FROM THE INSTITUTE OF MEDICAL CHEMISTRY, UPPSALA.
DIRECTOR: PROF. G. BLIX

THE CHEMISTRY OF SYNOVIAL FLUID WITH SPECIAL REGARD TO HYALURONIC ACID[1]

By

LARS SUNDBLAD

From a biochemical point of view, synovial fluid may be regarded as a plasma dialysate, containing varying amounts of protein and a specific component—hyaluronic acid. The latter gives the fluid its high viscosity and thus its ability to form a lubricating film, separating the articular ends. The present survey will deal mainly with the question of the hyaluronic acid, as it is in this field that the greatest progress has been made in recent years.

Already in 1846, Frerichs had isolated a so-called mucin from synovial fluid by precipitation with acetic acid. He realized that it was a compound of protein and carbohydrate, but the nature of the carbohydrate remained unknown until the thirties of this century, when Meyer and his co-workers found it to be identical with the polysaccharide of the vitreous humour of the eye, from which source it was first isolated and given the name of hyaluronic acid by the same authors. Hyaluronic acid is a highly polymerized acidic polysaccharide, built up from units of glucuronic acid and N-acetyl glucosamine linked together so as to form a long chain molecule. It is thus closely related to the chondroitin-sulphuric acid found in cartilage, but the molecule of hyaluronic acid is even longer and contains no sulphate radical. Subsequent investigations have revealed its presence in other mucoid tissues, such as the umbilical cord, the connective tissue of the skin, tumours of mesenchymal origin, and also the capsule of group A and C hemolytic streptococci. With regard to the well-known "spreading" activity of hyaluronidases in various mesenchymal tissues, it is generally assumed that hyaluronic acid, chondroitin-sulphuric acid or other closely related polysaccharides form main constituents of the basic

[1] Read at the meeting of the Svenska Ortopedföreningen, November 1949, Stockholm.

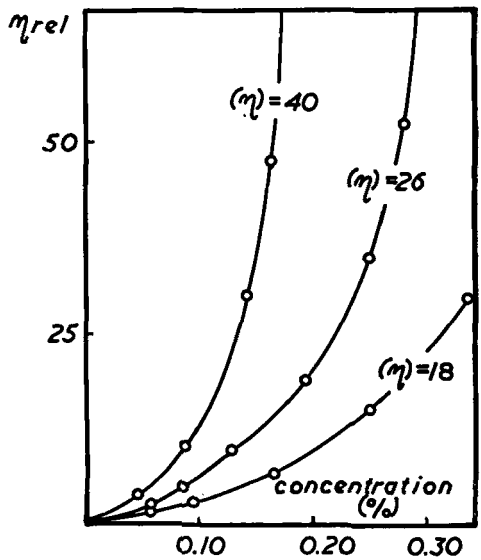
substance of all connective tissue, evidently acting as cement in the shape of a gel between the collagen fibres. (References concerning hyaluronic acid and hyaluronidases are to be found in the review given by Meyer in 1947. The "spreading" phenomenon is treated at length in the article of Duran-Reynals in 1942).

The joint cavity is generally regarded as an enlarged tissue space, containing a comparatively large amount of the inter-fibrillary substance of ordinary connective tissue. The study of the polysaccharide contained in synovial fluid may thus provide information as to the state of the mesenchymal tissues in general. There is, however, no evidence that polysaccharides containing sulphate are present in joint fluid, in contrast to the existence of about equal proportions of sulphate-containing and sulphate-free carbohydrates in subcutaneous, connective tissue (Meyer 1947). It would seem a probable explanation that the former are more firmly bound to protein structures of the tissue than the latter.

It has been claimed that hyaluronic acid is a specific secretion from cells in the synovial membrane. In its native state, the acid probably forms a very loose compound with protein (Ropes et al. 1947). In electrophoresis, however, hyaluronic acid moves as an independent component, well separated from the proteins (Blix 1940, Hesselvik 1940). On acidification with acetic acid, it combines with the proteins present, and the "mucin" thus formed should accordingly be regarded as an artificial product. A solution of isolated hyaluronic acid also gives rise to a mucin-like precipitate, but only if proteins are present in the solution. The proportion of hyaluronic acid and protein in such a precipitate depends among other factors on the concentration ratio of the two components in the original solution. Degradation of the hyaluronic acid causes a progressive decrease of the amount of protein carried down by the polysaccharide, and also changes the appearance of the precipitate in a characteristic way. If highly polymerized hyaluronic acid from normal synovial fluid is used, a ropy mucin in a clear solution is obtained. If, on the other hand, the fluid is first treated with a minimal amount of hyaluronidase, acidification gives rise to either a flocculent precipitate in a cloudy solution or to a colloidal turbidity, depending on the degree of degradation. It has been found (Ropes et al. 1947) that pathological fluids in rheumatoid arthritis and also in specific forms of infective arthritis present a changed precipitability with acetic acid, similar to that produced by incubation of normal fluid with hyaluronidase. Pathological fluids in affections of traumatic origin, however, present the normal type of precipitate, which indicates the possible value of the

precipitation test in differential diagnosis between these two types of joint affections.

A quantitative approach to the same problem may be made by means of viscosity determination. The high viscosity characteristic of synovial fluid is due to its hyaluronic acid content and depends on the elongated form of the molecules of this substance. Incubation of



Relative viscosity as a function of concentration in solutions containing hyaluronic acid of varying particle length. Relative values of intrinsic viscosity [η] are also given.

the fluid with hyaluronidase reduces the viscosity to approximately that of water, whereas protein-splitting enzymes leave the viscosity essentially unchanged. Although the concentration of protein in normal synovial fluid is almost ten times that of hyaluronic acid, the latter component is responsible for more than 99 per cent of the viscosity of the fluid.

The viscosity of a fluid containing chain-shaped molecules increases greatly with the increasing length of the chain. This is illustrated by fig. 1, which gives the relation between relative viscosity and concentration in solutions containing hyaluronic acid, the particles of which are of varying length. It appears that there is no linear relation between viscosity and concentration, and also that the viscosity/concentration ratio must increase more rapidly with increasing concentration in the solution containing the longest molecules. The ratio of viscosity to concentra-

tion as the latter approaches zero may be inferred from viscosity determinations in dilute solutions by means of graphic extrapolation. The limiting value thus found is generally called the *intrinsic viscosity,* and its numerical value is a function of the ratio of length to thickness of the dissolved molecules. In this way, viscosity determination may be used for the evaluation of relative molecular dimensions, provided that the concentration of the solute is known. The numerical values of intrinsic viscosity depend upon the conditions under which the measurements are performed, with regard to the presence of inorganic salts, the temperature and the pH. On account of the pronounced anomalous viscosity of synovial fluid, the rate of flow must be kept essentially constant in the determinations, or the relation of the relative viscosity to the rate of flow must be known. A linear relation between intrinsic viscosity and particle length calculated from double-refraction data was found for hyaluronate preparations by Blix and Snellman in 1945.

In the tables 1 and 2, some preliminary results are given of determinations of the hyaluronic-acid content, viscosity and protein content of synovial fluids, carried out at the Institute of Medical Chemistry in Uppsala[1]. The concentration of hyaluronic acid is calculated from determinations of the glucosamine component, which is usually found as the difference between the total amount of glucosamine and the amount of protein-bound glucosamine, by estimation for instance, of the loss of glucosamine content brought about by elimination of the polysaccharide through enzymatic degradation and dialysis[2].

Table 1 shows clearly that the concentration of hyaluronic acid in different varieties of synovial fluid varies greatly, approximately in inverse proportion to the volume of the fluid. To judge from the values of the intrinsic viscosity, the average length of the polysaccharide particles is of the same magnitude in the various groups. A comparison with the data given by Blix and Snellman in 1945, concerning hyaluronate preparations, would reveal the approximate molecular weight of the acid in its native state to be between 1 and 1.5 million. The relative viscosity of undiluted fluid obtained from horses was within the range 2.6-28.0 and calculation from dilution curves proved it to vary between 50 and 300 in undiluted fluids of the other groups. The wide variation as to hyaluronic-acid content and relative viscosity in different types of fluid is probably partly due to the varying secretive

[1] A short report of the first results obtained was given by Blix in 1948.

[2] The details of the technique and calculations employed will be communicated elsewhere.

TABLE 1

Hyaluronic acid and total protein concentration in some varieties of normal synovial fluid.

Species	Number of cases	Fluid volume ml	Hyaluronic acid g per 100 ml	Intrinsic viscosity 37°	Total protein g per 100 ml
Human[1]	4	0.2-1	0.285	43.2	2.54
	8	4-10	0.183	42.0	3.58
Horse[2]	7	15-40	0.061	48.0	2.00
Calf[2]	11	1-3	0.193	46.2	1.74

The last three columns give the average values obtained.

capacity of the synovial tissue, but also depends upon the degree of dilution by plasma dialysate of the hyaluronic acid produced. According to Ingelmark and Sääf, 1948, the amount of synovial fluid seems to increase when the joint bears weight, which may explain the fact that the relative viscosity in human beings is generally lower in the joints of the lower limb.

In table 2, some analyses of knee effusions in various articular disorders are assembled. The grouping of the material may of course be open to criticism and is to be regarded as provisional.

The material was divided into the following five groups:

1. Effusions of traumatic origin, in which the available clinical information did not indicate coincident joint disease.
2. Cases with radiological evidence of arthrosis deformans of varying degrees. In some of these cases, trauma was also given as the cause of the effusion.
3. Cases in which arthrography or subsequent operation revealed lesions of the menisci. Most of these fluids were obtained some time after the actual injury.
4. Effusions in rheumatoid arthritis of varying degrees of activity. Most of these cases were diagnosed as chronic primary polyarthritis, but some of them were designated as secondary chronic polyarthritis.

[1] Only two samples of these fluids were obtained by aspiration of normal knee joints. The rest are cases with small effusions of the knee joint after minor injuries, in which the clinical examination did not reveal other signs of joint affection.

[2] These fluids were obtained after slaughter.

5. In this group, cases of infective arthritis of the knee in the acute or subacute, usually febrile, stage were collected. The erythrocyte sedimentation rate was highly or moderately increased in all these cases. The usual diagnosis was "acute synovitis of uncertain etiology".

TABLE 2

Hyaluronic acid and total protein concentration and changes of intrinsic viscosity in effusions of the knee joint.

Group		Fluid volume ml	Hyaluronic acid g per 100 ml	Intrinsic viscosity 37°	Total protein[1] g per 100 ml
Traumatic synovitis (27 fluids)	I	15- 85	0.101 ± 0.008	41.0 ± 1.2	4.42 ± 0.17 (23)
Arthrosis deformans (16 fluids)	II	2- 90	0.146 ± 0.014	27.2 ± 2.2	3.70 ± 0.25 (15)
Lesions of the menisci (13 fluids)	III	1- 35	0.175 ± 0.017	29.4 ± 1.6	3.46 ± 0.46 (8)
Rheumatoid arthritis (37 fluids)	IV	10-200	0.150 ± 0.005	26.6 ± 1.1	5.45 ± 0.12 (22)
Acute and subacute infective arthritis (15 fluids)	V	40-180	0.153 ± 0.020	18.5 ± 1.6	5.30 ± 0.35 (9)

The values given in table 2 are the arithmetical means with their standard errors. As is seen from the table, the content of hyaluronic acid is lowest in the traumatic effusions, although the variation is great. The protein content is low in degenerative joint disease, moderately increased in the traumatic group, and strongly increased in the effusions caused by rheumatoid and infective arthritis. To judge from the average value of intrinsic viscosity, the length of the hyaluronic-acid particles is essentially the same in the fluids found in traumatic disorders as it is in the normal fluids of table 1. In all the other groups, the acid is more or less degraded, most markedly in acute febrile synovitis. In group 2, the changes are more pronounced in advanced arthrosis deformans, but the average degree of hyaluronic-acid polymerization is also significantly lower than normal in fluids from joints with radiographic evidence of early arthrosis deformans or lesions of the menisci. In rheumatoid arthritis, the degradation was more marked in the acute febrile stages of the disease.

[1] The total protein was not determined in all samples. The figures given in parentheses indicate the number of fluids from which the averages were obtained.

Chemical analysis of joint fluids, as described above, thus seems to be of value in differential diagnosis of articular disorders. The finding of degraded hyaluronic acid in an effusion of supposed traumatic genesis thus definitely suggests the existence of an underlying joint disease, especially if the concentration of hyaluronic acid is high. High protein content further indicates that the disease is of an infective type, and low protein content indicates a degenerative type of joint affection.

The cause of the change of hyaluronic acid, stated above, may be degradation due to the activity of enzymes or other factors known to depolymerize hyaluronic acid, or due to incomplete synthesis of the polysaccharide. The latter view is favoured by the fact that active hyaluronidase or free hexosamine have not been found in the pathological fluids. Liberation of low-molecular polysaccharides from the cartilage would be a possible explanation in some cases, but it is unlikely that this is a factor of any great importance.

It has not yet been established whether the lowered viscosity, due to chemical degradation of hyaluronic acid, may cause insufficient stability of the lubricating films in some cases. Previous experimental investigations, however, have shown that even synovial fluid of low viscosity forms films that do not break down, although much heavier loads than those encountered under physiological conditions have been used in the experiments (Ropes et al. 1947).

The osmotic pressure exerted by mucin has been determined indirectly, by Ropes and his co-workers in 1939, as the difference betwen the measured colloidal osmotic pressure of normal cattle fluid and the pressure calculated from the protein content. The writers mentioned found that the osmotic effect per gram of mucin is nine times that of serum albumin, and concluded that mucin is likely to be of importance to the water metabolism in the joint cavity, in spite of its low concentration.

If synovial fluid is compared with plasma with respect to other constituents than hyaluronic acid and proteins, the results are in accordance with the view that synovial fluid is a plasma dialysate. (For references, see the review by Bauer and his co-workers, 1940, and the monograph by Kling, 1938). The distribution of electrolytes between plasma and fluid is in harmony with the Donnan equilibrium theory. The calcium content, however, has been found to be much higher than expected, which has been explained by the fact that mucin has a calcium-binding power, about ten times as great as that of serum albumin (Ropes et al. 1947). The pH value is usually somewhat higher than in plasma, but it may be acid in septic arthritis. Non-electrolytes

occur in approximately the same concentration as in serum, except that the sugar content is lower, most markedly so in fluids with high cell counts.

SUMMARY

Synovial fluid may be regarded as a plasma dialysate, containing some protein and a specific component—hyaluronic acid. The latter component is responsible for the high viscosity of the fluid and is, therefore, probably of great importance to its function as a lubricant. The content of hyaluronic acid is between 0.2 and 0.3% in normal human fluid, and its average molecular weight is estimated to be more than one million. Effusions in traumatic disorders contain about 0.1% of hyaluronic acid, the polymerization degree of the latter being approximately normal. Degenerative as well as inflammatory conditions present a relatively high concentration of hyaluronic acid in a partly depolymerized state. The protein content is low in arthrosis deformans, moderately increased in traumatic disorders, and high in rheumatoid and infective arthritis. The value of chemical analysis in differential diagnosis of joint diseases is discussed briefly. Finally the significance of the findings in joint diseases is touched upon.

RESUME

La sérosité des articulations peut être considérée comme une dialyse du plasma contenant un peu d'albumine et un composant spécifique — l'acide hyaluronique.

L'acide hyaluronique donne à la sérosité sa haute viscosité et présente sans doute pour cette raison une grande importance pour sa fonction de lubrifiant.

Le taux d'acide hyalurique varie entre 0,2 et 0,3 % dans la sérosité normale chez l'homme et le poids moyen moléculaire des polysaccharides peut être calculé à plus d'un million. L'exsudat d'origine traumatique contient 0,1 % d'acide hyaluronique d'un degré de polymérisation à peu près normal. Dans les maladies articulaires aussi bien de type dégénératif qu'inflammatoire, l'exsudat contient une concentration relativement élevée d'acide hyaluronique qui est partiellement dépolymérisé. Le taux d'albumine est bas dans l'exsudat de l'arthrose déformante, lègèrement augmenté dans l'exsudat traumatique et élevé dans l'exsudat des arthrites rhumatismales et infectieuses. La valeur des analyses chimiques pour le diagnostic différentiel des maladies arti-

culaires est sommairement discutée. Enfin, est abordée la question de l'importance des modifications constatées pour la pathophysiologie de l'articulation.

ZUSAMMENFASSUNG

Die Gelenksflüssigkeit kann man als ein Plasmadialysat, das etwas Eiweiss und eine spezifische Komponente — Hyaluronsäure — enthält betrachten. Die Hyaluronsäure gibt der Gelenksflüssigkeit ihre grössere Viskositet und ist darum wahrscheinlich wichtig für deren Funktion als Gleitmittel (im Englischen „Lubrication"). Der Hyalonsäureinnhalt wechselt zwischen 0,2 und 0,3 % in der normalen Gelenksflüssigkeit beim Menschen. Das durchschnittliche Molekülgewicht der Polysaccharide kann man berechnen als über eine Million. Exsudat traumatischer Genese enthält ungefähr 0,1 % Hyaluronsäure mit einem ungefähr normalen Polymerisierungsgrad. Exsudat bei Gelenkskrankheiten sowohl degenerativen als entzündlichen Ursprungs enthält Hyaluronsäure in relativ hoher Konzentration, die in diesen Fällen teilweise depolymerisiert ist. Der Eiweissinhalt ist niedrig in Exsudaten bei Arthrosis deformans, mässig erhöht bei traumatischen Exsudaten und hoch in Exsudaten von chronischer Polyarthritis oder Infektarthritiden. Der Wert chemischer Analysen für die Differentialdiagnose wird in Kürze diskutiert. Zum Schluss wird die Bedeutung der gefundenen Veränderungen für die Patophysiologie der Gelenke kurz besprochen.

REFERENCES

Bauer, W., Ropes, M. W., and *Waine, H.:* Physiol. Rev. *20*, 272, 1940.

Blix, G.: Acta Physiol. Scand. *1*, 29, 1940.

— Acta Orthopaed. Scand. *18*, 18, 1948.

Blix, G., and *Snellman, O.:* Arkiv för Kemi, Mineralogi och Geologi *19 A*, No. 32, 1945.

Duran-Reynals, F.: Bact. Rev. *6*, 197, 1942.

Frerichs, F. T.: In R. Wagner, Handwörterbuch der Physiologie, *3*, Abt. 1, 463, 1846. Braunschweig.

Hesselvik, L.: Acta Med. Scand. *105*, 153, 1940.

Ingelmark, B. and *Sääf, J.:* Acta Orthopaed. Scand. *17*, 303, 1948.

Kling, D. H.: "The Synovial Membrane and the Synovial Fluid", 1938. Medical Press. Los Angeles.

Meyer, K.: Physiol. Rev. *27*, 335, 1947.

Ropes, M. W., Bennett, G. A., and *Bauer, W.:* J. Clin. Investigation *18*, 351, 1939.

Ropes, Robertson, W. v. B., Rossmeisl, E., Peabody, R. B., and *Bauer, W.:* Acta Med. Scand. Suppl. *196*, 700, 1947.