

Mode of aggregation of hyaluronic acid protein complex on the surface of articular cartilage

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There is a great deal of experimental evidence that the frictional forces between sliding cartilage surfaces lubricated with synovial fluid are very low under normal conditions. Considering coefficient of friction as the meaningful measure of friction (*i.e.* tangential force resisting sliding divided by the normal load forcing the sliding surfaces together), typical values for cartilage on glass have been found to be 0.002 to 0.01 (McCutchen, 1962; Walker, 1969). A similar range was found for the ankle joints of dogs (Linn, 1968). However, under certain conditions, the coefficient of friction can be much higher than the range quoted. For instance, a test on a dog ankle joint was run for up to 6 hours under conditions of starved lubrication (Linn, 1968), and the coefficient of friction was found to be 0.1. For cartilage on glass, even higher figures have been obtained (Walker, Dowson, Longfield, and Wright, 1968).

So far, the broad conclusions have been that low friction is associated with a full film of lubricant completely separating the surfaces, whether of synovial fluid or a derivative, whilst high friction is associated with thin films and localized boundary friction due to asperity contact.

The object of this paper is to show some characteristics of the fluid film formed between cartilage surfaces. Friction and squeeze-film tests were conducted for several samples of cartilage and synovial fluid running against glass and the resulting fluid films examined in the scanning electron microscope. Theoretical analysis has then been undertaken to assess the significance of the experimental observations in terms of joint lubrication.

Material and methods

Specimens of articular cartilage were obtained from the femoral condyles of knee joints from cadavers. Extraction of the specimens was usually carried out at the same time as the necropsy within 12 hours of death.

Some complete condyles were removed; other specimens were cut from the condyles with a scalpel and then stored in Ringer's solution adjusted to pH 7.8 with Triss. While the joint was open, as much synovial fluid as possible was obtained, and in the cases where the volume was sufficient, it was used with its own cartilage for friction tests. The experiments were carried out as soon as possible after removal but some specimens had to be stored at 2°C. until used.

FRICION EXPERIMENTS

A reciprocating friction machine was used to measure the coefficient of friction between cartilage and glass, the moving part being a flat glass plate. The reciprocating motion had an amplitude of 1 cm. and completed the full cycle in two seconds. This gave a maximum sliding speed of 3 cm./sec.

The specimen was held at one end of a pivoted arm so that it could be loaded against the glass plate while the other end of the arm contacted a force transducer which recorded the frictional force at the cartilage-glass interface and displayed this on a chart recorder. The accuracy of the recording was 0.1 ± 0.002 kg.

The cartilage specimen was cut in such a way that the bone remained attached to give good backing, the contact area being trimmed to 0.13 cm.². Thus, when a load of 1.8 kg. was applied, the nominal contact pressure was 14 kg./cm.². Synovial fluid was deposited on the glass plate and several minutes were allowed with the cartilage in light contact with the fluid. This allowed the fluid time to cover and possibly impregnate the cartilage surface.

The tests had two main objectives: to see how friction

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varied with time under load and to measure transient variations in friction which occurred as a result of momentarily lifting the cartilage specimen from the glass plate and then lowering it again. The duration of each test was 10 to 15 minutes.

To obtain a range of results, three different types of cartilage and synovial fluid were used; normal, soft (showing mild fibrillation), and osteoarthrotic. Three types of synovial fluid were also used: very thick, normal, and 'watery' (from a patient with rheumatoid arthritis).

SCANNING ELECTRON MICROSCOPY OF SYNOVIAL FLUID FILMS

From the friction experiments it became clear that, on lifting the specimen, the fluid film, was restored between the cartilage and glass. This fluid film was then investigated at various stages by scanning electron microscopy, using the Stereoscan manufactured by Cambridge Instruments Ltd.

(a) Normal loading only (i.e. no sliding motion)

Specimens of healthy cartilage of area 0.18 cm^2 and thickness 0.1 cm . were placed into an apparatus (Fig. 1) which pressed a glass surface on to the cartilage with synovial fluid interposed. Contact pressures were nominally 4, 8, and 16 kg./cm^2 . After a given time under load, the apparatus was plunged into liquid nitrogen maintaining movement in an effort to keep fresh liquid washing against the sides of the specimens. The weights were carefully removed leaving only the aluminium lever arms loading the specimens, and then the apparatus was dried in a Pearce-Edwards tissue drier at about -50°C . After drying, the specimens came free from the glass plate leaving no trace of residue. The surfaces of the cartilage were then coated with about 500\AA silver and examined in the Stereoscan.

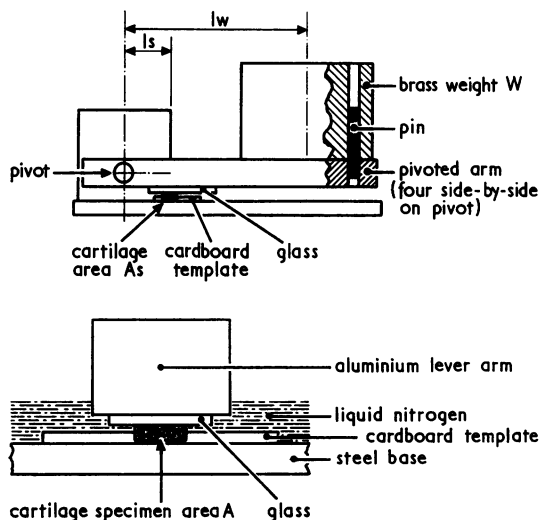


FIG. 1 *Static loading apparatus.*

(b) Sliding motion in addition to normal loading

Specimens of cartilage from the load-bearing region of the femoral condyles were cut in the form of discs of

0.65 cm . diameter and 0.2 cm . thick and used in the reciprocating friction machine. These were 'stuck' into the specimen holder and laid in a pool of synovial fluid on the glass plate for pre-soaking. A load of 1.8 kg ., giving a nominal contact pressure of 5.4 kg./cm^2 , was applied to the cartilage and the sliding action was started. After the required sliding time, the specimen was lifted from the glass plate at the centre of its stroke and immediately lowered into a small tray containing Arcton 22* which was held at a temperature just above its freezing point of -160°C . by surrounding it with liquid nitrogen. In this way ultra-rapid freezing was obtained. The specimens were then dried as described before.

Most specimens were sliding for time ranging from 2 seconds to 10 minutes, but a few were lifted and lowered after an initial sliding time.

Results

FRICITION EXPERIMENTS

The results of the friction tests are plotted as frictional shear stress against time after lift. The graphs for normal cartilage are shown in Fig. 2, and those for normal synovial fluid in Fig. 3.

The most obvious characteristic of these curves is that the frictional shear stress increased with time under load but reduced suddenly when the specimen was lifted from contact and then re-lowered. It is also quite evident that combinations of different qualities of cartilage and fluid give different ranges of frictional stress. Considering normal cartilage (Fig. 2), when thin fluid was used, the frictional shear stress was higher than for more viscous fluid. In fact, the rheumatoid ('watery') fluid gave frictional stresses twice as great as those obtained when normal fluid was used. Conversely, very viscous fluid produced frictional stresses between a half and a quarter of those for normal fluid.

Fig. 3 shows that, when normal fluid was used on different types of cartilage, frictional shear stress varied according to the condition. Fairly soft cartilage gave frictional stresses about twice as great as those obtained from normal specimens, and osteoarthrotic cartilage produced between five and ten times the shear stresses of normal cartilage.

SQUEEZE FILMS

Scanning electron microscopy was used to examine the surfaces of cartilage lubricated with synovial fluid and subjected to various loads. The results have been described recently by the present authors (Walker, Sikorski, Dowson, Longfield, and Wright, 1970). The main observations were as follows:

(a) No load condition

A thick deposit was seen attached to the surface of the cartilage, but this deposit showed no preferred orientation.

* Chlorodifluoromethane, supplied by I.C.I. Ltd.,

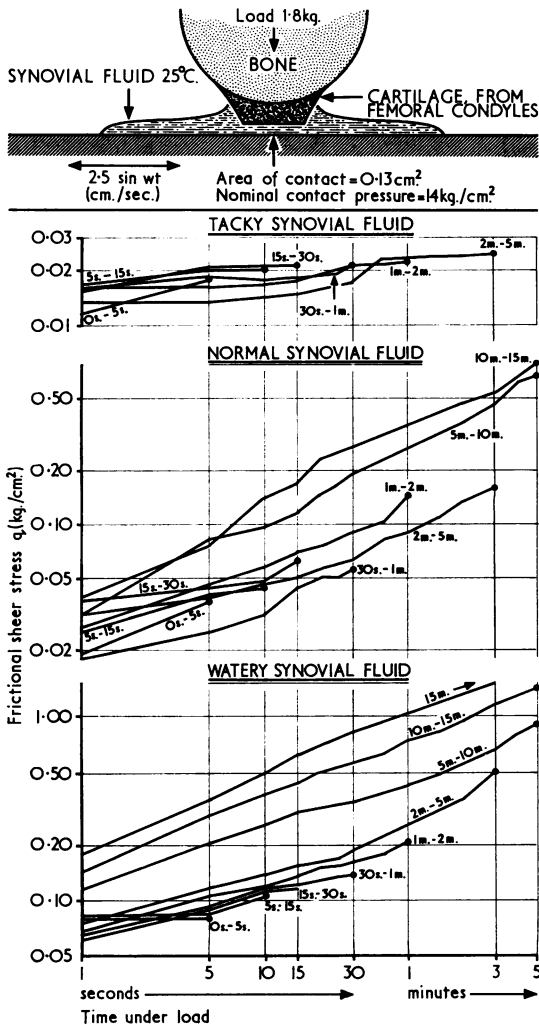


FIG. 2 Graph of frictional shear stress against time under load for normal cartilage.

(b) *Light-load condition*

The thick deposit was seen to have definite aligned aggregate which corresponded to the flow pattern of the fluid (Figs 4 and 5, overleaf).

(c) *Medium-load condition*

The 'aggregate' was still evident but its height was much less than before. It also showed signs of collapse and gross inclination to the surface (Fig. 6).

(d) *High-load condition (16 kg./cm.² for 6 sec. duration)*

The structures appeared to collapse into a continuous skin which covered the cartilage surface (Fig. 7).

Many examples of complete skin formation have been obtained and in some cases several layers of skin have been seen (usually in obvious depressions

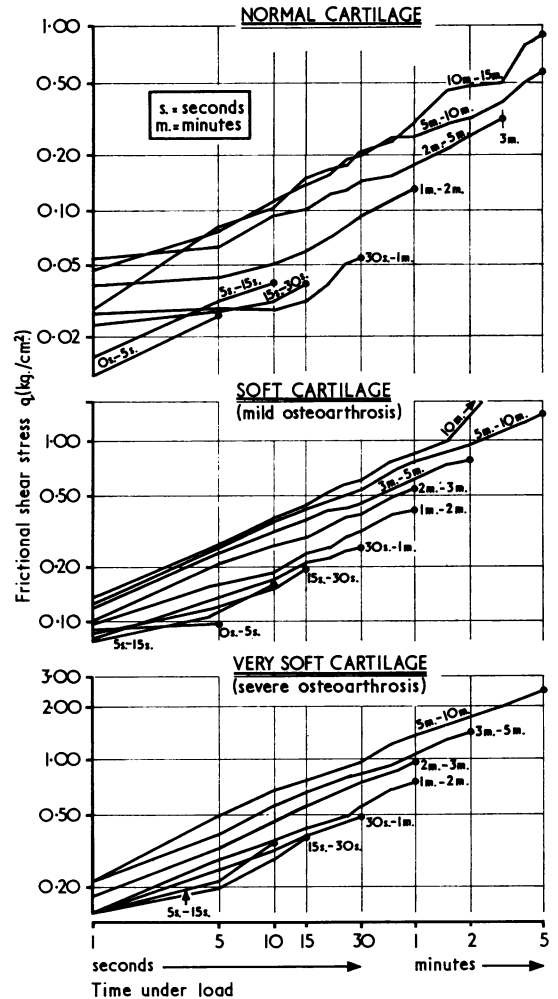


FIG. 3 Graph of frictional shear stress against time under load for normal synovial fluid.

in the cartilage surface). However, under extreme conditions, 16 kg./cm.² for 90 sec., the 'skin' showed evidence of penetration by asperities on the cartilage surface.

A summary of three separate tests is given in Table I (overleaf). There were two specimens prepared at each contact pressure and deposit thickness was estimated from representative micrographs.

The squeeze-film tests referred to previously were conducted in the absence of sliding, but similar results were obtained when sliding was superimposed. In some cases different types of deposit were seen on the same specimen and this was thought to be due to uneven load distribution resulting from the waviness of the cartilage surface. On several specimens,

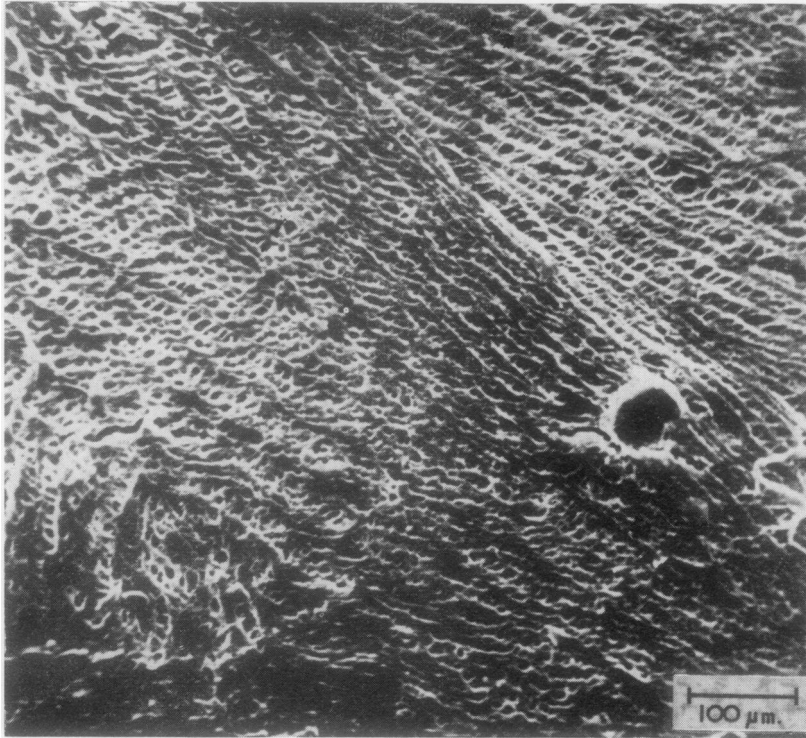


FIG. 4 Alignments of structures on cartilage surface, formed under a squeezing load of 4 kg./cm.² applied for 2 sec.

Table I Summary of squeeze-film experiments, with observations from the scanning electron microscope, including 'deposit thickness'

<i>Details of specimen (femoral condyle)</i>	<i>Nominal contact Pressure (kg./cm.²)</i>	<i>Time under load (sec.)</i>	<i>Type of surface deposit</i>	<i>Average thickness of deposit (μ)</i>
Healthy 55 yrs Area 0.18 cm. ²	4	6	Complete thin skin, with many 'double layers' over depressions in cartilage	0.4
	16	6	Complete thin skin very flat Areas of wrinkles	0.25
Healthy 46 yrs Area 0.18 cm. ²	4	2	Mainly aligned structures; some frothy non-aligned structure; some collapsed structures	10
	8	2	Tilted structures, with some areas of thick skin	5
	16	2	Complete skin, with several remnants of structures in 'low lying' areas	1
Healthy Area 0.18 cm. ²	8 and 16 (cartilage on glass and cartilage on cartilage)	90	Very thin skin over whole surface Some double layers, and evidence of some skin penetration	0.25

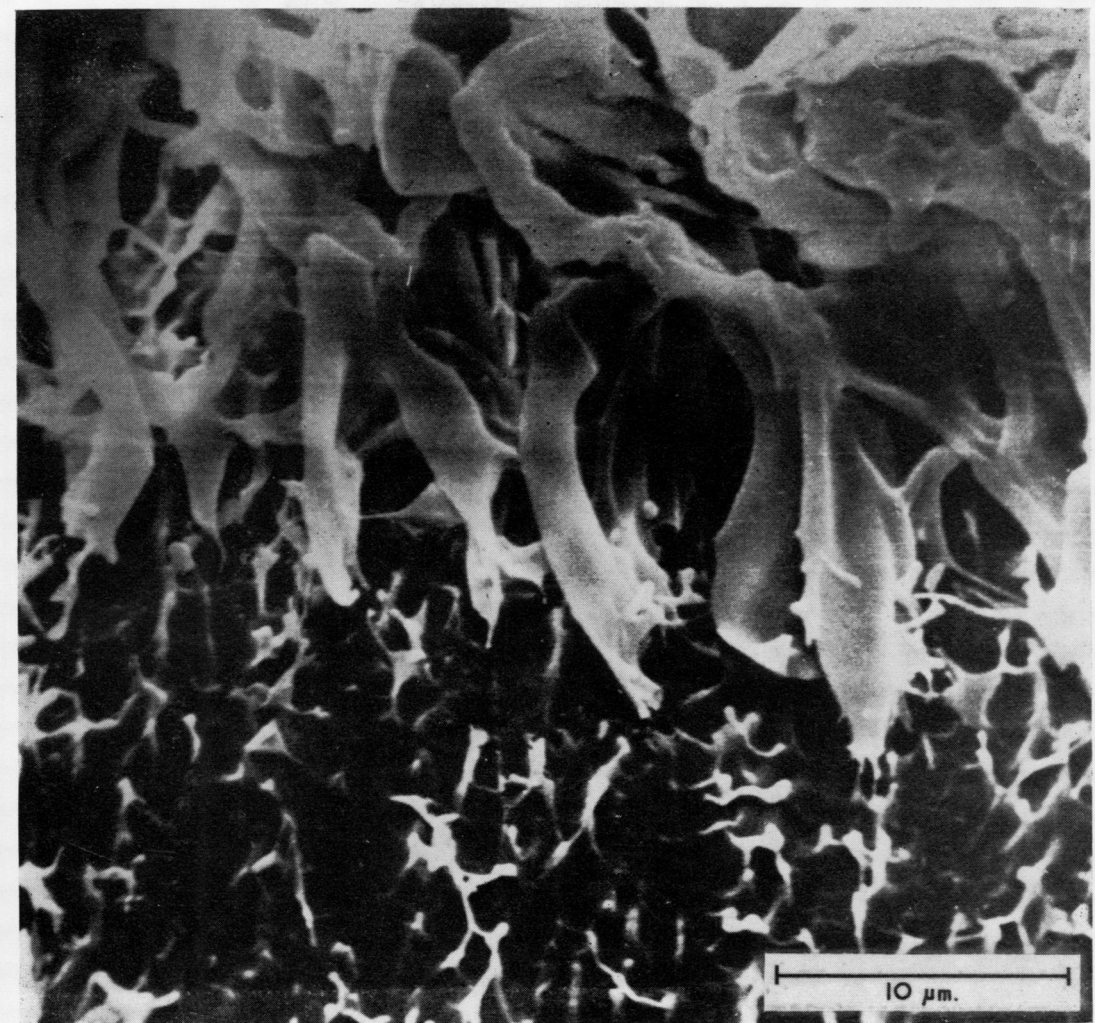


FIG. 5 Side view of aggregate of hyaluronic acid-protein complex on surface of cartilage.

a very uneven deposit was seen. This was probably due to severe fluid disruption when lifting the specimen from the glass plate.

One specimen was under load for 5 minutes, and then lifted and lowered for a further 3 seconds. The deposit formed was uniform and thick (Fig. 9). The drying cracks which were obvious from the micrograph, revealed a structure very similar to that of Fig. 5, and also allowed the deposit thickness to be measured.

A scanning electron micrograph shows an area where the sliding direction was clearly indicated by lines and there were many bright patches (Fig. 9). High magnification showed the alignments to be partially collapsed structures, while the bright patches were depressions in the cartilage which were covered with deposit. (The micrograph density

to some extent represents a contour map of the cartilage surface, dark areas being the higher regions.)

The third example (Fig. 10) represents a case where two patches appear to have taken most of the load. The fluid deposit was in the form of a thin skin in these areas whilst elsewhere a thick structured layer was seen.

Table II (overleaf) gives a summary of the results from sliding experiments.

THEORETICAL ANALYSIS

The scanning electron micrographs show the presence of aggregate adsorbed on to the articular surfaces. Fig. 8 shows how these can be represented, for the sake of analysis, as membranes inserted between the sliding surfaces. Fig. 11 shows this diagrammatically.



FIG. 6 *Structures collapsed on to cartilage surface.*

The terminology used in the analysis is as follows:

- h = thickness of solvent film between two membranes
- p = pressure at any point in the film
- P_z = load carrying capacity of the fluid film
- r = the radial co-ordinate
- R = radius of the specimen
- t = time
- W = velocity of approach of the two surfaces

- δ = structure thickness
- η = absolute viscosity of solvent

Consider the squeeze-film action of the circular specimen against the plate. Since there is no wedge within the film, no load-carrying capacity can come from the sliding action.

Consider the total radial flow of fluid at any radius r .



FIG. 7 Complete thin skin covering cartilage surface; the result of a loading of 16 kg./cm². for 6 sec.

Table II Summary of sliding experiments, showing measured friction values

Time under load (sec.)	Type of surface deposit	Average thickness of deposit (μ)	Average frictional shear stress (kg./cm. ²)	Coefficient of friction
300, then lift and lower for further 3	Complete, uniform, thick deposit with structure	10	0.034	0.006
2	Fairly uniform, partly collapsed structures, 'trapped pools'	2	0.038	0.007
5	Various: Very thin skin 25 per cent. of area; Medium skin 25 per cent.; Thick with structure 50 per cent.	0.3-4.3 mean 1.5	0.045	0.008
30	—	—	0.063	0.011
60	—	—	0.074	0.013
600	—	—	0.27	0.048

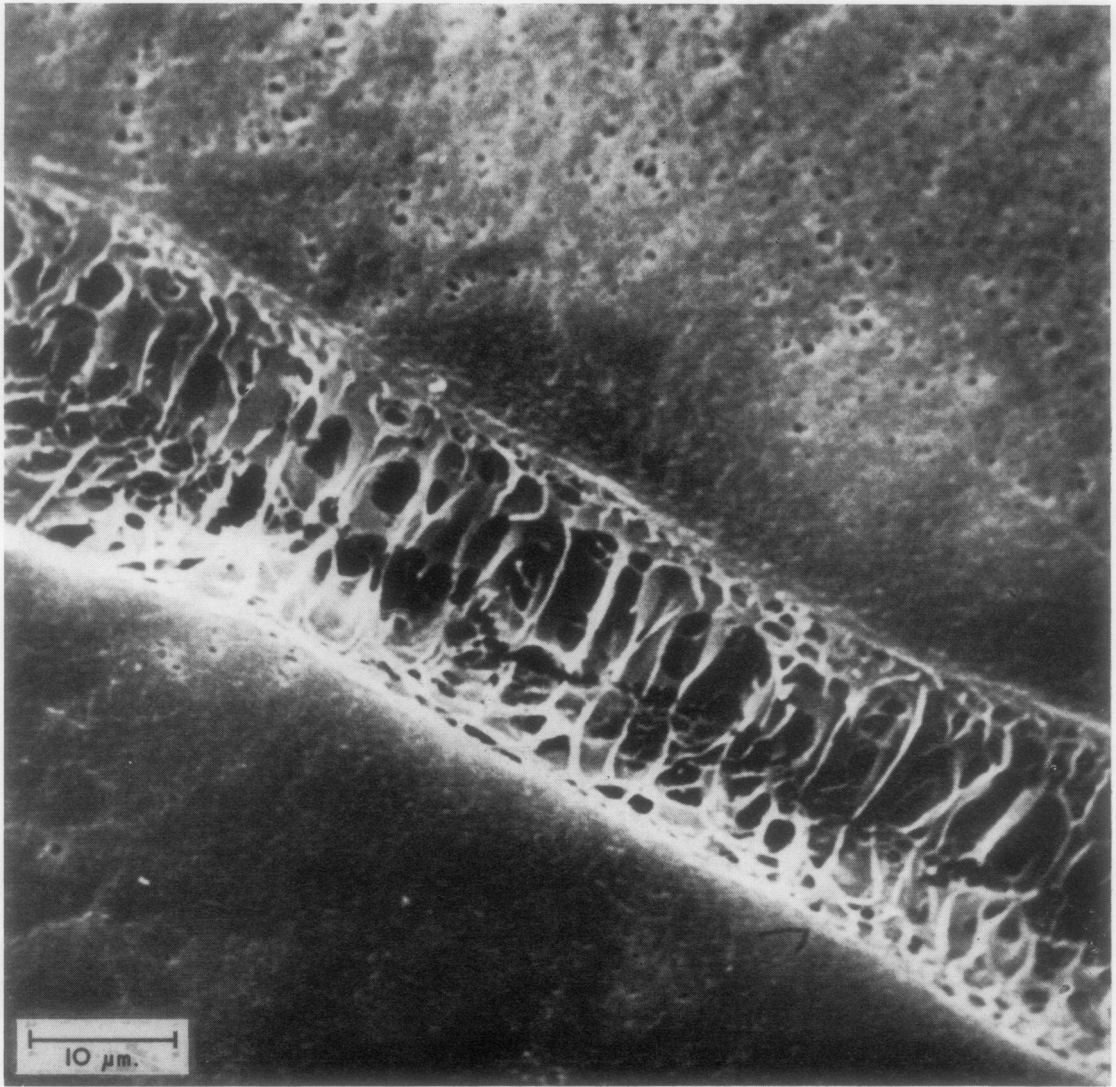


FIG. 8 Uniform thick film deposit on a sliding specimen. The drying crack shows the structure and the thickness of the deposit.

$$\text{Then } \frac{-\delta^3}{12\eta} (4\pi r) \frac{\partial p}{\partial r} - \frac{h^3}{12\eta} (2\pi r) \frac{\partial p}{\partial r} = \pi r^3 W$$

where η is the absolute viscosity of the solvent, $\partial p/\partial r$ is the partial derivative of pressure with respect to radius, and W is the velocity of approach of one plate relative to the other.

$$\therefore Wr = \frac{-\partial p}{\partial r} \cdot \frac{1}{6\eta} [2\delta^3 + h^3] \quad \dots (1)$$

integrate with respect to r , and apply boundary conditions that

$$p = 0 \text{ when } r = R.$$

$$\therefore p = \frac{3\eta W}{(2\delta^3 + h^3)} \cdot (R^2 - r^2) \quad \dots (2)$$

$$\text{But the load capacity } P_z = \frac{3\pi\eta WR^4}{2(2\delta^3 + h^3)} \quad \dots (3)$$

and $W = \partial/\partial t (2\delta + h)$, i.e. time derivatives of the total separation of the plates.

Substituting this into Equation 3 and integrating gives:

$$\int_{t_1}^{t_2} dt = \frac{3\pi\eta R^4}{2P_z} \int \frac{1}{(2\delta^3 + h^3)} d(2\delta + h) \quad \dots (4)$$

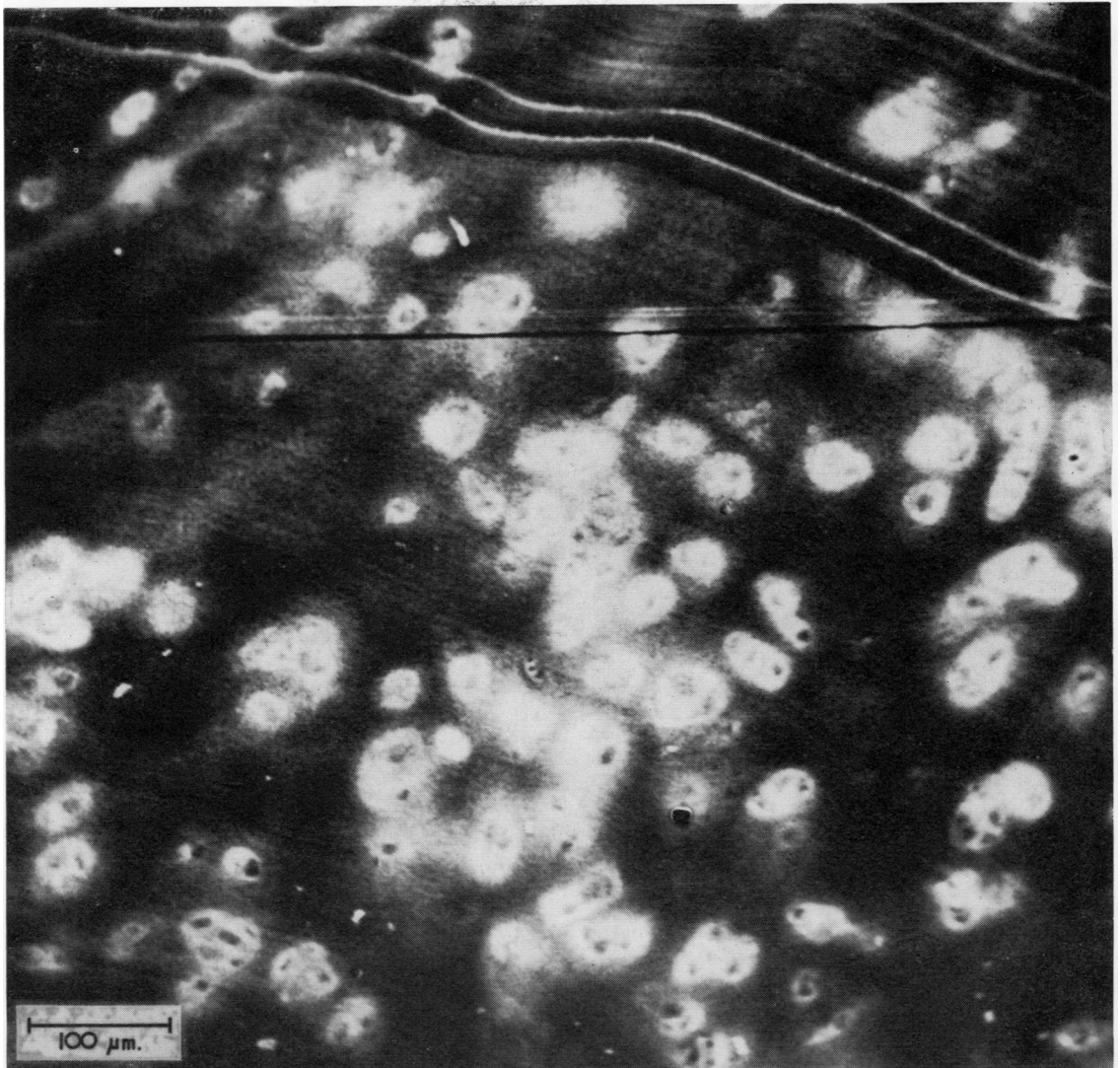


FIG. 9 Low magnification view of a sliding surface. Light patches are representative of 'trapped pools' or depressions on cartilage surface between 'humps'.

The solution of Equation 4 gives the squeeze-film time. Clearly, unless a relationship is known between δ and h , Equation 4 cannot be solved.

Thus, the following has been assumed:

[1] When the two plates are separated by a thick film of fluid, say 20μ , the structure height δ remains constant, this height being determined by the amount of complex and its molecular weight. Therefore, any reduction in total film thickness is a reduction in h .

[2] Some stage will be reached when h is zero and the two layers of structures will contact and squash each other. In this case any reduction in surface separation takes place in the δ region only. Therefore two distinct regions are assumed:

(a) Solution when $h \geq 0$; i.e. δ is constant

It can be shown by integration by partial fractions and substitution that Equation 4 becomes:

$$\Delta t = \frac{3\pi\eta R^4}{2P_z} \left[\frac{1}{3x^2} \log(x+h) - \frac{1}{6x^2} \log(x^2 - xh + h^2) + \frac{\tan^{-1} \left\{ \frac{2(h-x/2)}{x\sqrt{3}} \right\}}{x\sqrt{3}} \right]_{h_1}^{h_2} \dots (5)$$

where $x = \sqrt[3]{2\delta}$

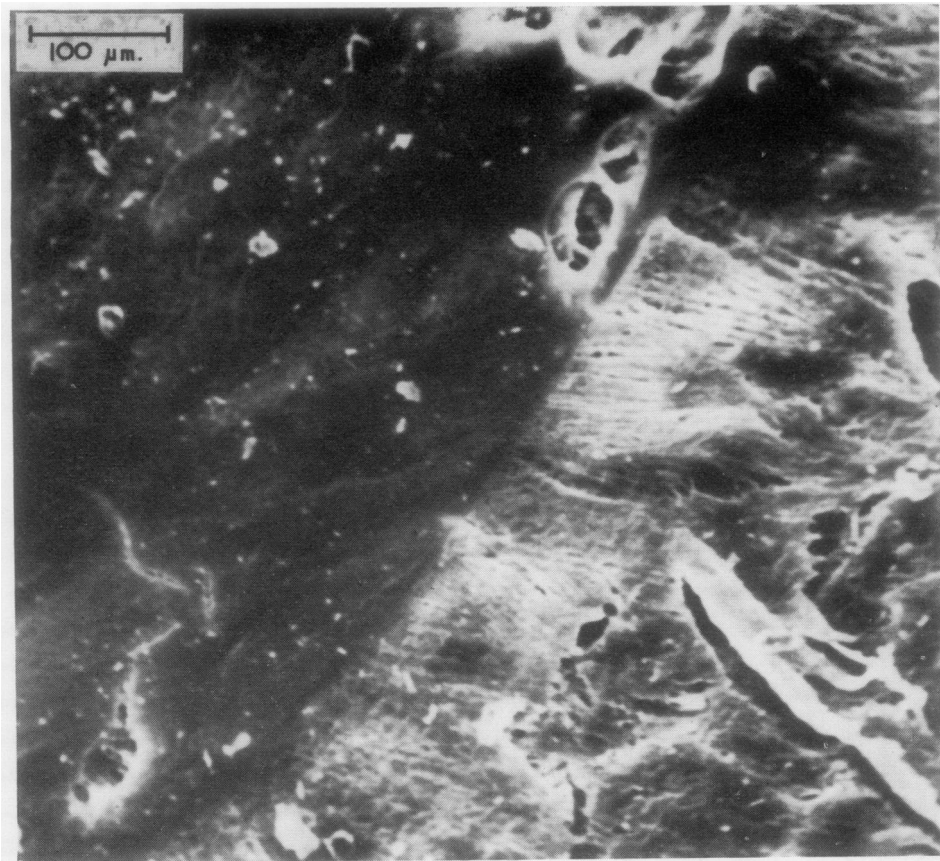


FIG. 10 Part of a patch (dark area at left) which had been carrying load under sliding, and a non-load-bearing area with thick structure.

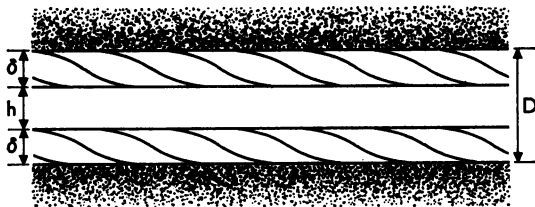


FIG. 11 *Idealized 'structure' formation.*
D = separation of cartilage surfaces.
h = separation of structure layers.
 δ = aggregate thickness.

(b) *Solution when $h = 0$; δ variable*

$$\int_{t_1}^{t_2} dt = \frac{3\pi\eta R^4}{2P_z} \int \frac{1}{\delta^3} d\delta$$

$$\therefore \Delta t = \frac{3\pi\eta R^4}{4P_z} \left[\frac{1}{\delta_2^2} - \frac{1}{\delta_1^2} \right] \dots (6)$$

If we now consider the calculated film thicknesses, based on the above expressions, we find that the results are as shown in Table III.

Table III *Comparison of theoretical and experimental film thickness*

Test No.	Calculated film thickness (μ)	Measured structure height (film thickness μ)
1	2.5	10
2	3.0	2
3	1.9	1.5
4	0.78	—
5	0.56	—
6	0.17	—

Discussion

When considering microscopical evidence, it is important to know how the specimens have been prepared. In general, if materials with a high water content are frozen, the best structural preservation is obtained when cooling is very rapid. The aim is to achieve full 'vitrification' of the water to prevent large crystal formation altering the material structure (see Sjöstrand, 1967). The higher the water

content, the faster the cooling rate required to achieve this.

By comparison with the controlled experiments of Luyet (1960) on solutions of, for example, polyvinyl pyrrolidone, it would be expected that some crystallization patterns could be introduced in our films of synovial fluid. However, it is likely that the patterns would be of the type in which these were exhibiting no particular orientation. This would mean that any orientation of the molecular aggregate under observation would not be affected by the water crystallization. To test this a drop of synovial fluid was allowed to run down a glass slide and then frozen in liquid nitrogen. After drying it was observed that the orientation was in the direction of flow. This suggests that the crystallization had not masked the aggregate structure.

The sliding tests were carried out and subsequent freezing was very rapid. Even so it is possible that some crystallization occurred, because Luyet found that materials containing 70 to 80 per cent. of water could be 'vitrified' only for a depth of about 0.1 mm.

With knowledge of some of the dangers of interpretation of micrographs, the significance of Figs 4 to 7 will be explained.

The polymer molecules took on definite alignments in the direction in which the fluid was being squeezed from the fluid film, and formed an aggregate. To some extent, the solvent and polymer phases separated out, so that the former was being preferentially squeezed out from the film. Further squeezing caused the aggregate to collapse (Fig. 6), so that the individual structures merged together to form polymer 'skins' between the cartilage surfaces. It seemed likely that thin films of low viscosity solvent would be present adjacent to the skins. With either structures or skins, the resistance to reduction in film thickness would be enhanced by the narrow channels through which the fluid, mainly solvent, had to escape. Furthermore, when only a skin remained, this would have the maximum concentration of polymer to give the best protection possible to the cartilage surfaces.

The situation in sliding displayed many similarities. Once again, the fluid film deposits ranged from aligned aggregate to thin skins, but further evidence was provided about the type of fluid film present in relation to the friction measured. (See Table II.)

Low frictional shear stress was associated with a thick 'structured' fluid deposit, and this friction was attributed entirely to shearing of the viscous film. Only a 16 per cent. increase in friction resulted from about a 5-fold decrease in film deposit thickness, whereas calculations of friction based on bulk viscosity at a range of shear rates (Davies and Palfrey, 1968) predicted a very much greater in-

crease in friction. This suggests that the synovial fluid in the thin films between the sliding cartilage surfaces did not behave in the same way as the bulk fluid.

A further increase in friction of about the same magnitude was measured for a specimen which showed two areas of thin skin deposit. Presumably the further increases in friction resulted from further areas of thin skin developing both because of the fluid film squeezing down, and because of the cartilage surfaces becoming gradually more conformable to one another.

The depressions suggested from Fig. 9 are doubtless due to the undulating nature of the cartilage surface, about which further information was provided by Gardner and Woodward (1969). Presumably, under lubricated sliding conditions, they form trapped pools, which act as a constant recharging source for the fluid film.

Conclusions

Aggregate of hyaluronic acid protein complex, adhering to the surface of cartilage, modifies conventional squeeze-film theory, so that longer squeeze-film times may be obtained, while allowing the low viscosity solvent to be sheared, so generating low frictional resistance.

In the normal walking cycle, where the load is applied for about 0.5 sec., the squeeze film generated would be of the order of 6 μ . This is quite sufficient to allow asperities on normal cartilage to slide freely over each other. Thus, during the worst conditions, some fluid film protection is provided. At the end of the loaded cycle, the relatively unloaded cycle is ideal for regeneration of a thick fluid film which will provide a re-charging mechanism for the next loaded cycle.

After standing still for say 10 minutes, the fluid film has reduced to 0.2 μ . Clearly, this is smaller than the surface asperities, but cartilage being elastic will deform somewhat to smooth the surface roughness. Even so, when the sliding is re-started, one layer of aggregate slides on the other layer and, since this mechanism will destroy the structures in preference to the surfaces of cartilage, protection is gained. Once the fresh supply of fluid is allowed to contact the surfaces, more hyaluronic acid-protein complex absorbs on the surface providing the next protective layer.

Therefore, in conclusion, the adsorbed 'structures' of complex not only increase the conventional squeeze-film time, but also provide a boundary lubrication mechanism, when asperities come into contact.

Summary

Scanning electron microscopy and friction tests are used to show that surface aggregation of hyaluronic acid protein complex can form on cartilage surfaces and give enhanced lubrication properties. A theoretical analysis is used to show how the structures can help to increase squeeze-film times whilst reducing frictional shear stress. Also, under conditions of

extreme load, it is shown that this aggregate collapses to form a highly protective skin over the cartilage surface which will be destroyed in preference to the cartilage when severe sliding conditions are present.

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