CURRENT TOPICS IN CONNECTIVE TISSUE DISEASE

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Edited by

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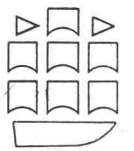

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PREFACE

The production of a book should represent one's own interests. In planning this book I have tried to bring together a series of subjects which I believe to be not only of great interest at this time but where progress is occurring. Thus I hope the result will appeal to practising clinicians and help to smooth the transition from today's research to tomorrow's clinical practice. For, although much clinical work can be done by rote, the best must be based on a determination to understand at an ever more finite level exactly what is happening.

The chapters have been constructed to introduce their subject matter so that aspects the reader may not be familiar with fall into place.

No attempt has been made to cover the whole field of rheumatology or the 'connective tissue' diseases since this would have led to an unwieldy book.

It is a pleasure to thank my fellow authors and my secretaries Mrs J. Andrews and Mrs E. Ramsbottom.

Finally I hope that readers will gain as much interest and benefit as I have.

Manchester 1975 P.J.L.H.

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CONTENTS

Chapter 1

THE PHYSIOLOGY OF THE SYNOVIAL CELL AND ITS CONTRIBUTION TO DISEASE PROCESSES

C. WILLIAM CASTOR

INTRODUCTION

The synovial membrane is a specialized connective tissue lining the capsule of diarthrodial joints, bursae and tendon sheaths. It serves primarily to form joint fluid which possesses useful biomechanical properties and provides nutrition for articular cartilage.

The specialized structural and functional characteristics of this tissue render it a sensitive herald of many systemic diseases, and in some circumstances a primary focus of disease, as in rheumatoid arthritis, infectious arthritis, and gout. Just as distinguishing 'synovial membrane' from subjacent joint capsule may be a difficult and arbitrary task, so too the designation of specific 'synovial cells' in the heterogeneous cell mixture present in the membrane is not only arbitrary, but largely unjustifiable on present evidence. Normal synovial tissue function probably requires an integrated effort by intimal lining cells, stromal cells, vascular, and, perhaps, neural components. A brief examination of the origin of the synovial membrane illustrates the interrelated nature of its several components.

STRUCTURAL ORGANIZATION

Embryologic origin of synovial membrane

Synovial tissue arises from the primordial skeletal blastema, especially the interzone between cartilaginous primordia destined to oppose each other in the formation of a diarthrodial joint. The cellular constituents of this avascular interzone appear to elaborate an intercellular substance having histochemical characteristics consistent with chondroitin-4- and/or -6-sulphate (Andersen, 1964), although analytic confirmation of this is not yet available. At approximately 8 weeks gestation, *joint cavity formation begins* in the central portion of the articular interzone between the chondrogenic primordia, *leaving vascular areas at the periphery of the interzone which are the anlage of*

the synovial membrane. As the joint cavity increases, vascular tissue grows into the synovial primordia from the periarticular mesenchyme. Accompanying the vascular tissue are large pale cells resembling tissue macrophages. Flattened superficial cells facing the primitive joint cavity represent the early synovial intimal cells and are characterized by an acid phosphatase reaction of moderate intensity. More deeply situated macrophages exhibit a stronger acid phosphatase reaction and contain granules resembling hemosiderin. At approximately 12 weeks mast cells appear, initially in a perivascular location, and a sparse deployment of collagenous fibres is noted. No major histochemical differences can be detected between the synov al intimal lining cells and their related mesenchymal derivatives located deep to the surface layer (Andersen, 1964).

Studies carried out in developing chick synovial membrane (Hendrikson and Cohen, 1965) make it clear that the synovial membrane is not a continuous structure, and ultrastructural studies reveal no evidence of desmosomes. The cells appear to be imbedded in the rich collagenous matrix. Synovial blood vessels have a complete-non-fenestrated endothelium which on occasion lies virtually contiguous to the joint space.

Structure of adult synovial membrane

Cellular components. The synovial membrane is arranged in folds and villi, with a condensation of specialized connective tissue cells on the surface, forming a loose layer one to four cells in depth (Fig. 1.1a) (Castor, 1960a). These intimal synovial cells are usually ellipsoidal and frequently exhibit long processes extending from the body of the cell toward the joint cavity (Fig. 1.1b). Commonly a network of branching cell processes with finely granular cytoplasm forms the innermost portion of the cellular lining layer. The lining layer appears to be discontinuous and lacks a clear basement membrane, although the surface cells appear to be enmeshed in a reticulin network (Fig. 1.1c). A survey of the frequency of various cell types to a vertical depth of 70μ beneath the joint lining surface disclosed that approximately 49 per cent of the cells were intimal synovial lining cells, 3 per cent were mast cells, 9 per cent were endothelial cells, and 39 per cent were unclassified connective tissue cells. This latter group included macrophages, primordial mesenchymal cells in the vascular adventitia, and cells with a fibroblastic appearance.

Electron microscopic studies of the intimal cell layer suggest that two primary cell types are present. The most common cell type, A, is distinguished by large vacuoles, numerous filopodia, vesicles, mitochondria, and branched aperiodic fibrils in the cytoplasmic ground substance. Cell type B is characterized by abundant endoplasmic reticulum, smaller mitochondria, and fewer vacuoles and vesicles. In this study of human material, no true cell junctions such as desmosomes or adhesion plates were found, and it appeared that the intercellular matrix was in continuity with the adjacent joint cavity (Barland, Novikoff and Hamerman, 1962). Other investigators (Roy, Ghadially and

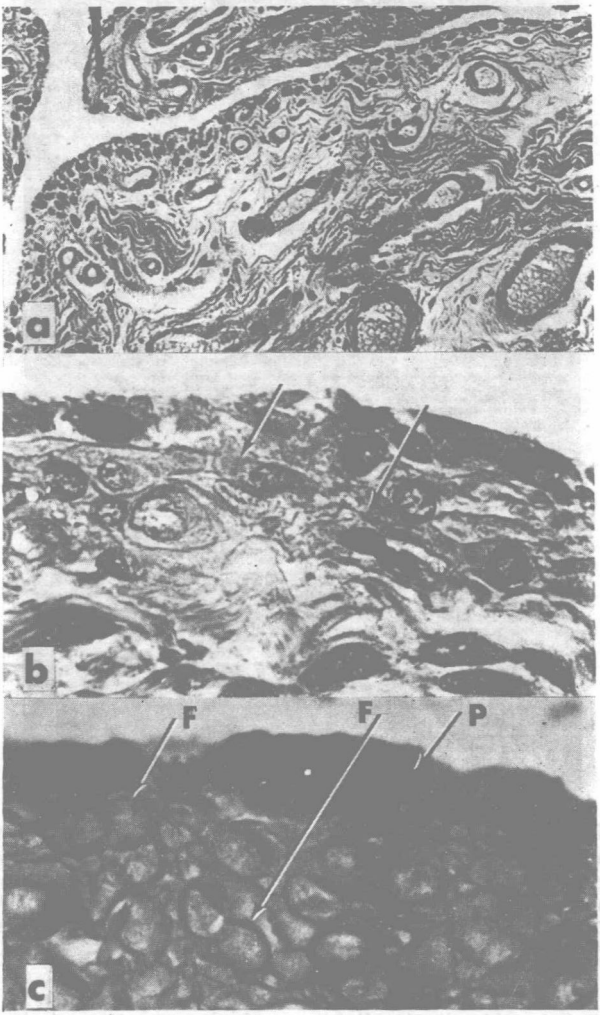

FIGURE 1.1. *Panel 1.1a* shows areolar synovium from the knee of a 58-year-old male, and illustrates the superficial intimal cell zone and subjacent vascular network. Masson trichrome stain X 148. *Panel 1.1b* illustrates the long cytoplasmic process of synovial intimal cells (arrows). Masson trichrome X 1080. *Panel 1.1c* demonstrates the branching argyrophilic fibrillar network (arrows, F) enmeshing surface synovial cells. Precipitated joint fluid denoted at P. Wilder silver stain, hematoxylin, and eosin, X 1080. Reproduced from Castor, 1960a, with permission of the publisher.

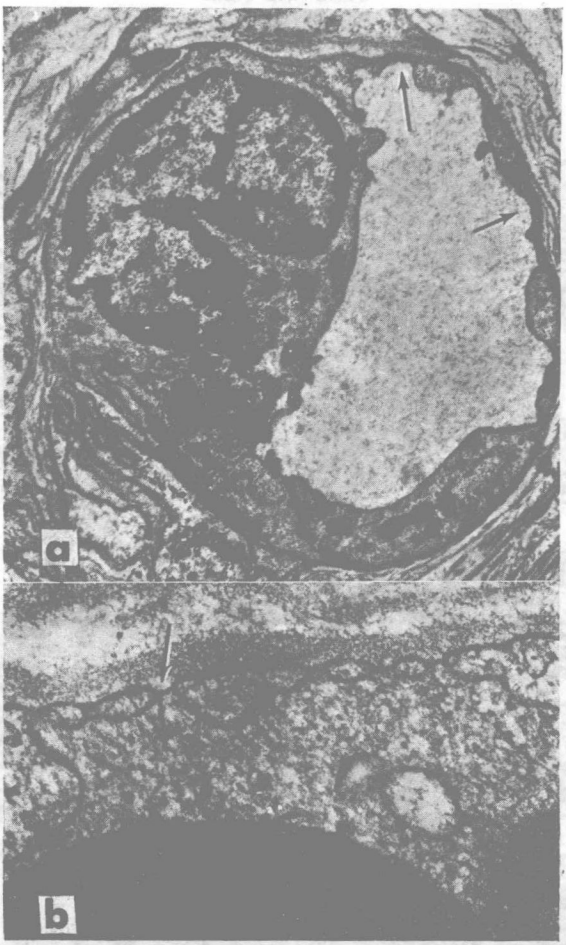

FIGURE 1.2. Electron micrograph of normal synovial capillary, 1.2a, showing fenestrae (arrows) in attenuated areas of the endothelium. In 1.2b the arrow indicates a thin diaphragm bridging an endothelial fenestra in a normal human synovial capillary. These figures were published with the kind permission of Dr C. C. Clawson, Dept. of Pediatrics, University of Minnesota, U.S.A. Fig. 1.2a was previously published (Clawson, Lounberg and Good, 1967).

Crane, 1966) have described cell forms which are intermediate in the morphology between the cell types A and B, a finding which is characteristic of cells grown from synovial tissue in cell culture (Castor and Muirden, 1964). While two distinctive cell types in the synovial lining layer have been recognized in several species, the possibility remains that these images may merely reflect temporal differences in function of one primary cell type. Alternatively, it is possible that a given cell, if reconstructed from serial sections, might show both types of EM appearance.

It is of interest that synovial membranes from patients with traumatic

effusions showed many cells intermediate in structure between the A and B morphologic types, as well as an increase in the number of type B electron microscopic images (Roy *et al.*, 1966). Histochemical studies on synovial membranes from patients with traumatic synovitis revealed increased amounts of RNA. In the same vein, an increase in the number of type B cells has been described in the synovial membrane of patients with hemarthrosis of short duration (Roy and Ghadially, 1967). In rheumatoid arthritis there are more normal-appearing type B cells than type A cells, the latter appearing unusual by virtue of large quantities of membrane-limited dense bodies, presumably reflecting accelerated phagocytic activity (Wyllie, Haust and More, 1966). It is noteworthy that in rheumatoid synovitis increased numbers of collagen fibrils were found in the intercellular spaces between the intimal lining cells, suggesting increased synthesis of this fibrous protein.

Vascular components. Vascular and lymphatic channels are prominent, frequently lying in close proximity to superficial lining cells, and on occasion appearing almost contiguous with the joint cavity. Vessels in synovial tissue are disposed in parallel layers with an innermost subintimal plexus of net-like vascular loops exhibiting many interconnections. In rat, monkey, and human synovial membrane, unlike the chick, fenestrated capillaries have been identified, usually showing the fenestrae bridged by a thin membrane (Fig. 1.2) (Suter and Majno, 1964; Schumacher, 1969; Clawson, Lounberg and Good, 1967). Arteriolovenular anastamoses, which may act as short-circuit vessels, have been described at the base of synovial villi (Fig. 1.3). The innermost synovial vascular bed is said to communicate frequently with periosteal,

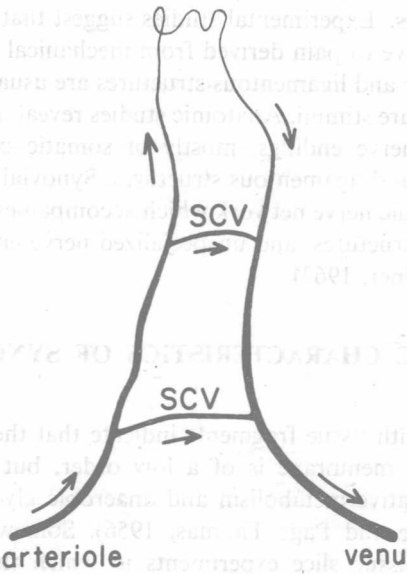

FIGURE 1.3. This schematic villous vascular pattern was redrawn after Lindström, 1963. Arrows indicate the direction of blood flow. Short-circuit vessels are labelled: SCV.

subchondral, and medullary vessels (Lindström, 1963). It is of interest that with elevation of the temperature of normal synovial tissue (in the range of 41 to 42 C in the rabbit) most corpuscular blood flow is shunted away from the villi through the short-circuit vessel system. While the short-circuit vessels may serve under normal conditions to modulate hydrodynamic distortion of fragile villous structures, it is clear that *the inflammatory state with its associated increase in joint temperature may lead to shunting of blood away from the villous tips and to promotion of glycolytic metabolism.* Studies of regenerating rabbit synovial membrane following synovectomy indicate prompt restoration of not only the intimal cell layer from subsynovial anlage, but also restoration of the capillary bed to something approximating the normal vascular architecture.

Studies of the effects of catecholamines on the clearance of ^{133}Xe (a measure of synovial perfusion) in normal and traumatized canine joints have been interpreted as suggesting that both α- and β-adrenergic receptors act to control synovial perfusion in both the normal and inflamed state (Dick, Provan and Pond, 1970). The vascular lesions in human synovial tissue from patients with osteoarthritis show dilated venules and capillaries associated with oedema and erythrocyte extravasation, but without thrombosis. Synovial membranes from patients with rheumatoid arthritis revealed the same features in more intense form, but also presented evidence of atonic dilatation of venules and vascular obliteration (Goldie, 1970). The overall impression was that *microvascular abnormality led to slowed circulation through the venular and capillary nets with preferential blood flow through the short-circuit vessels.*

Neural components. Experimental studies suggest that synovial membrane is relatively insensitive to pain derived from mechanical or chemical stimuli. In contrast, capsular and ligamentous structures are usually exquisitely sensitive to pain or pressure stimuli. Anatomic studies reveal numerous specialized and unspecialized nerve endings, mostly of somatic origin and primarily located in capsular and ligamentous structures. Synovial membrane is served by a largely autonomic nerve network which accompanies vessels, terminating in loops, globular structures, and unspecialized nerve endings (Kellgren and Samuel, 1950; Gardner, 1963).

METABOLIC CHARACTERISTICS OF SYNOVIAL TISSUE

Energy metabolism

In vitro studies with tissue fragments indicate that the energy metabolism of normal synovial membrane is of a low order, but that in rheumatoid arthritis, both oxidative metabolism and anaerobic glycolysis are markedly accentuated (Dingle and Page Thomas, 1956). Somewhat different results were obtained in tissue slice experiments in which the metabolic pattern observed was corrected for activity contributed by the infiltrating erythrocytes and leucocytes of the heterogeneous tissue (Roberts, McLees and Kerby,

1967). These experiments showed accelerated production of lactate by rheumatoid synovial tissue, a marked increase in $^{14}CO_2$ production from glucose-1-^{14}C, but little evidence of increased CO_2 production from glucose-6-^{14}C. The data were interpreted as showing a marked increase in both glycolysis and pentose shunt activity in the inflamed rheumatoid tissue, as compared to normal, but with little increase in the tricarboxylic acid cycle activity. It was speculated that the stimulation of the pentose shunt cycle might be of importance in the synthesis of lipids, including fatty acids and sterols. More recent studies using monolayer cultures of dispersed synovial connective tissue cells from normal and rheumatoid synovial membrane disclose that the rate of glucose consumption and lactate formation per cell is greater in rheumatoid cells than in normal control cells (Castor, 1971b).

Clinical studies of synovial fluid tend to agree with the general thrust of the *in vitro* studies. Severe joint disease was associated with a fall in pO_2, a fall in pH, and a rise in pCO_2 and the concentration of lactic acid (Falchuk, Goetzl and Kulka, 1970; Lund-Olesen, 1970). This suggests that, *although the regional blood flow is increased in rheumatoid arthritis, it is insufficient to meet the large metabolic demands incident to the disease process*, thus leading to the fall in pO_2 and pH in the diseased synovial fluids. The evidence suggests that these studies of synovial fluid did in fact reflect the conditions in the synovial membrane rather than a result of activity of the leukocytes in the synovial fluid. A more recent refinement of this approach measures the rate of fall of intra-articular saline pO_2 as a function of time after the circulation to the joint is interrupted by a tourniquet (Goetzl *et al.*, 1971). With this technique rheumatoid patients show a two- to threefold higher oxygen uptake rate and a tenfold higher lactate appearance rate in the joints with clinical evidence of severe synovitis.

Extracellular macromolecule synthesis

Hyaluronic acid is the extracellular acid glycosaminoglycan contributed by synovial cells to the interstitium of synovial tissue and to the fluid in the *adjacent specialized connective tissue space, the joint cavity*. Hyaluronic acid is a complex carbohydrate, a heteropolymer of N-acetyl-D-glucosamine glycosidically linked to D-glucuronic acid. Its high molecular weight and random coil structure account for its marked viscosity. In normal human knee joints the hyaluronic acid concentration is 3.5 to 4 mg/ml in individuals under 40 years of age, while concentrations in the range of 2 to 2.5 mg/ml are noted in persons in the 60 to 80 age group (Castor, Prince and Hazelton, 1966). Usually less than 1.0 ml of fluid is found in a normal human knee joint. Intrinsic viscosity measurements on normal joint fluid from persons of all ages are usually 38 to 40 dl/gm, corresponding to a molecular weight of approximately 2.8×10^6.

Biosynthesis of hyaluronic acid from glucose is accomplished by synovial cells via a pathway that involves production of two activated sugar nucleotides, uridinediphospho-N-acetylglucosamine and uridinediphosphoglucuronic acid,

which are added alternately to the growing carbohydrate chain by the polymerase, 'hyaluronate synthetase'. In rodent studies the biologic half-life of hyaluronic acid in skin tissue is estimated at 2 to 4 days (Schiller *et al.*, 1955, 1956). While there are no reports of the half-life of hyaluronate formed by human synovial membrane, preliminary observations on the turnover of hyaluronate in calves indicated that ^{131}I-labelled 'hyaluronate protein' of bovine origin had a half-life of one day or less (Sandson, 1966). The normal route for removal of hyaluronate from the joint is not known, although lymphatic and venous channels or local destruction all must be considered. Clearly, the manner and fate of hyaluronate removal from the joint is still a matter for speculation and of importance in understanding the normal and abnormal physiology of joint function.

There is evidence that the hyaluronate found in some synovial fluids is polydisperse with respect to molecular weight, but whether this is due to the synthesis of chains of non-identical length or to some as yet unrecognized extracellular degradation process is unknown. Although it is clear that many of the sulphated glycosaminoglycans (e.g., chondroitin-4-sulphate, dermatan sulphate, and heparin) are covalently linked to protein 'core molecules' via specific linkage areas, the situation regarding a specific hyaluronate-protein linkage is much less clear. While hyaluronate of high molecular weight may be isolated with as little as 2 or 3 per cent protein, it is not known whether this is in fact covalently linked, a naturally occurring complex, or an artifact of isolation procedures. The detailed chemical structure of the presumed linkage area between protein and hyaluronate has not yet been defined. In fact, if such a linkage area does exist, it may vary from one tissue source to another.

In synovial inflammation there is usually *an increase in the amount of joint fluid and in the total hyaluronic acid content*, in spite of a decrease in the hyaluronic acid concentration to the range of 1 to 1.5 mg/ml. Inflammatory fluids exhibit decreased intrinsic viscosity measurements consistent with molecular weight estimations of 1 to 2 million. Although the total amount of hyaluronate synthesized by the inflamed synovial membrane is usually markedly increased, the combination of low concentration and inferior molecular weight explains the decreased viscosity and poor mucin clot test which is observed in clinical practice. Inflammatory joint conditions yielding this pattern of hyaluronate abnormality include traumatic synovitis, ankylosing spondylitis, ulcerative colitis, Reiter's syndrome, and rheumatoid arthritis. On the other hand, the bland effusions found with degenerative joint disease tend to show near-normal concentrations of hyaluronate of normal molecular weight and characteristically are viscous and produce a normal mucin clot reaction.

Collagen formation is clearly a synthetic capacity retained by the superficial cells of the synovial membrane. The surface cells are enmeshed in a fibrillar network having the histochemical characteristics of reticulin, and fibres with the EM characteristics of collagen are frequently seen in the

superficial layers of the membrane. Further, cells grown from normal synovial membrane which synthesize high molecular weight hyaluronic acid also synthesize collagen fibres, as well as soluble collagen with an intrinsic viscosity similar to that of tropocollagen (Castor, 1970). It is clear that rheumatoid pannus leads to the deposition of increased amounts of fibrous collagen. Recent evidence indicates that the protocollagen proline hydroxylase activity in biopsy specimens from rheumatoid synovial tissue is twice as great as that from control non-rheumatoid specimens when related to the protein in the extracts (Uitto *et al.*, 1970). It is not yet certain that this enzyme activity per unit of cell mass has been altered. An intriguing report suggesting qualitative modifications of the collagen in rheumatoid synovial membrane has yet to be confirmed (Steven, 1965).

Ferritin, a non-collagen protein, is formed by synovial cells which phagocytose haemoglobin *in vitro* (Muirden, Fraser and Clarris, 1967). Electron micrographs of rheumatoid synovium disclose substantial quantities of ferritin in the lysosomes and cytoplasm of synovial lining cells, especially type A cells (Muirden, 1966). Chemical measurements reveal that rheumatoid synovial tissue may contain 20 times as much iron as normal tissue, a finding of possible significance in the pathogenesis of the anaemia of rheumatoid arthritis (Muirden and Senator, 1968; Senator and Muirden, 1968). The iron was believed to be derived from haemoglobin after bleeding in the vascular granulation tissue.

Antisera to the 'species-specific component' of human cartilage protein polysaccharide are reported to detect substantial amounts of this antigen in synovial membrane cells of normal persons, patients with Reiter's disease and pigmented villonodular synovitis, but not in rheumatoid synovial membranes (Janis *et al.*, 1967; Becker *et al.*, 1969). Interestingly, monolayer cultures of non-rheumatoid and rheumatoid synovial cells show evidence of this antigen in both cells and medium after several generations in culture, although cultivated rheumatoid synovial cells appear to form less of this material. The biologic significance of this interesting antigen, believed to be a glycoprotein, is as yet unknown.

There is good evidence, based on electrophoretic and immunologic assessments of synovial cell homogenates, that a protein similar to the serum *α_2-macroglobulin* is closely associated with these cells (Williamson *et al.*, 1966), but it is not known whether this protein is formed by the cells or adsorbed from the circulating plasma, although experiments with carefully washed cells favour the latter idea. Fibrinolytic activity has been attributed to the synovial membrane of rats and guinea pigs, raising the interesting possibility that synovial cells may synthesize a *plasminogen activator* capable of converting plasminogen to the fibrinolytic enzyme, plasmin (Myhre-Jensen, Larsen and Astrup, 1969).

Immune globulins are synthesized in small amounts by normal synovial membrane, presumably by lymphoid constituents normally present in this tissue (Jasin and Ziff, 1969). Inflammatory synovial reactions to known

antigens were accompanied by synovial formation of specific antibody directed against these antigens. Immunofluorescent studies of rheumatoid synovium have disclosed deposits of IgG, IgM, β1C, and nucleoprotein (Brandt, Cathcart and Cohen, 1968). Immunoglobulins and complement were found in the synovial interstitial tissue, the cytoplasm of infiltrating inflammatory cells, and in and near blood vessel walls. ^{14}C-labelled amino acids incubated with minced rheumatoid synovium were incorporated to a significant extent into immunoglobulins, largely IgG, and in lesser amounts into IgA and IgM (Smiley, Sachs and Ziff, 1968). Rheumatoid factor accounted for less than 10 per cent of the newly synthesized IgM. The levels of isotope incorporation into immunoglobulins appeared similar to that accomplished by similar masses of splenic and lymph node tissue.

Cortisol metabolism

In view of clinical evidence that supraphysiologic concentrations of cortisol given to rheumatoid patients suppress inflammatory activity in synovial membrane, and because *in vitro* evidence shows that rheumatoid synovial connective tissue cells are hyporesponsive to the regulatory actions of glucocorticoids (Castor and Dorstewitz, 1966; Castor, 1971c), the characteristics of cortisol metabolism in this tissue are of considerable interest. Tissue culture studies indicate that both normal and rheumatoid synovial cells in monolayer culture remove cortisol from the nutrient medium, and both normal and pathologic cells have been shown by radioautographic techniques to take up ^{3}H-cortisol (Wynne-Roberts and Castor, 1972). Normal and rheumatoid synovial tissue were incubated *in vitro* with cortisol-4-^{14}C, the sterols extracted, fractionated, and identified. Cortisol, cortisone, 20-α-dihydrocortisol, 20-β-dihydrocortisol, 20-α-dihydrocortisone, and 20-β-dihydrocortisone were identified, making it clear that significant catabolism of radioactive cortisol occurred in the synovial tissue. Approximately 50 per cent of the radioactivity was present in metabolites. This study indicated that rheumatoid tissue tended to convert unusual amounts of cortisol to cortisone, possibly leading to a decrease in the effective concentration of the active agent in the inflamed tissue (Murphy and West, 1969).

Regulatory mechanisms

Connective tissue activating peptide (CTAP) is a polypeptide with a molecular weight between 4 000 and 10 000 which induces hypermetabolism in synovial connective tissue cells. CTAP is found in human leucocytes, platelets, fibroblasts, and several other cell types (Yaron and Castor, 1969; Castor and Yaron, 1969; Castor, 1971a). *In vitro* experiments demonstrate that the active principle may be transferred from lymphocytes to the surrounding medium, and then to sensitive synovial cells. When synovial cells are exposed to CTAP *in vitro*, they promptly exhibit *enhanced energy metabolism* as measured by increased glucose consumption and increased lactate production. Of greater importance is the fact that there is also a *marked increase in*