

The Physical Properties of Synovial Fluid and the Special Role of Hyaluronic Acid



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This chapter was written with two aims in mind. First, to give a brief review of the chemical composition and rheological properties of the synovial fluid in normal and pathological joints; and second, to present, with critical comments, various current hypotheses on the importance of this fluid in the function of the joint with special emphasis on the biological role of hyaluronic acid.

There is considerable data available in the literature on the chemical composition and rheological properties of synovial fluid of various species. This review does not intend to be all-inclusive, rather it presents primary data on human joint fluids only. A large number of speculations and hypotheses are recorded in the literature on the lubricating and nutritive functions of the synovial fluid in the joint. This review aims to be selective in this area as well and present only the most important recent findings and speculations on this subject.

SYNOVIAL FLUID AS AN INTERCELLULAR MATRIX

From a rheological point of view, the natural environment of cells in a tissue can be liquid or solid. The liquid or solid extracellular matter around and between cells is called matrix. In the solid matrix, certain macromolecular components (mostly colla-

gen or elastin, or both) and their aggregates form a continuous solid phase. The rheological qualities of the solid matrix, that is, the rigidity, elasticity, and viscosity, vary considerably as exemplified by the differences between cartilage and the vitreous of the eye.

The solid matrix consists of fixed and fluid components. The fixed components are made up of such microscopic elements as collagen and elastin fibrils, basal laminae, and the structural network of proteoglycans. The fluid component comprises water and those solutes (salts, small organic molecules, peptides, proteins, etc.) that, dissolved in water, are distributed between the fixed components of the solid matrix.

The liquid matrix, on the other hand, is water in which molecules of various sizes are dissolved or dispersed. The rheological qualities of liquid matrices also vary considerably, as exemplified by the differences between the aqueous humor of the eye and synovial fluid.

The joint contains both solid and liquid matrices. From the four tissue elements forming a joint: articular cartilage, synovial tissue, intraarticular ligaments, and synovial fluid, the first three have solid matrices and the last is a liquid matrix.

It is of primary importance to recognize that all three solid matrix compartments of

TABLE 5-1. Proteins and Hyaluronic Acid in the Synovial Fluid of Knee Joints of Human Subjects of Various Ages

Age Group (yrs.)	Knees Investigated	Protein (mg./ml.)	Hyaluronic Acid mg./ml.	Limiting Viscosity Number (ml./g.)
18-20	36	20.5 $\pm$ 0.8	3.8 $\pm$ 0.1	5200 $\pm$ 100
21-23	24	21.0 $\pm$ 0.9	3.8 $\pm$ 0.2	5300 $\pm$ 100
24-27	20	19.4 $\pm$ 1.3	3.4 $\pm$ 0.1	5000 $\pm$ 150
28-35	18	18.5 $\pm$ 2.6	2.5 $\pm$ 0.04	5800 $\pm$ 300
52-78	34	18.5 $\pm$ 1.6	2.5 $\pm$ 0.2	5400 $\pm$ 200

(Data taken from unpublished work of E. A. Balazs, N. W. Rydell, P. O. Seppälä, I. F. Duff, E. W. Merrill and D. A. Gibbs)

the joint are directly adjacent to the liquid matrix compartment, the synovial fluid. Another important morphological fact is that the solid matrix compartments of the articular cartilage, ligaments, and synovial tissue are not separated from the fluid matrix compartment by a continuous cell layer or basal lamina. Therefore, no visible, morphological barrier separates these two types of matrix compartments.

Other adjacent liquid and solid matrices such as are present in the peritoneal, pericardial, and pleural spaces and in the anterior chamber of the eye, are separated by microscopically recognizable cellular (epithelium) and matrix (basement membrane) barriers. The joint, however, is not the only tissue in which the liquid and solid matrices are not separated by these barriers. Other tissues in the musculoskeletal systems are similarly structured, such as the tendons and their sheaths and the space between fasciae and the bursal space.

CHEMICAL COMPOSITION OF THE SYNOVIAL FLUID

Proteins

The protein content of normal synovial fluid in all species studied is much lower than in serum.³⁴ The general statement can be made that the very large protein molecules of the serum, such as γ -1 macroglobulin, β -lipoprotein, fibrinogen, and α -2 macroglobulin, are absent, and others, such as α -1 antitrypsin and plasminogen, are present in traces in the normal synovial fluid.

In addition, some smaller proteins, such as haptoglobulins and prothrombin present in the serum are also absent from normal synovial fluid.^{3,14,18,33,39} Because of the absence of fibrinogen and prothrombin, the normal fluid does not clot.

The total protein content of synovial fluid aspirated from normal human knee joints does not change with age of the donor (Table 5-1). In all inflammatory joint diseases, however, the concentration of protein in synovial fluid increases, and the missing plasma proteins appear. Fibrinogen appears, and, after aspiration, the fibrin precipitates, or often, the fluid clots. From the point of view of protein content, inflammatory synovial fluid is more "plasmalike" than normal synovial fluid.

In most degenerative type joint diseases, the protein concentration also increases in the fluid, but it does not necessarily become "plasmalike." While there is no clear proof, there are indications that in these cases, proteins appear in the synovial fluid that originate from the cells and matrix of neighboring tissues.³⁸

Proteins with enzymatic activity also appear in the inflammatory synovial fluid. Enzymes present in the lysosomes of leucocytes, such as acid phosphatases, β -glucuronidase, and β -N-acetylglucosaminidase, are released from the destroyed cells. Other enzymes, such as lactic dehydrogenases, collagenase, and muramidase (lysozyme) are also found in pathological synovial fluids.^{1,16,21}

Most importantly, inflammatory synovial

fluid contains immune complexes and antibodies that are not present in the normal fluid. In rheumatoid arthritis, the appearance of the so-called rheumatoid factors (primarily antibodies to γ G-globulin) and their complexes with immunologically active proteins, as well as activation of components of the complement system are typical examples of the drastic changes occurring in the protein composition and immunological characteristics of synovial fluid during inflammation.^{29,37,43-45} The extremely complex alteration in the immunologically active proteins of the synovial fluid indicate that the neighboring tissues—first of all, the synovial tissue—are the sites of very intensive protein synthesis during inflammation.

Hyaluronic Acid

The viscoelastic nature of synovial fluid is due to its hyaluronic acid content. This polyanion is present in the synovial fluid of all species investigated. In equine, bovine, and human joints a considerable variation was found in the hyaluronic acid concentration of synovial fluid collected from various joints of the same subject.⁴⁰

Hyaluronic acid concentration also varies with age.³⁹ In the synovial fluid of human knee joints the hyaluronic acid concentration is highest between 18 and 25 years, after which it decreases. Between the ages of 30 and 80, no change could be observed in normal joints.⁶

The size of the hyaluronic acid molecules in the synovial fluid of human knee joints was determined by various physicochemical methods. The results vary somewhat, depending on the method used for the purification of the hyaluronic acid and for the determination of the molecular weight.

This large molecule occupies an extremely large volume when dissolved in water that contains a physiological concentration of salts and hydrogen ions. A single molecule of Na-hyaluronate, with a weight of 5 million, occupies a spheroidal domain with a diameter of 0.5 μ . This means that one gram dissolved in physiological saline fills

3 liters of solvent. In other words, the individual molecules, in close contact with one another but without overlapping, occupy the whole volume of solution at a concentration of only 0.33 mg./ml. The concentration of hyaluronic acid in synovial fluid of the human knee joint is 2 to 3 mg./ml. This means that the molecules are "crowded"; they overlap, and, therefore, such a solution must be considered as a continuous network of interacting, entangled molecular chains. Any other molecules, large or small, dissolved in the solution, are within the domain of this hyaluronic acid network, and any molecule or particle that moves in it must pass through this feltlike molecular network. The chemical activity of the molecules that can penetrate this network may be changed, and other large molecules cannot find space to penetrate the network. In this sense, one speaks about the exclusion effect and the dynamic filtration effect of hyaluronic acid solutions.²⁶

Experiments *in vitro* carried out by many investigators, have demonstrated that large protein molecules can be filtered from solution by passage through a filter layer of synovial fluid or hyaluronic acid. Furthermore, due to the exclusion effect, molecules dissolved in hyaluronic acid solutions are altered in their chemical activity (solubility, aggregation, osmotic effect, charge effect, etc.).²³

Limiting viscosity number (intrinsic viscosity) is a widely-used index for characterizing polymers such as hyaluronic acid. The limiting viscosity number of hyaluronic acid in synovial fluid can be measured without separating the polysaccharide from proteins. This measurement gives a meaningful parameter of one hyaluronic acid molecule which expresses the size, volume, and shape of the molecule as well as its interaction with the solvent (water with ions dissolved in it^{8,14,36}). The limiting viscosity number of the synovial fluid of normal human knee joint does not change with age (Table 5-1).⁶ This indicates that the molecular size of the hyaluronic acid in the joint remains the same

during a lifetime, but the concentration of this polymer drops suddenly around 28 to 35 years of age.

THE ELASTOVISCOUS NATURE OF SYNOVIAL FLUID

Normal Fluid

Synovial fluid exhibits viscous and elastic properties. Both depend on its size, conformation, interactions, and number of hyaluronic acid molecules present in the fluid.

In recent years, a considerable amount of work has been reported on the elastoviscous nature of human knee synovial fluid obtained from normal and pathological joints.^{5,6,12,14} These studies clearly show that, from a rheological point of view, the synovial fluid and the protein-free (<1.0 per cent) hy-

aluronic acid prepared from other tissues (umbilical cord and rooster comb) are identical. Up to now, no evidence has been found that would indicate that the presence of proteins in the synovial fluid significantly alters the elastoviscous properties of the pure sodium salt of hyaluronic acid. Of course, this does not eliminate the possibility that some interaction may occur between proteins and Na-hyaluronate molecules, which could cause minor modifications in the viscoelastic properties of synovial fluid. The important fact is, however, that Na-hyaluronate without proteins exhibits the same molecular relaxation mechanism as synovial fluid when it is exposed to strain of various frequencies.²⁰

To demonstrate the elastoviscous nature of synovial fluid, one has to measure the

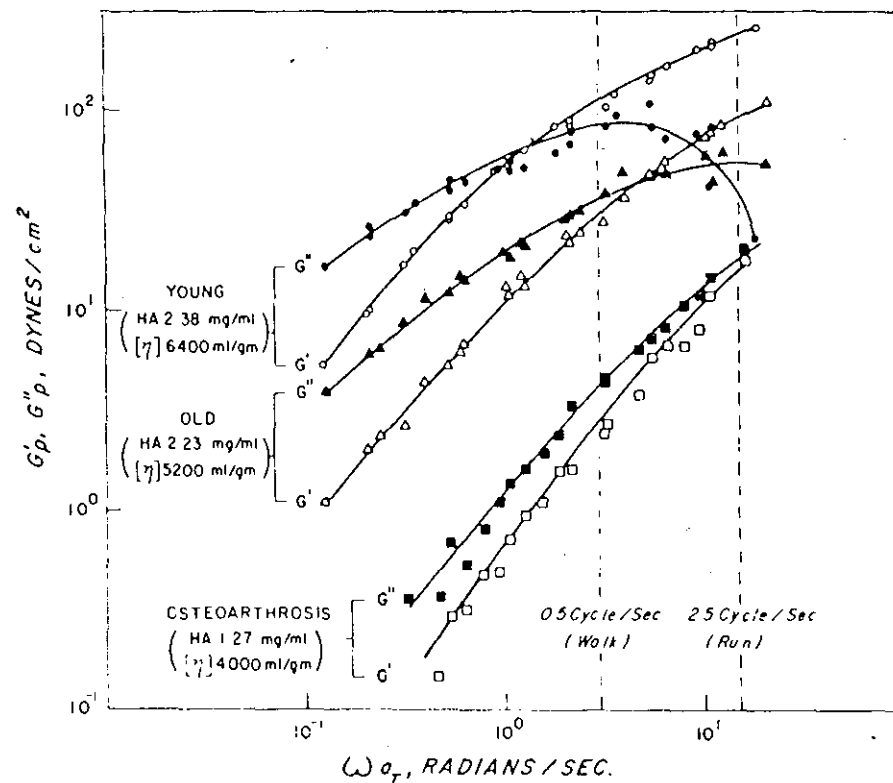


FIG. 5-1. Dynamic elastic modulus, G' (open symbols) and dynamic viscous modulus, G'' (filled symbols) of three human synovial fluid samples aspirated from the normal knee joint of one young (20 yrs.) and one old (67 yrs.) subject and from the osteoarthritic knee joint of an old subject (63 yrs.), plotted against strain frequency. The broken vertical lines indicate the frequencies that correspond approximately to the movement of the knee joint in walking and running. The concentration (HA) and limiting viscosity number ($[\eta]$) of hyaluronic acid in the aspirated fluid are given in parentheses. (From Balazs, E. A.: Univ. of Michigan Med. Ctr. July, [Special Arthritis issue]: 225, 1969.)

dynamic shear moduli at various strain frequencies and at various temperatures.²⁰ The dynamic rigidity of synovial fluid (G^*), which is the ratio of the peak stress to the peak strain in the fluid, can be divided vectorially into two components ($G^* = \sqrt{G''^2 + G'^2}$). One of these components is called the dynamic loss module (G'') or viscous module because it represents the energy that is dissipated as heat when the molecule is submitted to strain. The other component is called the dynamic storage module (G'), or elastic module, because it represents the energy stored when the molecule is submitted to strain. This energy stored in the molecules for a short period of time imparts an elastic nature to the fluid. This elasticity is similar to that described in rubber solutions, and it is based on the interaction between various segments of the molecular chain of hyaluronic acid. It is also called entropy elasticity because it depends on the states of order and disorder in the arrangement of the molecular chains of hyaluronic acid.

A typical set of values of the storage and loss moduli as a function of strain frequency is shown in Figure 5-1. The three sets of curves of dynamic shear moduli are selected as representative samples of synovial fluid obtained from the normal joint of a young (20 yrs.) and an old (67 yrs.) donor and from an osteoarthritic joint of an old (63 yrs.) donor.

In all three fluids, as the strain frequency increases, both the loss and the storage modules increase. The absolute values of these moduli at low frequencies vary considerably. The fluid obtained from the normal young joint has the highest value and that obtained from the osteoarthritic joint the lowest. Most importantly, as the frequency increases, the curves representing the loss and storage moduli cross each other in the two normal fluids, but not in the pathological fluid. This means that the normal fluids are predominantly viscous at low strain frequency and predominantly elastic at high strain frequency. Since this crossover is not observable in the patho-

logical fluid, one has to conclude that this fluid behaves in the entire frequency range, as a predominantly viscous rather than an elastic fluid. It is important to note that the strain frequencies at which these measurements were made are within the range to which the fluid is exposed in the course of the normal movement of the knee joint (flexing under no load, walking, running).

These observations can be explained on the molecular level by configurational adjustments of the hyaluronic acid chains. At low strain frequencies, the configurational adjustments of the chains, owing to thermal (Brownian) motions are rapid enough to allow the molecule to maintain its original conformation. That is, under imposed strain, the chains slip by each other, which results in a viscous flow. Therefore, the rheological properties of the fluid are predominantly viscous. At high strain frequency, the configurational readjustment of the chains cannot occur between the short periods of the oscillating strain, and the molecules cannot maintain their original conformation. That is, under the imposed strain, the molecules deform sinusoidally and alternately store the mechanical energy and then release it elastically. Under these conditions, the fluid's rheological behavior is predominantly that of an elastic body.

The most remarkable behavior of synovial fluid under increasing strain frequency is its rapid transition from viscous fluid to elastic body. This transformation is reversible and has no deteriorating effect on the molecule. Natural rubber and industrial polymers with elastoviscous properties show considerably different behavior in that this transition occurs during a considerably extended frequency range.

What are the biological implications of this frequency-dependent viscoelastic transformation of synovial fluid? The synovial fluid occupies narrow channels between the soft tissues of the joint, and it is sandwiched between the two cartilage surfaces. The measurements of dynamic shear moduli tell us that the fluid will move as a viscous liquid in these channels when the joint moves at

TABLE 5-2. Rheological Properties of Synovial Fluids Aspirated from the Knees of Human Subjects of Various Ages.*

Age Group (yrs.)	Subjects	Elastic Modules G' (dyn./sec. ⁻²)†	Viscous Modules G'' (dyn./sec. ⁻²)†	Crossover Point of Two Moduli (G', G'') (dyn./sec. ⁻¹)	Two Moduli (G', G'') at Crossover Point (dyn./sec. ⁻²)
18-27	16	1170 ± 130	450 ± 82	0.13 ± 0.02	332 ± 41
27-35	18	226 ± 7	72 ± 8	0.21 ± 0.004	59 ± 6
52-78	26	189 ± 33	101 ± 12	0.41 ± 0.12	61 ± 7

* Data taken from the unpublished work of E. A. Balazs, N. W. Rydell, P. O. Seppälä, I. F. Duff, D. A. Gibbs and E. W. Merrill.

† Measured at 2.5 cycle/sec.⁻¹

low shear frequency. Under high shear frequency movements, the fluid does not move in the channels; rather it behaves as an elastic solid, storing the mechanical energy.

But the fluid also impregnates the surface layer of articular cartilage and synovial tissue. Thus, it separates cells (synovial cells) and protein fibrils (collagen), preventing them from direct contact with each other. This means that under slow mechanical loading of the joint, when articular cartilage and synovial tissue are exposed to low frequency strain, hyaluronic acid behaves as a viscous oil. The fluid between the cells and collagen fibrils is displaced (flows) under the mechanical forces and the tissue itself deforms. On the other hand, at high mechanical loading rates of the joint, when these tissue layers are exposed to high frequency strain, hyaluronic acid transforms into a highly deformable elastic system. Therefore, the fluid, which contains hyaluronic acid, is not displaced, and the tissue itself is not deformed. This means that the hyaluronic acid in the surface layers of articular cartilage and synovial tissue absorbs mechanical stress thereby protecting the cells and collagen network from mechanical shock and deformation. In other words, this polysaccharide serves in these tissue layers as a shock absorber.

The cells, which are extremely sensitive to mechanical stress, and the rigid, load-supporting collagen fibrils are surrounded by the elastoviscous hyaluronic acid solution. There is a mechanical coupling between the

rigid system of cells and fibrils and the energy-storing and energy-dissipating system of hyaluronic acid. Therefore, a large part of the stress imposed on the entire system is converted to elastic deformation of the hyaluronic acid. By this mechanism, the stress-sensitive elements of the system (cells) are protected and the structural integrity of the tissue (special organizational pattern of the fibrils) is maintained.

Effects of Aging*

The rheological properties of synovial fluid change considerably during aging. The elastic (storage) modulus (G') and viscous (loss) modulus (G'') decrease sharply after the age of 27 years. The elastic modulus drops further after the age of 52 years (Table 5-2). As we pointed out above, synovial fluids, like solutions of pure hyaluronic acid, show a rapid transition from viscous to elastic behavior when the strain frequency increases. The frequency at which this transition occurs or, more precisely, where the curves of the storage and loss moduli cross one another and both moduli have the same numerical value, is specific for a given fluid (Fig. 5-1). This frequency value at the cross-over point increases with aging (Table 5-2). The value of the two moduli at the cross-over point drops sharply after the 27th year but does not change

* The data reported here are from the work of Balazs, Rydell, Seppälä, Duff, Merrill and Gibbs. While the details of this work are as yet unpublished, a brief review can be found in Balazs, 1969.⁶

TABLE 5-3. Concentration and Limiting Viscosity Number of Hyaluronic Acid and the Rheological Properties of Synovial Fluids Aspirated from Human Pathological Knees.*

Pathological Condition	Fluids Analysed	Volume Fluid Collected (ml.)	Hyaluronic Acid		Elastic Modules G' (dyn./sec. ⁻²)	Viscous Modules G'' (dyn./sec. ⁻²)	Crossover Point of Two Modules (G', G'') (cycles/sec. ⁻¹)
			mg./ml.	ml./g.			
Osteoarthritis	11		1.55 ± 0.14	3800 ± 350	85 ± 54	48 ± 28	4.7 ± 1.9
Traumatic Arthritis	3	7-20	0.69-1.76	2100-4200	2-41	2-29	1.3-2.9
Gout	4	3-5	1.28 ± 0.14	3500 ± 690	30 ± 10	15 ± 7	0.9 ± 0.3
Chondrocalcinosis	2	2.5	0.75	3700	5	5	1.7
			1.22	3900	22	13	0.9

* Data taken from unpublished work of E. A. Balazs, P. O. Seppälä, N. W. Rydell, I. F. Duff, E. W. Merrill and D. A. Gibbs.

later. Thus, the rheological properties of synovial fluids in all three age groups studied are significantly different. The fluid from young subjects is very highly elastic and rigid at relatively low frequencies. The fluid from middle-aged subjects is less rigid but still highly elastic at higher frequencies. The synovial fluid from older subjects is less rigid, less viscous, and less elastic at all frequencies.

The frequencies at which these measurements were carried out were in the same range as the frequencies at which the joints are loaded and flexed during natural movements of the body. Therefore, some conclusions can be drawn about the rheological behavior of the fluid in the joint submitted to various rates of strain. Between the ages of 18 and 39, the synovial fluid undergoes a substantial decrease in rigidity, but retains its generally elastic character. With further aging the elasticity decreases in such a way that under the frequency conditions of normal knee motion the synovial fluid changes from the highly elastic fluid in the young to a nonelastic viscous fluid in the old.

Since the concentration, size, shape, and limiting viscosity number of individual hyaluronic acid molecules does not change in the synovial fluid of normal human knee joint between the ages of 27 and 78 but the elastoviscous properties of the fluid radically decrease, one has to assume that the interaction between the chains of the neighbor-

ing molecules is altered. Recent studies on hyaluronic acid, carried out using X-ray diffraction and optical rotation measurements, indicate that a certain amount of the individual polysaccharide chains form double helical junction points or crosslinks that increase the elastoviscous properties of the polymer. It is possible that during aging the amount of these double helical crosslinks between the chains of neighboring molecules decreases. This in turn would make the molecular chain segments less stiff and the solution less elastic.

Pathological Fluids*

In the synovial fluids aspirated from joints with osteoarthritis, traumatic arthritis, gout, chondrocalcinosis, and rheumatoid arthritis, the concentration, limiting viscosity number, and molecular weight of hyaluronic acid is lower than in normal joints.^{14,35,40} Consequently, all rheological properties of the fluid, such as the dynamic viscous and elastic moduli and the crossover point of the two moduli are also much below normal values (Table 5-3; Fig. 5-1). Thus, in the pathological joint, the synovial fluid does not have those rheological properties that protect the synovial tissue and cartilage against mechanical stress.

* Data reported here are from the work of Balazs, Seppälä, Rydell, Gibbs, Duff and Merrill. While the details of this work are not yet published, a brief review of it can be found in Balazs [1968].⁵

THE SURFACE OF THE ARTICULAR CARTILAGE

There is some indication that hyaluronic acid is not evenly distributed in the entire joint space. On the surface of the articular cartilage and on the surface of the synovial tissue, a hyaluronic acid layer was observed which cannot be easily washed away from these tissue surfaces. The concentration of the hyaluronic acid in these layers is higher than in the fluid aspirated from the joint. It is not clear what kind of molecular interaction is responsible for the "accumulation" of hyaluronic acid on these tissue surfaces.

Electron microscopic studies showed that a 1- to 2- μ -thick layer of hyaluronic acid-protein complex covers the surface of the articular cartilage. This layer is anchored to the fibrillar collagen matrix of the cartilage and can be removed from it by treatment with proteolytic enzymes or hyaluronidase. With aging, and in osteoarthritic cases, this layer becomes thicker. It is now known, however, how the hyaluronic acid-and protein content of the layer change with aging and with various pathological conditions.⁸

Histochemical and chemical analyses carried out on the cartilage close to the articular surface indicate the presence of hyaluronic acid 50 to 100 μ deep into the cartilage where it shares the space between the collagen fibrils with sulfated proteoglycans.⁸ The deep layers of the cartilage matrix contain only sulfated proteoglycans (proteoglycans of chondroitin 4-sulfate and keratan sulfate) but no hyaluronic acid.

Scanning electron microscopy also showed an accumulation of synovial fluid (hyaluronic acid and proteins) on the surface of articular cartilage^{19,24,42} (1970). Under conditions of extreme load, experiments *in vitro* show, this fluid layer protects the cartilage surface.⁴²

One can only speculate about the importance of this layer in the normal function and pathology of the joint. Since it represents the only morphologically visible barrier between the cartilage matrix and the

joint space, it is tempting to assume that it is responsible for the protection of the cartilage surface. Since the synovial fluid of the normal joint does not contain appreciable amounts of sulfated proteoglycans, it is possible that the surface layer of the cartilage impregnated with hyaluronic acid forms an effective barrier against diffusion or flow (under pressure caused by compressing the cartilage when the joint is loaded) of the sulfated proteoglycans into the synovial fluid. In various pathological conditions sulfated glycosaminoglycans were found in the synovial fluid,³⁵ suggesting that the integrity of the surface layer of the articular cartilage impregnated with hyaluronic acid is responsible for the normal maintenance of the cartilage by preventing the leakage of proteoglycans into the synovial space and the subsequent loss of this important component of the cartilage matrix. The missing link in this argument, of course, is the complete lack of knowledge of the chemical composition of the cartilage surface layer in pathological conditions.

THE EFFECT OF HYALURONIC ACID ON CELL ACTIVITIES

Recently, several effects of hyaluronic acid on cells *in vitro* and *in vivo* have been reported. None of these effects have been directly connected to the pathological processes in the joint. Nevertheless, the effect of hyaluronic acid on cell activities is so general that the assumption that it is operative in wound healing and inflammation of the joint is justified.¹²

Na-hyaluronate was found to be a cell-immobilizing agent. The movement of cells of the lymphomyeloid system (lymphocytes, granulocytes, macrophages) is inhibited by this biopolymer and by the tissue fluids that contain this molecule (synovial fluid, liquor vitreus). The fast-moving cells of the lymphomyeloid system exhibit different sensitivities to hyaluronic acid. This cell-immobilizing effect is specific for these types of cells because cells that move slowly in

vitro (fibroblasts) are not affected. The effect of hyaluronic acid on the motility of cells is not directly related to the bulk viscosity of the solution in which the cells are moving. The effect is dependent on the limiting viscosity number of the hyaluronic acid used. Hyaluronic acid preparations with low limiting viscosity numbers are less effective cell movement inhibitors than preparations with high limiting viscosity numbers. Pathological synovial fluids, with low limiting viscosity numbers, are less effective than normal fluids.¹³

Hyaluronic acid also inhibits the modulation of lymphocytes to lymphoblasts. When blood lymphocytes, stimulated by mitogens (phytohemagglutinin, pokeweed mitogen, streptolysin O or purified protein derivative of tuberculin) are placed in viscous hyaluronic acid solution, the transformation of lymphocytes to lymphoblasts and the subsequent mitosis is prevented as long as the cells are surrounded and separated from each other by viscous Na-hyaluronic solutions.¹⁷

Na-hyaluronate can also prevent the targets cells from killing sensitized lymphocytes. The cytotoxic effect of lymphocytes is inhibited when the Na-hyaluronate concentration in the medium separating the target cells from the lymphocytes reaches a certain concentration.¹¹

The graft-versus-host reaction could be inhibited with Na-hyaluronate when the donor cells are injected into the peritoneal cavity. Apparently, the spleen cells injected with Na-hyaluronate do not find their target organ (spleen, liver); or, if they do, their proliferation is inhibited.¹³

Na-hyaluronate seems also to influence the wound healing of articular cartilage, tendons, and fasciae. Relatively few experiments have been reported in this area and therefore, one has to regard these results as preliminary. When the dorsal fasciae of rabbits and guinea pigs and the long extensor tendons of the legs of rabbits were traumatized by mechanical damage and, after the trauma, viscous Na-hyaluronate solution (sterile and pyrogen-free) was

applied to the damaged area, the subsequent formation of granulation tissue and fibrous adhesions was considerably suppressed. Na-hyaluronate also suppressed formation of granulation tissue around foreign bodies (polyethylene).^{17,30,32} All these investigations suggest that hyaluronic acid has a cell regulatory function which specifically affects the lymphomyeloid system during the inflammatory process.

Effect on Frictional Resistance

It has been generally accepted for a long time that the frictional resistance of those parts of the joint that move adjacent to each other (articular cartilage, synovial tissue, ligaments, tendons within their sheaths, walls of bursae) is decreased by the viscous synovial fluid. The viscosity of the fluid has been regarded as the key to this lubricating effect, and the high-molecular-weight hyaluronic acid as the essential component of the lubricating fluid.^{25,27,42}

Recently, this joint lubrication was further defined by separating it into two problem areas: the lubrication of the "soft tissues" (ligaments and synovial tissue) and the lubrication of the cartilage surfaces.²⁷ The role of hyaluronic acid in diminishing the frictional resistance of "soft tissues" sliding across each other was confirmed. On the other hand, the same action of hyaluronic acid on cartilage sliding over cartilage was questioned. A glycoprotein fraction was found in the synovial fluid that decreased the coefficient of friction between moving cartilage surfaces.⁴¹ The importance of hyaluronic acid as an agent that reduces the frictional resistance between the moving surfaces inside the joint, and between tendons and their sheaths, is still not fully understood.

Role of the Cartilage Surface

As described above, the surface layer of articular cartilage is impregnated with hyaluronic acid. One can visualize the two opposing cartilage surfaces and the thin layer of synovial fluid between as a con-

tinuous hyaluronic acid network. The hyaluronic acid network is anchored onto the collagen fibrillar matrix of the surface layer of cartilage of both sides. The space between the two collagen matrices is filled with the same hyaluronic acid molecular network that impregnates the collagen matrix. Therefore, dislocations between the two moving cartilage surfaces occur not between two rheologically different systems (solid cartilage and synovial fluid), but within the hyaluronic acid network. This concept has two important biological implications. One, there are no asperities or ripples on the sliding surfaces. That is, the beautiful scanning electron micrographs showing the unhydrated cartilage-synovial fluid surface with its many crevices do not picture the real sliding surface. The real sliding surface is not that which one exposes by breaking the continuity of the hyaluronic acid network and its dehydrated picture in the electron microscope does not give the proper impression of a highly hydrated hyaluronic acid molecular network. Two, the hyaluronic acid impregnated cartilage surface that serves as a barrier against the movements of macromolecules in and out of the cartilage is not distributed by the movements in the joint. Therefore, the integrity of this layer and the composition of the cartilage matrix is maintained. According to this hypothesis, the major role of hyaluronic acid on the cartilage surface is to provide the real sliding surfaces and to maintain the integrity of the cartilage matrix.

Control of Cell Invasion

There is no epithelial barrier on the surface of the synovial tissues that would prevent cell migration from these tissues into the synovial space and on the surface of the soft tissues and the cartilage of the joint. It was proposed that hyaluronic acid prevents the invasion of cells into joint space.^{7,9} This concept is especially important in view of the fact that in all acute or chronic inflammatory processes of the joint, both the concentration and size of the hyaluronic acid

molecules decrease and, at the same time, the cell population in the joint space increases. It is important to note that the pathological fluid is less effective in preventing the migration of the lymphomyeloid cells *in vitro* than the normal fluid. These findings, while suggestive, do not present direct proof of the role of hyaluronic acid as a cell movement controlling factor in the joint.

HYALURONIC ACID AS A THERAPEUTIC AGENT

In acute and chronic inflammation and in most degenerative processes of the joint, the concentration and molecular size of hyaluronic acid decreases in the synovial fluid. Consequently, the viscosity and elasticity of the fluid also decreases. We suggested that intraarticular application of highly purified (protein content <0.3%) concentrated (10 to 20 mg./ml.) Na-hyaluronate that contains fairly large molecules (molecular weight 1 to 3 million) of this biopolymer can influence the healing and regeneration of the cartilage and soft tissues of the joint. The rationale for this suggestion is that the injected hyaluronic acid will accumulate on the articular and synovial tissue surfaces, thereby "reinforcing" the natural barriers which are most probably deteriorated in the course of the pathological process. Thus, the injection of hyaluronic acid into a diseased connective tissue compartment in which it is normally present can properly be called a macromolecular implantation. The main objective of the implantation is to increase the hyaluronic acid concentration in the joint well above the pathological and even the normal level. Since this biopolymer is a natural component of the joint, it metabolizes by diffusion and probably by phagocytic activity of macrophages. Consequently, the elevated concentration in the joint caused by the injection decreases to normal level within days. Nevertheless, one expects that the invasion of the lymphomyeloid cells into the joint space is halted by the temporary increase of hyaluronic acid

concentration by the same mechanism as the movement of these cells is inhibited by this biopolymer *in vitro*. Furthermore, the hyaluronic acid accumulated on the surface of the cartilage and soft tissues may block inflow of proteins (immune complexes) and proteoglycans into the joint space, thereby triggering a healing process in the cartilage and decreasing inflammation in the synovial tissue.

Experiments carried out in dog and rabbit joints indicate that intraarticular cartilage heals better when the Na-hyaluronate concentration of the synovial space is increased after wounding by implantation of this biopolymer.³² It was also found that the granulation reaction in subcutaneous tissue after surgical wounds³⁰ and in adhesion formation between tendon and tendon sheaths after mechanical damage is decreased when Na-hyaluronate is applied to the wounded surfaces.³²

Treated with intraarticular administration of Na-hyaluronate traumatic arthritis in horses, rapidly improved and, in most cases, the normal function of the joint was restored after one or two treatments.^{31*}

Na-hyaluronate was administered intra-articularly for human osteoarthritis by several investigators. (Rydell, Helfet, Peyron). Results of these investigations are reported elsewhere in this book (see p. 142).¹⁷⁹

Na-hyaluronate was also implanted into human vitreous during surgical procedures for retinal detachment. Since hyaluronic acid is present in the vitreous in its highest concentration adjacent to the retina, it was thought that in chronic inflammation caused by retinal wounds, the healing would be promoted by implantation of this biopolymer. Furthermore, it was stipulated that viscoelastic Na-hyaluronate solution would facilitate the reattachment of the retina in cases where other surgical techniques failed.⁴ Several investigators found

that Na-hyaluronate implanted into human vitreous in complicated cases of retinal detachment facilitated the reattachment of retina to choroid, thus improving the healing of the vitreoretinal wound.^{2,15,22,28}

One has to point out that the Na-hyaluronate used for implantation into the joint, vitreous, or other connective tissue compartments must be free from impurities that can cause immunological reaction or tissue irritation. Furthermore, the preparation must exhibit specific biological activity on lymphomyeloid cells. Such Na-hyaluronate fractions have been prepared from both human (umbilical cord) and avian (comb) tissues;[†] it was used in most of the experimental and clinical work cited above.

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