating similar chondrocyte characteristics. Note, fatty accumulation in cells without uptake and glycogen (clear spaces) of cells with decreased ^3H proline uptake.

peculiar to the upper radial zones (fig. 22c). Here, however, control and treated animals appeared different. Though it could not be quantitated, the cell population of the treated animals seemed healthier. There were fewer degenerating cells. The matrix of the cartilage stained well but appeared to have a coarse fibrillar appearance more so in control animals. Staining was variable; halo-like rings appeared around some cells, other areas were relatively devoid of stain, other areas of stain appeared to highlight the fibrillar nature of the deeper zones of matrix (fig. 22b,c,d). Again though no clear pattern emerged, the signs of injury and cartilage decompensation observed was less severe in treated animals.

At 60 days, these observations persisted and were more obvious; control cells appeared degenerative and frankly necrotic. There was less intense uptake of ^3H -proline, and, at times, large areas were completely deficient of uptake (fig. 22d,e).

TEM studies confirmed impressions made by light microscopy of cellular detail. There were no cell characteristics peculiar to the ^{32}P treated animals. The changes observed in arthritic cartilage were evident: excessive accumulation of lipid, fibrillar material in the chondrocytes, frank necrosis, surface collapse and erosion coarse arrangement of matrix collagen, and a double tidemark (fig 23a-h). These changes were more obvious in the 8 week controls than in the treated animals (fig 23j-o).

Biochemistry:

Hexuronic acid and hydroxyproline analyses measured in micrograms per milligrams dry weight of cartilage as indirect measure of proteoglycan and collagen content respectively, showed a gradual decline in concentration consistent with degenerative arthritis (fig 24a,b). Values varied and no pattern in treated animals was evident different from controls. Specific activities of digest and purified samples were measured but values were too scattered to interpret reliably.



fig 23(a) Transmission electron micrograph. Cartilage with antigen-induced arthritis 4 weeks after 0.1 mCi ^{32}P intra-articular injection. Surface collapse is evident with appearance of transitional cells near the surface.



fig 23(b) Transitional zone chondrocyte with tremendous lipid accumulation (Li) and lysosomes (arrow).



fig 23(c) Radial zone "ageing" cell. Note fibrillar material (F).

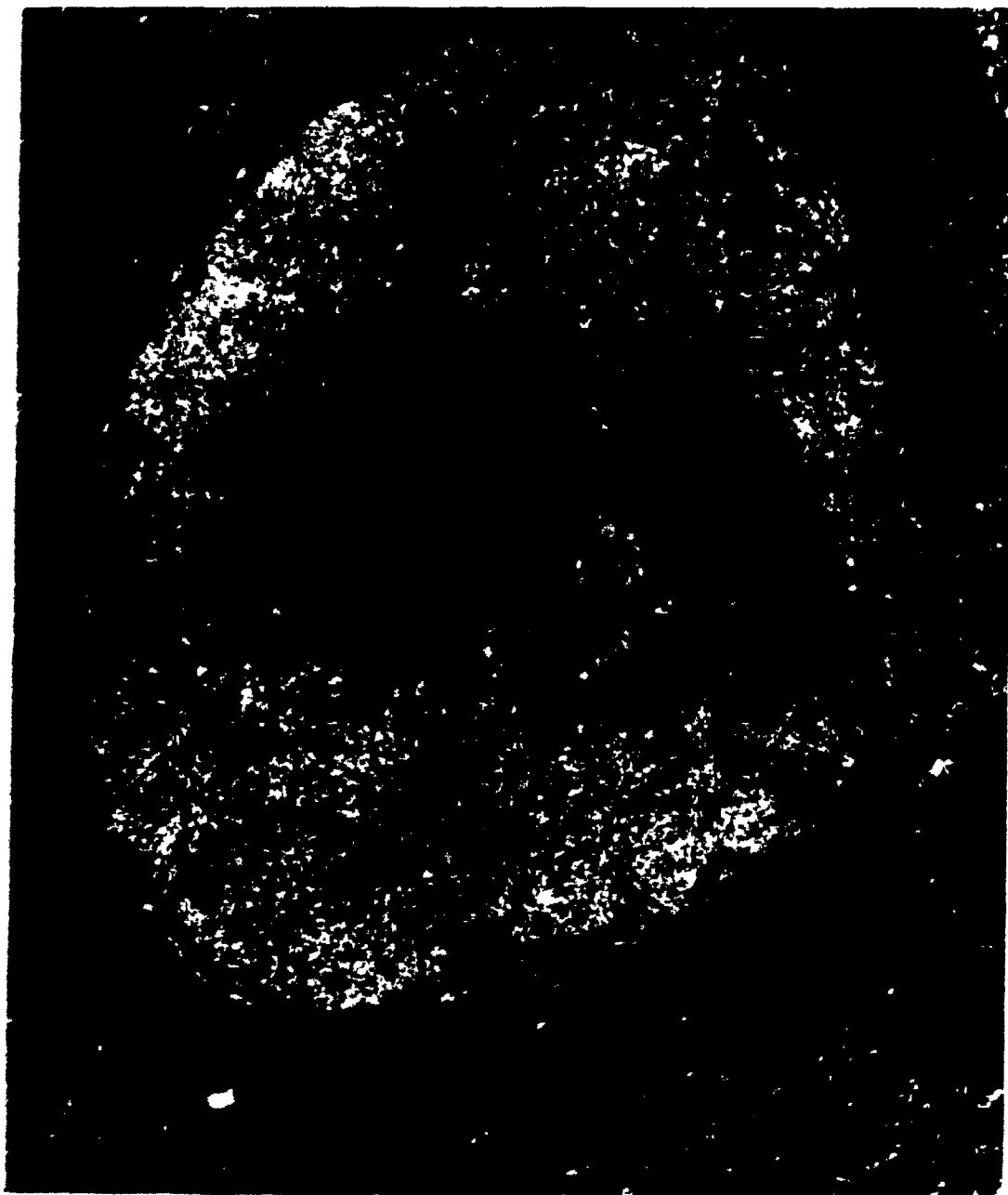


fig 23(d) Another "ageing" cell. (F) Fibrillar material.



fig 23(e) A healthy pair in the radial zone.



fig 23(f) Midzone lipid filled cell and matrix kinking. Upper left, a cell "ghost" (arrow).

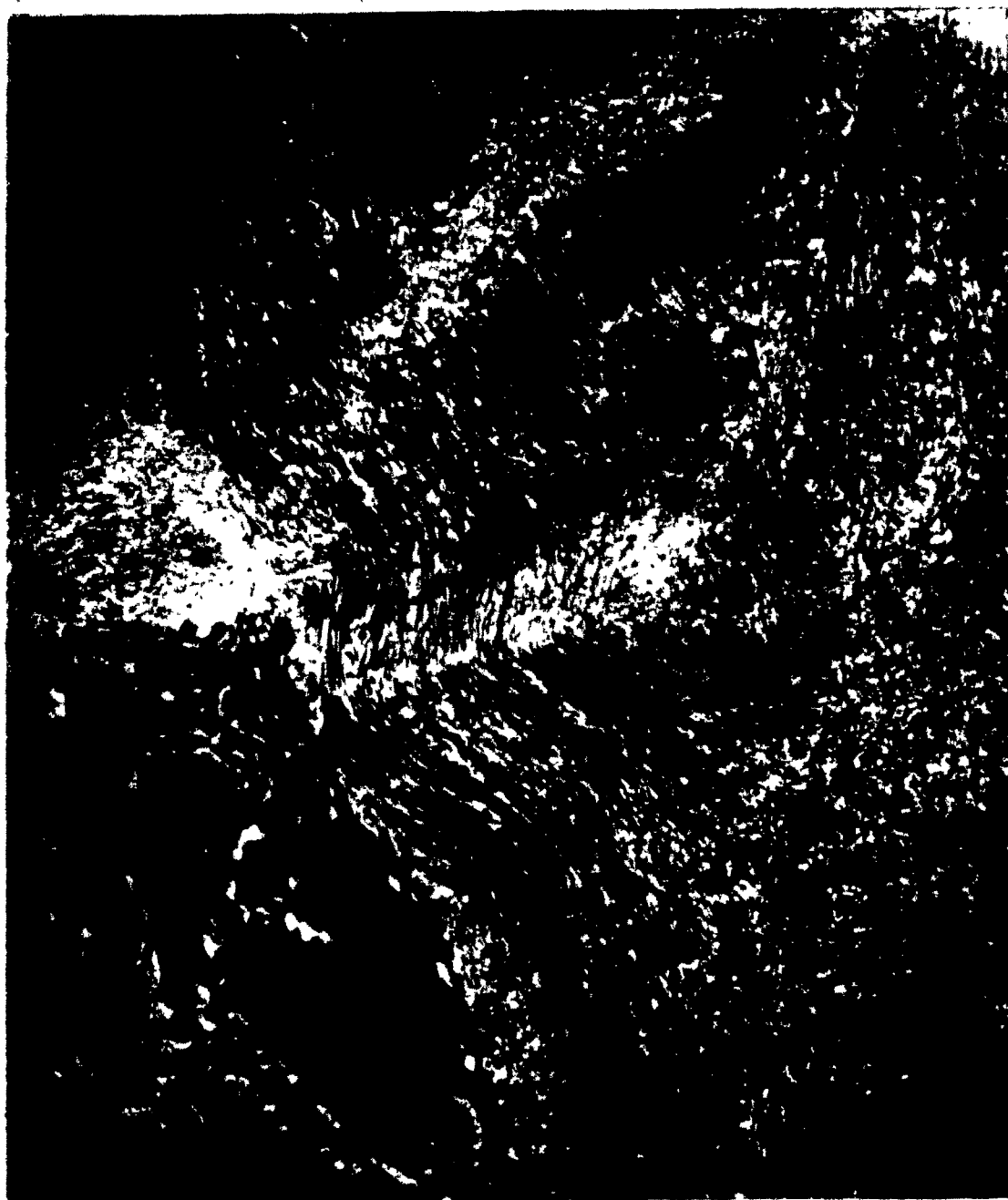


fig 23(g) Midzone degenerate chondrocyte and matrix irregularity.

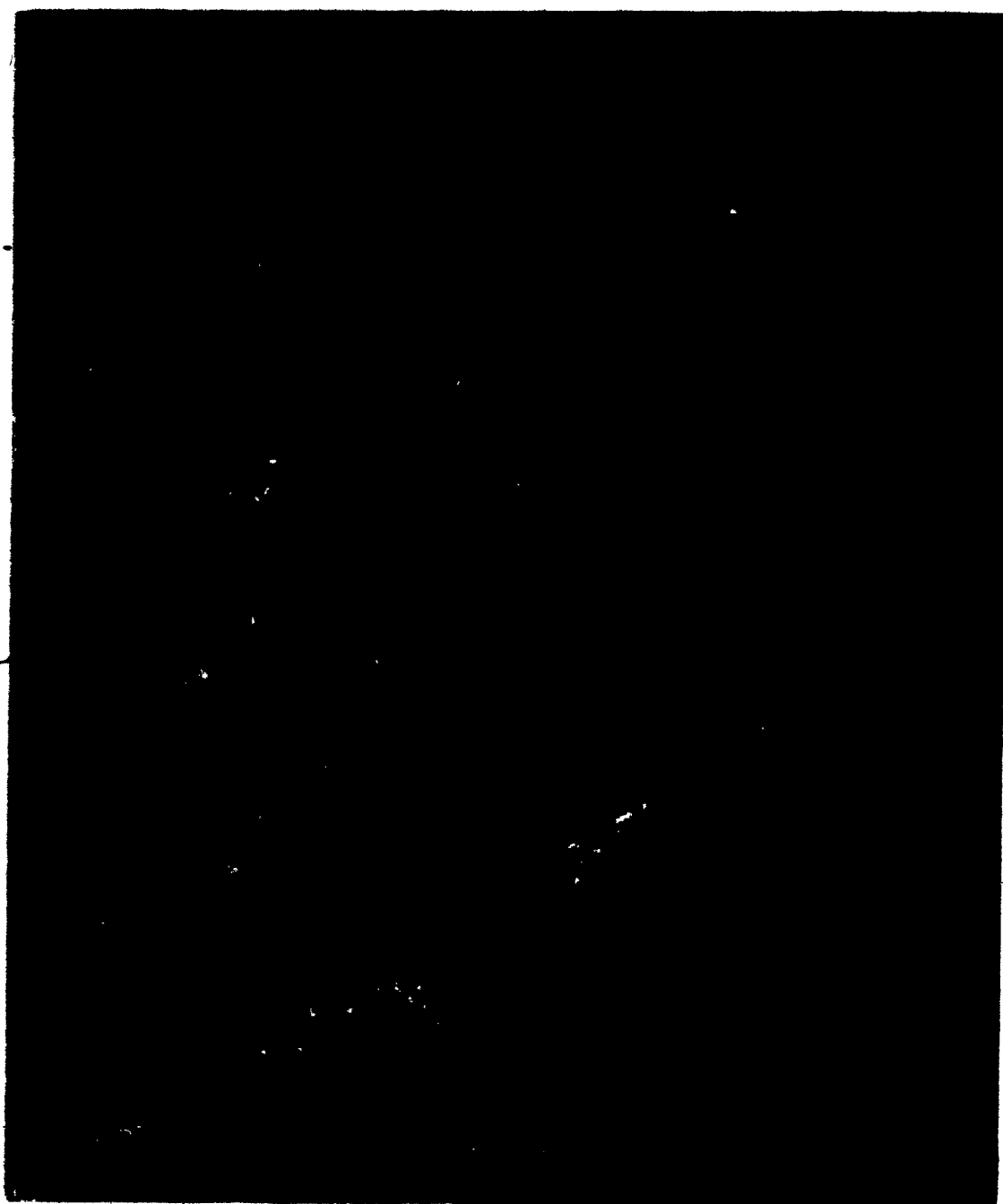


fig 23(h) Matrix streaks (kinking) deep in cartilage.



fig 23(i) Tidemark with coarse "kinked" matrix.



fig 23(j) Cartilage with antigen-induced arthritis, 8 weeks after 0.1 mCi ^{32}P intra-articular injection. Surface cell and matrix changes are more obvious than in the 4 week control group. Changes, however, of these treated animals were comparable with controls. Note surface irregularity and proximity of round cell to the surface.



fig 23(k) Here, the surface matrix is disorganized. The cells necrotic.



fig 23(l) A bizarre chondrocyte in the radial zone : the egg cell. Lipid droplet (L) with surrounding fibrillar material (F). Pericellular area filled with proteoglycan (arrow) and dense spots which are thought to be proteoglycan precipitates or breakdown product.



fig 23(m) A cell pair in the radial zone. Note the "halos" of dense proteoglycan (arrow).



fig 23(n) The tidemark area. Cells are round, ageing and degenerative.



fig 23(o) Atypical degenerating cells in the tidemark area. Note again arrangement of coarse collagen fibrils.

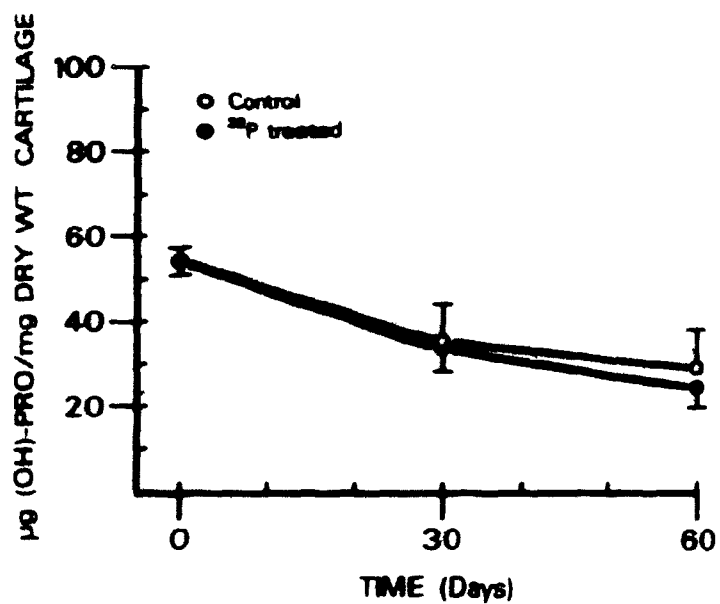


fig 24(a) Hydroxyproline content in µg/mg dry weight of cartilage of antigen induced arthritis, control vs. antigen induced arthritis, treated with 0.1 mCi ³²P.

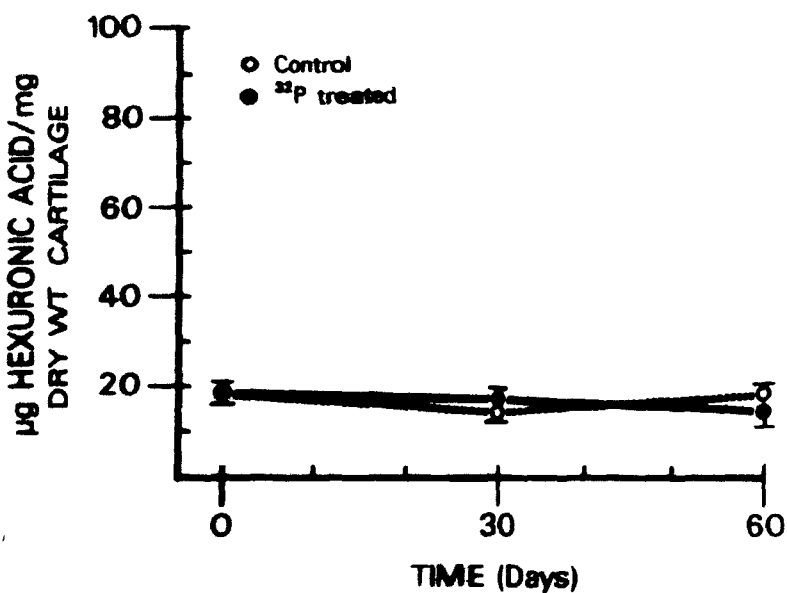


fig 24(b) Hexuronic acid in µg/mg dry weight of cartilage of antigen induced arthritis, control vs. antigen induced arthritis, treated with 0.1 mCi ³²P.

3.3.3 DISCUSSION:

The concept of radiation synovectomy, essentially a sound one, is based on the supposition that one can control or predict the effect and therefore depth of radiation damage to a tissue.

It is moreover, very difficult to predict in vivo what damage radiation will have on a tissue as discussed in chapter 1.3. Physical principles can only be a rough guide. The doses used clinically and experimentally seem to be estimated roughly by different methods and with different results. It has been calculated that the beta dose of Au^{198} is 76 rads/gm/microcurie (67). Ten μCi applied to an adult knee of 300 grams would expose 6300 rads to the synovium (51). Chromic phosphate dose rate is 885 rads/gm/ μCi and thus has a ten fold activity (67). The average dose used clinically is 2-4 μCi in the knee joint (125). A similar therapeutic dose is estimated based on a hollow sphere and on the surface area. The estimate of surface area (S.A.) in an adult knee is 153 cm^2 and for the interphalangeal joint of the hand, 4 cm^2 . The rabbit knee S.A. is approximately the same as the finger joint and therefore is approximately $1/40$ that of a knee. Thus 0.05 to 0.1 μCi should be an appropriate dose to the rabbit knee.

Our studies on synovitic joints treated with ^{32}P did not demonstrate a synovectomy nor reproduce the findings of Pavelka (109) with 0.1 mCi ^{90}Y in arthritic rabbits, of fibrosis, diminished number of cells in the surface, and thrombosis of vessels in synovium. Was the dose too low? We believe not, since evidence of some effect on cartilage was demonstrated. Was the dose so severe that what we observed was a completely regenerated synovium? If so, then the damage could not extend below the surface since the deeper layer did not show the expected radiation effects on vessels and connective tissue. However, it is possible that radiation effect occurred without obvious vascular damage. In a pilot study where 10 times the therapeutic dose (1.0 mCi) was injected into an animal with acute synovitis, 7 and 21 days later, there were dead cells, vacuolated cells, and loss of cell to cell contact on the surface, but no vasculitis.

Vessel thrombosis and vasculitis, did not reliably indicate a state of radiation damage in our studies.

Regardless of whether a synovectomy occurred, however, cartilage degeneration continued on its slow course regardless of the events taking place in the synovium. The difference seemed to be one of degree. Chondrocyte and matrix degeneration was demonstrated in the treated animal and controls but to a lesser degree in the former. It is import to note that one did not see the effects on cartilage as one did with high dose radioactive gold on growing bone in rat (Rubin 126) nor as Umbios (148) described, in cartilage with high dose ^{32}P in rats nor as one did with previous agents used for synovectomy such as osmium (83,92,96).

Thus from our shortterm, but in depth study of ^{32}P in arthritic joints it would appear that treated joints were healthier. The discrepancy between the effect on normal tissue and arthritic tissue may be conjectured. Perhaps the increased thickness of the pathologic synovium acted as a barrier to the penetration of the radiation into the articular cartilage, while normal synovium is too thin to absorb the effects.

Long term studies presently under way should clarify whether this apparent improvement in expected degree of arthritic degeneration is temporary or lasting.

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CHAPTER 4
SUMMARY

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Though the studies reported leave many questions unanswered, they are informative on several aspects.

First the study of ^{32}P on arthritic rabbits in doses comparably to human treatment regimens supports the enthusiastic clinic findings of radiosynoviorthesis. The synovium, if effected appeared to have recovered and the cartilage seen less degenerated than control sites with arthritis. However, the study was relatively short term and may not have reflected what might be occurring in joints a year or more later.

Secondly, the enthusiasm of radiosynovectomy must be sharply tempered by the suggestion that while the same isotope in the same therapeutic dose produced synovial fibrosis, a preferred result, cartilage damage was also clearly evident.

Perhaps then radioisotopes are safe in joints where the synovium is pathologically thickened? This is perhaps so, but then, the margin of safety in using such an agent must be narrow as it harms normal joints but is helpful with pathological joints.

"Despite uncontrolled evidence that radiosynovectomy is a useful procedure for intractable synovitis (mainly of larger joints), both the short and long term benefits in RA and the seronegative arthritides need to be clarified through carefully controlled prospective studies." P. Lee (72)

The implication of this, is that extreme caution should be employed in even considering the use of this therapeutic modality clinically.

The comments of Dr. Peter Lee in his editorial are apropos and it is hoped that this thesis has presented some advance in the pool of knowledge.

Lastly, the light of studies of antigen induced arthritis which showed that by merely varying the challenge dose in a sensitized animal a tremendous difference in the arthritis produced is demonstrated. Caution must be employed in using this model and in the conclusions drawn from it.

APPENDIX I**HISTORICAL NOTES ON INTRACAVITARY USE OF RADIOISOTOPES**

The idea of placing solutions of radioisotopes on cavities was not new even in 1962 when Ansell reported her work. Müller in Switzerland is credited with first treating uncontrollable effusions (in this case neoplastic) with intracavitary injections of radioactive gold ^{198}Au , the only isotope likely available for therapeutic purposes at that time (4). Through the 1950's several groups used ^{198}Au but soon switched to ^{32}P chromic phosphate to treat malignant effusions, as the availability, ease of handling, ease of disposal, and wider margin of safety, and reduction in cost became evident (29,66,151,107).

Jaffe, who began his work with ^{198}Au around the same time as Müller and then switched to chromic phosphate in 1951, seems, to be the first, to have used it clinically (67). Root examined chromic phosphate as an alternative to gold for intra-cavitary use, but noted that although distribution was on the surface of the cavity, significant ionization of the phosphate occurs and was detected in the red blood cells. Also, he found significant uptake by the local lymph nodes (122).

Others followed in Australia, and the U.S. in support of this isotope, and in working out methods of preparation to obviate the problems of the colloidal suspension -clumping, small particle size, large particle size and uneven distribution (29,66,151,107).

APPENDIX II

Isotope	Half-life (T1/2 days)	MeV (Energy of radiation particles)	E _b	Emittor	Particle size (μ)	Max. range in soft tissue(mm)	Max. range in cartilage
¹⁹⁸ Au(salt)	2.7	.96	.312	beta&gamma**	.02-.07	3.6	2.7
³² chromic phosphate (³² P)α α	14.3	1.70	.695	beta+	0.2-2.0α α α	7.9	?
⁹⁰ Y silicate	2.7	2.27	.917	beta+	.03-.06	11.0	8.5
⁹⁰ Y resin colloid	2.7	2.27	.917	beta+	.03-.05	11.0	8.5
¹⁶⁹ Erb	9.5	.34	.100	beta	.01-.03	0.7	0.7
¹⁸⁶ Re	3.7	.98	.333	beta&gamma**	.005-.01	3.6	3.1
¹⁶⁵ Dys	.097 (140 min)	1.30	.433	beta&gamma	0.5-5.0	5.7	?

*ref 84

**Significant gamma radiation

***Very low emission of gamma radiation

+Because of relative high energy, it has significant Bremsstrahlung when it is exposed to a high density material eg. aluminum, lead etc. In handling, it must be shielded with only low density material eg. plastic syringes to prevent production of Bremsstrahlung.

α α Prepared as ³²Cr³PO₄ solubilized in 30% dextrose and water with NaOH and HCl and 2% benzyl alcohol added (164). Cr³PO₄ molecules form macromolecules and remain as a colloid solution.

α α α Old preparations of CrPO₄ had particle sizes of 4 μ (range 0.5-10μ)(66,67)

New preparations of CrPO₄ colloid have a particle size of 2 μ with most larger than 0.5 μ (162,164).

Appendix III

LEAKAGE OF INTRA-ARTICULAR RADIOISOTOPES

There is great difficulty in assessing risk in the body because dose to total body, to lymph nodes, to bone and to joint after injection are all different; besides the amount of leakage being highly variable, there is no experience in the radiation effects of low parental doses of isotopes in a large group of patients. Most figures rest on X-ray beam therapy and the nuclear holocaust of Hiroshima. However the recommendation of the British Medical Association, Symposium of Radioactive colloids in the Treatment of Arthritis, convened in 1973 at Northwick Park Hospital, Harrow, England to study the problem stated:

"It may be argued that the treatment should normally be reserved for older patients unless no alternative is available. The leakage of radioactivity from the knee should be avoided or kept down to 2 to 3% of the injected amount, as the radiation dose to the lymphocytes and lymph nodes from larger leakages may have a biological significance". (31).

Moreover, most of the estimates of exposure to extraarticular organs are made by scanning the knee joint, serial counting the Bremsstrahlung emitted and extrapolating this value to determine the amount remaining in the knee. Other organs are scanned externally, for example over the groin lymph nodes, and the proportion of counts obtained over the knee joint shortly after injection, is taken as the percentage escaped. The actual amount found in the lymph nodes is, perhaps more germane, since the amount which has escaped may actually be mostly that which has entered the circulation and been cleared through the kidney very quickly - thus of relatively low risk.

Most studies have been with $^{90}\text{Yttrium}$ and estimates of leakage into lymph nodes have varied widely. Even when optimal immobilization and the addition of corticosteroids have been employed, leakage for $^{90}\text{Yttrium}$ from 0 to 45% has been

reported (121,114,103,48,23,24,115,102). Ferric hydroxide colloid, resin colloid, silicate and citrate particles of Yttrium have been prepared and used. The resin colloid and silicate preparations have had the best results but even here there are conflicting results and ranged from 10-50% (121,34,52,115,114,159). Gold leaks to a similar degree but the radiation to the gonads is far greater due to the gamma radiation it carries (50). A single report on eight patients of ^{32}P phosphates showed negligible leakage in all but one patient who had about 20% leakage to the lymph nodes. This would give him an exposure of about 12000 rads to 10 gms of nodes. Plasma and urine but not blood cells or bone were analyzed for ^{32}P (161). What is different about ^{32}P , however is its affinity to bone. U.S.N.R.C.'s maximum acceptable total bone burden is 6 μCi . One might ask what the total bone uptake with this 20% leakage is? This has not been calculated by any of the papers reviewed. In an old report (122) of intracavitary instillation for ^{32}P of treatment of malignant effusions the leakage into other organs was very small but interestingly, red blood cells were analyzed and ^{32}P was found in the cytoplasm suggesting that the phosphorus separated from the colloid. Thus besides the leakage problem of the radioactive macromolecules, some isotopes may separate from the macromolecule and may be absorbed selectively or non-selectively in indeterminate amounts by organs such as bone.

Appendix IV

RADIATION AND NUCLEAR PHYSICS

An atom, our elementary particle, consists of a nucleus surrounded by negatively charged electrons spinning in orbits about it. The nucleus has an uncharged particle (neutron) and may have one or more positively charged particles (as well protons). The atomic number relates to the number of protons in an atom eg. 6C. An isotope (nuclide) of an altered element with the same atomic number but a different atomic mass due to the number of neutrons (which have mass but no charge) in the nucleus. For whatever reason, if a proton neutron combination is unstable, the nucleus will adjust itself to achieve a more compatible combination by ejecting one or more of its charged particles (thereby changing its atomic number) which have energy therefore which can penetrate solid material. This is radioactivity. The process of achieving this stable state is called "decay" and actually is a measure of the number of disintegrations of nuclei and those remaining and indirectly gives a measure of the intensity of the emitting radiation still present. The rate of decay is inalterable and an integral property of the particle. There are as many different kinds of radiation as there are different kinds of charged particles capable of being emitted from the element. Alpha (α) radiation refers to that due to a positively double charged particle with a mass of 4 (helium). These particles are 7400 times larger than electrons and when emitted can travel only very short distances.

Beta (β) radiation of particular interest to us, refers to that due to electrons. A neutron in the nucleus can make the element unstable and so it changes into a proton and an electron pair and then emits the energetic electron with a definite energy and therefore a finite travelling range (this kind of radiation is easy to shield from). What complicates quantitations is that a beta radioactive substance may also emit gamma

energy as well.

Gamma radiation (γ) is due to the emission of mass-less energy called electromagnetic energy. This is due to the movement of electrons from one orbit to another in the shells. If the movements or changes produce an excess of energy for the needs of the electron spinning about, it will emit it as high energy electromagnetic energy. X-rays are identical to gamma rays.

Another type of radiation related to the beta radiation is *bremstrahlung*. This is electromagnetic radiation identical with X-rays or gamma rays produced when a stream of beta particles strikes a target. The yield is directly proportional to the energy of the particle and the density (atomic number) of the target material.

What then, do these energetic particles and rays do? The basic action is penetration of mass, removing electrons from the mass and in the course, using up the travelling particles' own energy allowing themselves to come to rest. The mass which has lost some electrons is then, "ionized". The word "ionization density" refers to the number of ionized atoms produced per unit length of the path of the particle. That is, how much effect it will have as it makes its way through a mass (for our purposes tissue). The greater the velocity of the particle the less the ionization density (it covers more territory and expends less energy in ionizing/distance travelled). The greater the charge on the particle the greater is the ionization density (it carries more energy in a given distance travelled). If the energy is not transferred as ionization, it may, instead, excite a molecule and then dissipate its energy as heat.

The quantity of ionizations that have occurred in a tissue is the roentgen and this term has equivalents in coulombs/kg. The quantitation of an amount of material containing radioisotope and detected by the number of decaying particles per second (or disintegrations per second (d.p.s.) or converted to disintegration per minute (d.p.m.'s.) has an equivalent in becquerels (B) and using an older term in Curies (Ci). The

measurement of the amount of radiation in terms of pure energy emitted is spoken of in terms of MEV. That is, for example, when a beta emitter emits a positron it is slowed down by other atoms, finally uniting with an electron with the emission of quanta of energy. The measure of the amount of energy of radiation that can be or is being transferred to the tissue to produce potential energy for work is the rad. A rad will produce 100 ergs of energy/gram.

One roentgen of gamma radiation or X-ray, for example, can produce approximately 93 ergs or about one rad. Rads have equivalents in Grays (gy), centigrays (dGy) and Joules/kg.

Three more terms should be mentioned: that of the RBE - "the degree to which any particular type of irradiation differs from standard X-ray of given energy" and therefore varies with the type of radiation and the tissue exposed. The REM is the dose in rads multiplied by RBE. The average person gets about 119 Mrem/year about 100 of it from the environment and about 19 from exposure to X-rays etc..

The Curie expresses the actual activity of a mass in terms of its decaying atoms. That is, it is equivalent to 3.7×10^{10} disintegrations/sec. The bequerel is the new term for disintegrations/sec.

How then does one determine how much energy of radiation is absorbed by a tissue? If the distribution is uniform throughout a tissue, rads = a constant X the energy of emission and recombination of a particular kind of particle (MEV) X its half-life X the number of disintegrations (C) divided by the weight of the tissue.

$$\text{Thus: Rads} = 73.8 \times \bar{E} \times T_{1/2} \times \frac{\mu\text{Ci}}{\text{gm}}$$

However if we are dealing with a hollow viscus or a flat surface, then the hypothetical isotope will be distributed on the surface and produce limited effects on the underlying tissue. So, half will project their particles away from the tissue and half will project

their particles into the given tissue. These particles will project themselves only a known range h (cm), over a given surface area SA (sq. cm.). So, the mass of tissue is given Ah (cm^3) - a volume, and the time element of this is represented by the half-life, so that:

$$\text{rads} = \frac{2.13}{2} \times \frac{\mu\text{Ci} \times \text{MEV} \times T_{1/2}}{S.A. \times h (\text{cm}^3)}$$

This presumes a perfect measurable hollow viscus but, in vivo the volume of cavities are often difficult to measure or even predict.

BIBLIOGRAPHY

1. Amini D, Daziani L, Gagnon J, Laurin CA: The use of intra-articular osmic acid to produce chemical synovectomy in rabbits. Clin Orth Res 79:164-172, 1971
2. Anderson RE: Radiation Injury. In Pathology ed. Anderson WAD and Kissani JM 7th ed. Mosby, St. Louis 1977, p. 326-368.
3. Andreis M, Stastny P, Ziff M: Experimental arthritis produced by injection of mediators of delayed hypersensitivity. Arthritis Rheum 17:537-551 1974
4. Andrews GA, Root SW, Kerman HD, Bigelow RR: Intracavitary colloidal radiogold in the treatment of effusion caused by malignant neoplasms. Ann Surg 137:375-381, 1953
5. Ansell BM, Crook P, Mallard JR, Bywaters EGL: Au 198 in the treatment of persistent knee effusions. Ann Rheum Dis 21: 218-219, 1962
6. Ansell BM, Crook A, Mallard JR, Bywaters EGL: Evaluation of intra-articular colloidal gold Au¹⁹⁸ in the treatment of persistent knee effusions. Ann Rheum Dis 22:435-439, 1963
7. Ansell BM: Characteristics of radio-isotopes and considerations involved in their use. Early studies of 198Au in the treatment of synovitis of the knee. Ann Rheum Dis 32 suppl 6:1-2, 1973
8. Barland P, Novikoff AB, Hamerman D: Electron microscopy of the human synovial membrane. J Cell Bio 14:207-220, 1962
9. Bitter T, Muir HM: A modified uronic acid carbozole reaction. Ann Biochem 4:330-334, 1962
10. Bridgman JF, Bruckner F, Eisen V, Tucker A, Bleehen NM: Irradiation of the synovium in the treatment of rheumatoid arthritis. Quart J Med 42:357-367, 1973
11. Caro LG, Tubergen RP: High resolution autoradiography. I Methods Cell Biol 15:173-188, 1962

12. Caruso I, Montrone F, Fumagalli M, Vernazza M, Santandrea S: Rheumatoid knee synovitis successfully treated with intra-articular Rifamian S.V. *Arthritis Rheum* 23(4): 518-519, 1980
13. Cayla J: L'Au colloidal¹⁹⁸ dans le traitement des hydarthroses au cours de la polyarthrite rhumatoïde. *Rev Rheum* 34:438-441, 1967
14. Consden R, Doble A, Glynn LE, Nind AP: Production of a chronic arthritis with ovalbumin. Its retention in the rabbit knee joint. *Ann Rheum Dis* 30:307-315, 1971
15. Coombs RRA, Poole AR: Rheumatoid-like joint lesions in rabbits injected intravenously with foreign serum: A histopathological and immunopathological study. *Rheumatoid Arthritis* Gordon JL and Hazelman BL eds. Amsterdam, Elsevier/North-Holland Biomedical Press, 1977, p 181-192
16. Coulton LA, Henderson B, Bitensky L, Chayer J: DNA synthesis in human rheumatoid and non rheumatoid synovial lining. *Ann Rheum Dis* 39:241-247, 1980
17. Cremer MA, Stuart JM, Townes AS, Kang AH: Collagen-induced polyarthritis in rats: a study in native type II collagen for adjuvant activity. *J Immunology* 124:2912-2918, 1980
18. Cruess RL, Hong KC: Effect of cortisone or collagenolytic activity in the rat. *Proc Soc Exp Biol Med* 148: 887-890, 1975
19. Cruess RL, Rennie W: Adult Orthopaedic Surgery. Churchill Livingstone, N.Y. in press
20. Cruess RL: The musculoskeletal system. Embryology, Biochemistry and physiology. Churchill Livingstone, N.Y. 1982
21. Curran RC, Harnden DG: Tissue response to ionizing radiation. In *Pathological Basis of Medicine*. WB Sanders Co. p. 323, 1972
22. Davis P, Jayson MIV: Acute knee joint rupture after Yttrium⁹⁰. *Ann Rheum Dis* 34:62-63, 1975
23. De la Chapelle A, Oka M, Rekonen A, Ruotsi A: Chromosome damage after intra-

- articular injections of radioactive Yttrium. Effect of immobilization on biological dose. *Ann Rheum Dis* 31:508-512, 1972
24. Deckart H, Tamaschke HJ, Ertl S, Tautz M, Knop G: Radiosynovectomy of the knee joint with ^{198}Au -colloid, ^{90}Y -ferric hydrate colloid and ^{186}Re -sulphide colloid. *Radiobiologia Radiotherapia* 3:363-370, 1979
 25. Delbarre F: La synoviorthese. *Rev Rheum Malosteoartic* 35:1, 1968
 26. Delbarre F, Roucayrol JC, Ingrand J, Cayla J, Aignan M: Interest of radioisotope synoviorthesis in the treatment of inflammatory rheumatism. In: *Proc Symp on Early Synovectomy in Rheumatoid Arthritis*. ed. Hymans W and Paul WD. Excerpta Medica Foundation 1969, p. 194-200
 27. Delbarre F, Menkes JC: Non-surgical synovectomy in rheumatoid arthritis. Results obtained by radio-synoviorthesis. *Adv Clin Pharm* 6:134-139, 1974
 28. Del Maestro RF: An approach to free radicals in medicine and biology. *Acta Physiol Scand Suppl* 492:153-168, 1980
 29. Doehner GA: New scintigraphic technique for demonstration of beta emitters following intracavitary instillation. *Radiology* 78:100-102, 1962
 30. Doherty M, Dieppe PA: Effect of Intra-articular Yttrium 90 on chronic pyrophosphate arthropathy of the knee. *Lancet* 8258:1243-1246, 1981
 31. Dolphin GW: Biological hazards of radiation. *Ann Rheum Dis* 32 suppl 6:23-28 1973
 32. Downie WW, Freeman PA, Dick WC, Deodhar SD, Nuki G, Whaley R, Buchanan WW: Synovectomy of the knee in rheumatoid arthritis: critical appraisal based on a long-term follow-up of 145 patients. *Acta Medica Polona* 14:233-252, 1973
 33. Dumonde DC, Glynn LE: The production of arthritis in rabbits by an immunological reaction to fibrin. *Br J Exp Path* 43:373-382, 1961
 34. Dunscombe PB, Battacharyya AK, Dale RG: The assessment of the body distribution of Yttrium -90 ferric hydroxide during radiation synovectomy. *Brit J Radiol* 49:372-

373, 1976

35. Editorial. Medical Synovectomy. Brit Med J 2: 682, 1974
36. Erwin BG, Stoschecki C, Florini JR: A rapid fluorometric method for the estimation of DNA in cultured cells. Anal Biochem 110:291-294, 1981
37. Fearn CBdA: Radioactive gold in the treatment of rheumatoid arthritis. Rheum Rehab 1979 suppl: 36-37
38. Fehr K, Velvart M, Boni A, Watanabe A, Spycher MA, Rüttner JR: Experimental arthritis of rabbits caused by intra-articular injection of autologous Fab2 produced by digestion of IgG with cathepsin D. I. Microscopical and immunohistochemical findings in short-term experiments. Ann Rheum Dis 35:85-96, 1976
39. Fine PH, Farrer PA, Albrecht M, Jacox RF: Intra-articular radioactive gold (^{198}Au) in the treatment of rheumatoid synovitis of the knee. Arthritis Rheum 10:278, 1967
40. Fox A, Glynn LE: Persistence of antigen in non arthritic joints. Ann Rheum Dis 34:431-437, 1975
41. Fox A, Glynn LE: Is persisting antigen responsible for the chronicity of experimental allergic arthritis? Ann Rheum Dis 36:34-38, 1977
42. Geens S: Synovectomy and debridement of the knee in rheumatoid arthritis. Part I. Historical review. Part II. Clinical and roentgenographic study of thirty one cases. J.B.J.S. 51-A:617-625, 626-643, 1969
43. Gertzbein SD, Goldfarb P, Cruikshank B, deDemeter D, Argue S, Kapasouri A: Altered lymphocyte response in adjuvant-induced arthritis. J Rheumatol 6: 629-635, 1979
44. Goldberg VM, Lance EM, Davis P: Experimental immune synovitis in the rabbit. Relative roles of cell mediated and humoral immunity. Arthritis Rheum 17:993-1005, 1974
45. Goldie I, Schlossman D: Radiologic changes in rheumatoid knee - joints before and

- after synovectomy. Clin Orthop 64:98-106, 1969
46. Goldie I: Pathomorphologic features in original and regenerated tissues after synovectomy in rheumatoid arthritis. Clin Orth Rel Res 77:295-304, 1971
 47. Goldlust MB, Rich LC, Brown WR: Immune synovitis in rabbits. Effects of differing schedules for intra-articular challenge with antigen. Am J Pathol 91: 329-344, 1978
 48. Goode JD, Howey S: Effect of premedication with intra-articular hydrocortisone on the retention of ^{198}Au in the knee joint in rheumatoid arthritis. Use of $^{99\text{m}}\text{Tc}$ in assessment. Ann Rheum Dis 32 suppl 6: 43-45, 1973
 49. Graham J, Checketts RG: Synovectomy of the knee joint in rheumatoid arthritis. A long term follow up. JBJS 55B:786-795, 1973
 50. Gumpel JM, Williams ED, Glass HI: Use of Yttrium 90 in persistent synovitis of the knee. I Retention in the knee and spread in the body after injection. Ann Rheum Dis 32: 223-227, 1973
 51. Gumpel JM: Discussion. Symposium on radioactive colloids in the treatment of arthritis. Ann Rheum Dis 32(6) Suppl 140-146, 1973
 52. Gumpel JM, Farran HEA, Williams ED: Use of Yttrium 90 in persistent synovitis of the knee. II Direct comparison of Yttrium colloid resin and Yttrium citrate. Ann Rheum Dis 33:126-128, 1974
 53. Gumpel JM, Beer TC, Crawley JCW, Farran HEA: Yttrium 90 in persistent synovitis of the knee - single center comparison. The retention and extra-articular spread of the four ^{90}Y radiocolloids. Brit J Radiol 48: 377-381, 1975
 54. Gumpel JM, Roles NC: A controlled trial of intra-articular radiocolloids versus surgical synovectomy in persistent synovitis. Lancet March 1: 488-489, 1975
 55. Gumpel JM, Matthews SA, Fisher M: Synoviorthesis with Erbium -169: a double-blind controlled comparison of Erbium -169 with corticosteroid. Ann Rheum Dis 38:341-343, 1979

56. Gynning I, Odelberg-Johnson O, Waldeskog B, Wendeberg B: Intra-articular treatment with colloidal ^{198}Au of persistent synovial effusion in the knee. *Acta Radiologica (Stockh)* 3: 409-417, 1965
57. Hasselbacher P, Nacht JL, Labosky DA, Steinberg ME: Antigen-induced arthritis: An immunohistologic study of articular tissue and synovial fluid using the horseradish peroxidase technique. *J Rheumatology* 7:596-608, 1980
58. Henderson B, Glynn LE: Metabolic alteration in the synoviocytes in chronically inflamed knee joints in immune arthritis in the rabbit: comparison with rheumatoid arthritis. *Br J Exp Path* 62: 27-33, 1981
59. Henderson B, Glynn LE, Bitensky L, Chayer J: Evidence for cell division in synoviocytes in acutely inflamed rabbit joints. *Annals Rheum Dis* 40:177-181, 1981
60. Hirohata K: Cartilage physiology and breakdown: Ultrastructure pathology. Presented at workshop. 16th Annual Meeting, Canadian Orthopaedic Research Society, Kingston Canada, 1982
61. Hnatowich DJ, Kramer RI, Sledge CB, Noble J, Shortkroff S: Dysprosium-165 ferric hydroxide macroaggregate for radiation synovectomy. *J Nucl Med* 19:303-308, 1978
62. Howson MP, Shepard NL, Mitchell NS: Acute cartilage necrosis in an antigen induced model of rheumatoid arthritis in the rabbit. Light microscopy and T.E.M. studies. In press
63. Hunneyball IM, Stanworth DR: The production of bilaterally symmetrical arthritis in rabbits. *Surgery and Transplantation* 7:515, 1979
64. Ingrand J: Symposium on radioactive colloids in the treatment of arthritis. Characteristics of radioisotopes for intra-articular therapy. *Arthritis Rheum* 32 suppl: 3-9, 1973
65. Isomäki AM, Inoue H, Oka M: Uptake of ^{90}Y resin colloid by synovial fluid cells and synovial membrane in rheumatoid arthritis. A radioautographic and electron

- microscopic study. *Scand J Rheum* 1:53-60, 1972
66. Jacobs ML: Radioactive colloidal chromic phosphate to control pleural effusions and ascities. *JAMA* 166:597-599, 1958
 67. Jaffe HL: Treatment of malignant serous effusions with radioactive colloidal chromic phosphate. *Am J Roentgenol* 74:657-666, 1955
 68. Jalava S: Irradiation synovectomy: clinical study of 67 knee effusions intra-articularly irradiated with ^{90}Y resin. *Current Therap Res* 15:395-401, 1973
 69. Johnson PM, Christian CC: Colloidal chromic phosphate P-32 for treatment of chronic synovial effusions. *J Nucl Med* 8:274, 1967
 70. Key JA: The reformation of synovial membrane in the knees of rabbits after synovectomy. *JBJS* 7:793-813, 1925
 71. Laurin CA, Desmarchais J, Daziano L, Garipey R, Derome A: Long-term results of synovectomy of the knee in rheumatoid patients. *JBJS* 56A:521-531, 1974
 72. Lee P: Editorial. The efficacy and safety of radiosynovectomy. *J Rheumatology* 9:165-168, 1982
 73. Lillie RD, Fullmer HM: Histopathologic technique and practical histochemistry. McGraw Hill book Co, New York 4th ed, 1977
 74. Lowther DA, Gillard GC: Carrageenin-induced arthritis. The effect of intraarticular carrageenin on the chemical composition of articular cartilage. *Arthritis Rheum* 19:769-776, 1976
 75. Lowther DA, Sandy JD, Santer VB, Brown HLG: Antigen-induced arthritis. Decreased proteoglycan content and inhibition of proteoglycan synthesis in articular cartilage. *Arthritis Rheum* 21:675-680, 1978
 76. Makin M, Robin GC: Chronic synovial effusions treated with intra-articular radioactive gold. *JAMA* 188:107-110, 1964
 77. Makin M, Robin GC: Intra-articular radioactive gold in the treatment of chronic

- synovial effusions. Proc Br Orth Ass JBJS :582, 1966
78. Makin M, Robin GC: Radioactive colloidal gold in the treatment of chronic synovial effusions. Proc Roy Soc Med 61: 908-910, 1968
 79. Manicourt D, Rao VH, Orloff S: Serum and synovial fluid hydroxyproline fractions in microcrystalline arthritis and osteoarthritis. Scand J Rheum 8:193-198, 1979
 80. Marmor L: Surgery of the rheumatoid knee: Synovectomy and debridement. JBJS 55A:535-544, 1973
 81. Martio J, Isomaki H, Heikkola T, Laine V: The effect of intra-articular osmic acid in juvenile rheumatoid arthritis. Scan J Rheumatology 1:5-8, 1972
 82. Meier-Ruge W, Muller W, Pavelka K: Effect of Yttrium 90 on experimental allergic arthritis in rabbits. Ann Rheum Dis 35:60-66, 1976
 83. Menkes CJ, Piatier-Piketty D, Zucman J, Delbarre F: Effects des injections articulaires d'acide osmique chez le lapin. Repercussion sur la croissance osseuse. Rev Rheum 39:513-521, 1972
 84. Menkes CJ, Tubiana R, Galmiche B, Delbarre F: Intraarticular injection of radioisotope beta emitters, application to the treatment of the rheumatoid hand. Orth Clin N A 4:1113-1125, 1973
 85. Menkes CJ, LeGo A, Verrier P, Aignan M, Delbarre F: Double-blind study of Erbium 169 injection (synoviorthesis) in rheumatoid digital joints. Ann Rheum treated with radioactive Yttrium. Scand J Rheumatol 3:33-38, 1974
 86. Menkes CJ: Is there a place for chemical and radiation synovectomy in rheumatic diseases. Rhuem and Rehab 18: 65-77, 1979
 87. Mitchell NS, Cruess RL: Synovial regeneration after synovectomy. Can J Surg 10:234-239, 1967
 88. Mitchell NS, Cruess RL: The effect of synovectomy on articular cartilage. JBJS 49A:1099-1109, 1967

89. Mitchell N, Blackwell P: The electron microscopy of regeneration synovium after subtotal synovectomy in rabbits. *JBJS* 50-A:675-686, 1968
90. Mitchell N, Shepard N: The ultrastructure of articular cartilage in rheumatoid arthritis. A preliminary report. *JBJS* 52-A:1405-1423, 1970
91. Mitchell N, Shepard N: The effect of synovectomy on synovium and cartilage in early rheumatoid arthritis. *Clin Orthop* 89:178-196, 1972
92. Mitchell N, Laurin C, Shepard N: The effect of osmium tetroxide and nitrogen mustard on normal articular cartilage. *JBJS* 55B:814-821, 1973
93. Mitchell N, Shepard N: Changes in proteoglycan and collagen in cartilage in rheumatoid arthritis. A study by light and electron microscopy. *JBJS* 60-A:349-354, 1978
94. Mitnick H, Hoffstein S, Weissmann G: Fate of antigen after intravenous and intra-articular injection into rabbits. *Arthritis Rheum* 21:918-929, 1978
95. Morgan K, Clague RB, Shaw MJ, Holt P: Native type II collagen-induced arthritis in the rat. I Incidence and humeral response to collagen. *Annals of Rheum Dis* 39:285-290, 1980
96. Mötönen M, Nevalainen T, Nieminen L: The effect of osmium tetroxide on synovitis in rabbits induced by immunization. *Scand J Rheum* 1:41-45, 1972
97. Mow V: Cartilage physiology and breakdown: Bioengineering studies. Presented at workshop, 16th annual Meeting, Canadian Orthopaedic Research Society, Kingston Canada, 1982
98. Muirden KD, Mills KW: Do lymphocytes protect the rheumatoid joint? *Brit Med J* 4:219-221, 1971
99. Muirden KD, Phillips M: Evidence for a direct effect on articular cartilage and its lysosomal enzymes in filipin-induced arthritis. *Ann Rheum Dis* 32:251-261, 1973
100. Oka M, Rekonen A, Ruotsi A, Seppälä O: Intra-articular injection of Y-90 resin

- colloid in the treatment of rheumatoid knee joint effusions. *Acta Rheum Scand* 17:148, 1971
101. Oka M, Hyphen M: Radiation synovectomy. Results in rheumatoid knee effusions treated with radioactive Yttrium. *Scand J Rheumatology* 3: 33-38, 1974
 102. Oka M: Experimental studies on osmic acid and Yttrium 90 in synovitis. 126-129, 1975
 103. Oka M: Radiation synovectomy of the rheumatoid knee with Yttrium 90. *Ann Clin Res* 7:205-210, 1975
 104. Okada Y, Naknishi I, Kajikawa K: Secretory granules of B-cells in the synovial membrane. *Cell Tissue Re* 216:131-141, 1981
 105. Onetti CM, Gutierrez E, Hlibia E, Aguirre CR: Synoviorthesis with ^{32}P -colloidal chromic phosphate in rheumatoid arthritis-clinical, histopathologic and arthrographic changes. *J Rheum* 9:229-238, 1982
 106. Ott H, Boussina I, Fallet GH: La synoviothese des articulations digitales de la main a l'erbium 169. *Schweiz med Wschr* 107: 1366-1371, 1977
 107. Overton MC, Sheffel DD: Recurrent cystic formation in craniopharyngioma treated with radioactive chromic phosphate. *J Neurosurg* 20:707-710, 1963
 108. Pappas PW: The use of a chrome alum-gelatin (subbing) solution as a general adhesive for paraffin sections. *Stain Technology* 46:121-124, 1971
 109. Pavelka K, Meier-Ruge W, Müller W, Fridrich R: Histological study of effects of colloidal 90 Yttrium on knee joint tissue of rabbits. *Ann Rheum Dis* 34:64-69, 1975
 110. Perryman CR, Pavsek EJ, McAllister JD: Clinical evaluation of radioactive chrome phosphate in the control of malignant pleural and ascitic effusions. *Radiology* 73: 865-870, 1959
 111. Poole AR, Coombs RRA: Rheumatoid-like joint lesions in rabbits injected intravenously with bovine serum. *Int. Archs Allergy Appl Immun* 54:97-113, 1977

112. Poole AR, Oldham G, Coombs RRA: Early rheumatoid-like lesions in rabbits injected with foreign serum: Relationship to localization of immune complexes in the lining tissues of joints and cellular content of synovial fluid. *International Arch. of Allergy and applied Immunology* 57:135-145, 1978
113. Poole AR, Golds EE, Champion BR: The immunology of inflammatory arthritis. *Surg Clin N.A.* 61 (2):353-368, 1981
114. Prichard HL, Bridgman JF, Bleehan NM: An investigation of radioactive yttrium (^{90}Y) for the treatment of chronic knee effusions. *Br J Radiol* 43:466-470, 1970
115. Ramsey NW: Retention of ^{90}Y in patients with rheumatoid arthritis. *Ann Rheum Dis* 32suppl: 38-40, 1973
116. Ranawat CS, Straub LR, Freyburg R, Granda J, Rivelis M: A study of regenerated synovium after synovectomy of the knee in rheumatoid arthritis. *Arth and Rheum* 14:117-125, 1971
117. Ranawat CS, Ecker ML, Straub LR: Synovectomy and debridement of the knee in rheumatoid arthritis: (a study of 60 cases). *Arth and Rheum* 15:571-581, 1972
118. Redler I, Zimny ML: Scanning electron microscopy of normal and abnormal articular cartilage and synovium. *JBJS* 52-A:1395-1404, 1970
119. Rekonen A, Oka M, Kuikka J: Measurement of synovial fluid volume by a radioisotope method. *Scand J Rheumatology* 2: 33-35, 1973
120. Richter CB, King CS: Formalin as a hardner of photographic emulsions to facilitate staining of epon sections after autoradiography. *Stain Technology* 47:268-269, 1972
121. Roberts SD, Gillepsie PJ: Effect of immobilization on retention of ^{90}Y . *Ann Rheum Dis* 32suppl:46-47, 1973
122. Root SW, Tyor MP, Andrews GA, Kniseley RM: Distribution of colloidal radioactive chromic phosphate after intracavitary administration. *Radiology* 63:251-257, 1954
123. Rosenberg L: Chemical basis for the histological use of Safranin-O in the study of

articular cartilage. *JBJS* 53-A:69-82, 1971

124. Rosenspire KC, Blaw M, Kennedy AC, Green FA: Assessment and interpretation of radiopharmaceutical joint imaging in an animal model of arthritis. *Arthritis Rheum* 24: 711, 1981
125. Rosenthal L: Use of radiocolloids for intra-articular therapy for synovitis. *Therapy in Nuclear Medicine*. ed. Richard P Spencer. Grune & Stratten Inc. p. 147-153, 1978
126. Rubin P, Casarett G, Farrer P: The effect of intra-articular ^{198}Au instillations on articular cartilage. *Radiol* 103:685-692, 1972
127. Ruotsi A, Hypen M, Rekonen A, Oka M: Erbium -169 versus triamcinolone hexacetone in the treatment of rheumatoid finger joints. *Ann Rheum Dis* 38:45-47, 1979
128. Sandy JD, Lowther DA, Brown HLG: Antigen-induced arthritis . Studies of the inhibition of proteoglycan synthesis observed in articular cartilage during short-term joint inflammation. *Arthritis Rheum* 23:433-447, 1980
129. Sechrist JW, Upson RH: Prevention of chemography in prestained eposy autoradiographs by an intervening carbon film. *Stain Technology* 49:297-300, 1974
130. Shepard N, Mitchell NS: The simultaneous localization of proteoglycan by light and electron microscopy using toluidine blue O. A study of epiphyseal cartilage. *J Histochem Cytochem* 24:621-629, 1976
131. Shurman DJ, Hirshman HP, Strober S: Total lymphoid and local joint irradiation in the treatment of adjuvant arthritis. *Arthritis Rheum* 24:38-44, 1981
132. Sledge CB, Noble J, Hnatowich DJ, Kramer R, Shortkroff S: Experimental radiation synovectomy by ^{165}Dy ferric hydroxide macroaggregate. *Arthritis Rheum* 20:1334-1342, 1977
133. Soren A: In *Anatomy and Histology of the Synovial Membrane*. Histodiagnosis and

- clinical correlation of rheumatoid and other synovitis. JB Lippincott Co, 1978 p.1-7
134. Spurr AR: A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Research* 26:31-43, 1969
 135. Stastny P, Cooke TD, Ziff, M: Production of a macrophage migration inhibitory factor in rabbits with experimental arthritis. *Clin Exp Immunol* 14:141-147, 1973
 136. Steffen C, Zeitlhofer J, Zielinski Ch, Menzel J, Smolen J. Collagenase-induced experimental arthritis. *Z Rheumatol* 38:1-10, 1979
 137. Stegemann H: Mikrobestimmung von hydroxyprolin mit chloramin-T und p-dimethylaminobenzaldehyd. *Hoppe-Seyler Zeitschrift fur physiologische chemie* 311:41-45, 1958
 138. Stein H, Yarom R, Levin S, Dishon T, Ginsburg I: Chronic self-perpetuating arthritis induced in rabbits by a cell free extract of group A Streptococci. *Proc Soc Exp Biol and Med* 143:1106-1112, 1973
 139. Steinberg J, Sledge CB, Noble J, Gillies HG: Dextran sulphate induced arthritis in rabbits. *Ann Rheum Dis* 39:387-391, 1980
 140. Stevenson AC: Chromosomal damage in human lymphocytes from radioisotope therapy. *Ann Rheum Dis* 32suppl 6:19-23, 1973
 141. Stevenson AC, Bedford J, Dolphin GW, Purritt RJ, Lloyd DC, Hill AGS, Hill HFH, Gumpel JM, Williams D, Scott JT, Ramsey NW, Bruckner FE, Fearn CBD a: Cytogenic and scanning study of patients receiving intra-articular injections of gold-198 and Yttrium-90. *Ann Rheum Dis* 32suppl:112-121, 1973
 142. Stuart JM, Cremer MA, Kang AH, Townes AS: Collagen induced arthritis in rats. Evaluation of early immunologic events. *Arthritis Rheum* 22:1344-1351, 1979
 143. Swett PP: Synovectomy in chronic infectious arthritis. *JBJS* 5: 110-121, 1923
 144. Swett PP: Synovectomy in chronic infectious arthritis (second paper). *JBJS* 6: 800-804, 1924

145. Szanto E: Long-term follow-up of 90 Yttrium treated knee-joint arthritis. *Scand J Rheumtol* 6:209-212, 1977
146. Tanner E, Hein G, Klumbies G, Stoll W, Wessel G: Zur intraartikulären therapie mit radionuckliden. *Z Ges Inn Med Jahrg* 34:636-640, 1979
147. Topp JR, Cross EG: The treatment of persistent knee effusion with intra-articular radioactive gold. Preliminary report. *CMAJ* 102:709-714, 1970
148. Umbios AM, Cabrini RL, Silberman FS, Miranda FF: Radiation effects produced by the intra-articular injection of ^{32}P . *Clin Orth Rel Res* 136:299-303, 1978
149. Van Beusekom HJ, van den Broek WJM, van de Putte LBA: ^{99}Tc pertechnetate uptake measurement in the rabbit knee joint. I Standardization of the method and measurements in experimental arthritis. *J Rheum* 8:31-39, 1981
150. Van Beusekom HJ, van de Putte LBA, van der Berg WB, van der Broek WJM: Antigen induced arthritis - inflammation and antigen handling after two different dose of intra-articularly injected antigen. *Immunology* 45: 193-198, 1982
151. Van den Brenk HAS, Clarke KH, Holman WP, Winkler C: Studies of the effect of a colloidal radioactive chromic phosphate ($\text{Cr}^{32}\text{PO}_4$) in clinical and experimental malignant effusions. *Br J Cancer* 13:181-194, 1959
152. Venable JH, Coggeshall R: A simplified lead citrate stain for use in electron microscopy. *J Cell Biol* 25:407, 1965
153. Virkkunen M, Krusius FE, Heiskanen T: Experiences of intra-articular administration of radioactive gold. *Acta Rheum Scand* 13:81-91, 1967
154. Von Reis G, Swensson A: Intra-articular injections of osmic acid in painful joint affections. *Acta Med Scand* 259 suppl:27-32, 1951
155. Warshawsky H, Moore G: A technique for the fixation and decalcification of rat incisors for electron microscopy. *J Histochem Cytochem* 15:542-549, 1967
156. Washburn LR, Cole BC, Gelman MI, Ward JR: Chronic arthritis of rabbits induced by

- mycoplasmas. I Clinical, microbiologic and histologic features. *Arthritis Rheum* 23:825-836, 1980
157. Webb FWS, Lowe J, Bluestone R: Uptake of colloidal radioactive Yttrium by synovial membrane. *Ann Rheum Dis* 28: 300-302, 1969
158. Weissman G, Pras M, Rosenberg L: Arthritis induced by filipin in rabbits. *Arthritis Rheum* 10: 325-336, 1967
159. Williams ED, Caughey DE, Hurle PJ, John MB: Distribution of Yttrium 90 ferric hydroxide colloid and gold¹⁹⁸ colloid after injection into knee. *Ann Rheum Dis* 35:516-520, 1976
160. Williams PL, Crawley JCW, Freeman AM, Lloyd DC, Gumpel JM: Feasibility of outpatient management after intra-articular yttrium-90: comparison of two regimens. *Brit Med J* 282: 13-14, 1981
161. Winston MA, Bluestone R, Cracchiolo A, Bland WH: Radioisotope synovectomy with ³²P-chromic phosphate-Kinetic Studies. *J Nucl Med* 14:886-889, 1973
162. Wolfangle R., Research and Development Dept., Mallinckrodt Inc., St. Louis, Missouri. Personal communication.
163. Yates DB: Arthroscopy of the knee after the injection of 90Y. *Ann Rheum Dis* 32 Suppl: 48-50, 1973
164. Product data. Phosphocol-P32. Mallinckrodt Diagnostics, Mallinckrodt Inc., St. Louis Missouri.