The effect of liquid crystals on joint lubrication

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Abstract

This investigation has studied a natural phenomenon – the low friction of living joints – and synthetic lubricants have been developed. The experiments revealed a previously unknown property of synovia, namely, that the low friction of cartilages results from liquid crystals in the lubricant. It is proposed that molecules of liquid-crystalline cholesterol compounds found in synovia are arranged with their longer axis along the direction of microgrooves on the cartilage surfaces. The resulting liquid-crystalline nematic phase reduces friction during relative motion of the contacting cartilages and leads to the therapeutic effect by experimentation.

1. Introduction

There is presently no agreed explanation for the low friction and low wear of cartilages over a wide range of dynamic loads and velocities [1]. Therefore, investigations in this area of tribology are important.

Of great interest in the study of solid friction is the state of the interphase layers. According to the latest data, ordered lubricants provide the lowest energy dissipation [2,3]. Such an ordered state can be realized by liquid crystals. Liquid crystals demonstrate unusual lubricating properties in many cases [2–5]. Since the liquid crystalline state is intrinsic to substances in living organisms [6], it is logical to assume that the low friction of joints results from a similar state of the synovial fluid. Lack of information supporting this hypothesis prompted the investigation of liquid crystalline compounds found in human and animal synovia, and their role in joint lubrication.

2. Materials and test procedure

2.1. In vitro tests

The tests were conducted using fluid aspirated from knee joints of human patients and samples from animal knee joints taken 12 h post mortem.

The total content of cholesterol and its compounds in synovial fluid was determined using the technique developed for the analysis of blood [7]. Cholesterol compounds in synovial fluid were identified by chromatography. For this purpose, synovial fluid was extracted using a mixture of chloroform and ethanol. From the obtained lipid extract, a blend of cholesterol esters was then separated by means of thin layer chromatography. The thin layer chromatography was conducted in a mixture of heptane–ether–acetic acid in the proportion 80:20:1. Cholesterol esters were eluted from the plate by heptane and then identified in a gas chromatograph on the corresponding carbolic acids.

Synovia samples dried on glass were examined by thermopolarization microscopy. The sample is observed under crossed polaroids as the temperature is varied.

Hyaluronic acid found in synovia was depolymerized by introducing 3 g l^{-1} of hyaluronidase into the fluid. The synovia viscosity was measured with a rotational viscometer.

Tribological measurements were performed using a pendulum tribometer (Fig. 1); a most suitable type for our purposes since it can provide motions characteristic of living joints. Specimens for the tribological testing were prepared from the head of the humerus (convex part) and from the glenoid cavity (concave part) of pigs 1 or 2 h post mortem. The head of the humerus was fastened to the pendulum and allowed to rest in the glenoid cavity, which was fixed on a rigid foundation. Before tests, the cartilages were placed into water for 24 h to be fully hydrated.

The joint was loaded by adding calibrated weights to the pendulum weight. The total load on the joint was 5.17 kgf. The period of the pendulum was 1.8 s at the pendulum length 850 mm. The friction force

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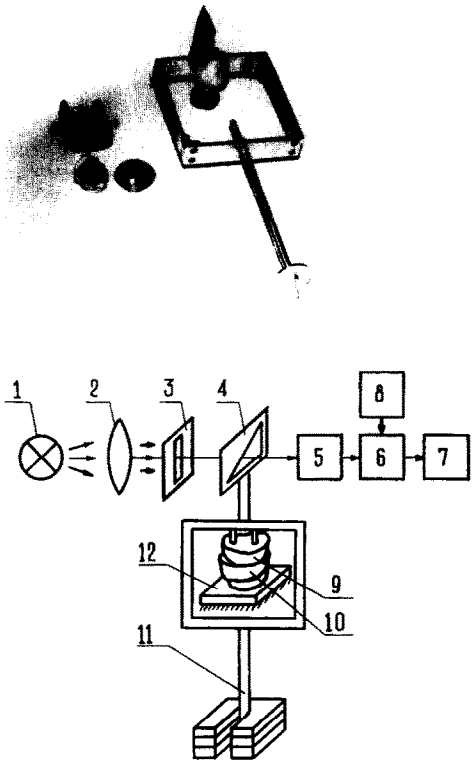

Fig. 1. Block diagram of the pendulum tribometer and a general view of the friction unit: 1, light source; 2, condenser; 3, slit aperture; 4, wedge-type aperture; 5, electron multiplier; 6, amplifier; 7, recorder; 8, power supply; 9, movable specimen; 10, stationary specimen; 11, pendulum; 12, support for specimens.

was estimated from the damping of the pendulum oscillations. The initial oscillation amplitude was the same in all experiments. The experiments were done seven times and their results averaged.

During the experiments, the following media were tested as lubricants. (1) Dimethyl sulphoxide (DMSO), widely used in medicine to treat living joints [8]. (2) Polymethyl siloxane fluid PMS-300 (IIMC-300). This substance was described in ref. 9. (3) Pseudosynovial fluid prepared according to ref. 10. It consisted of a water solution of carboxymethylcellulose sodium salt (2 wt.%) with addition of blood plasma inorganic salts. (4) Polymethyl siloxane fluid as in medium 2 doped with 2% of liquid crystals. (5) Pseudosynovial fluids as in medium 3 doped with 2% of liquid crystals. (6) Synovial fluid with a total cholesterol concentration of 0.9 mmol l^{-1}. (7) Synovial fluid with a total cholesterol concentration of 4.09 mmol l^{-1}.

Cholesterol in the last two contained esters forming a liquid crystalline phase in the temperature range of 25–40 °C. The amount of lubricant in each test was 1 ml.

Besides the pendulum tribometer, a face-friction device was employed in the tribological experiments. Specimens measuring 10 mm × 10 mm × 4 mm were cut from elastic cartilages of pork knee joints (*menisci medialis et lateralis*) kept in water for 24 h, arranged at vertices of an equilateral triangle and tested without storing. Glass petri dishes of diameter 0.1 m were used as the counterface. Each dish contained 20 ml of the lubricant tested.

Microstructures of the rubbed surfaces were examined using polarization and scanning electron microscopes.

2.2. *In vivo tests*

In the hope that liquid crystalline compounds could be introduced into a joint by applying them topically to the skin, the ability of the cholesterol liquid crystalline compounds to diffuse through skin was estimated experimentally using a radioisotope technique. For this purpose, 24 herd-bred male rats were used. Their weights were between 265 and 290 g. The animals were divided into two groups. One group (12 rats) was analysed for diffusion of liquid crystalline compounds through the skin under normal conditions. In the other group (12 rats), arthritis was simulated in left posterior limb joints by introducing 0.1 ml Freund adjuvant (Grand Island Biological Company, USA) under the plantar aponeurosis following the procedure described by Beneke and Nhol [11].

Investigation of diffusion of the liquid crystalline compounds through the animal skin in the second group was begun 14 days after the injection if inflammatory changes were observed in the joints under consideration. Applications in first and second groups were made daily onto animals' skin in the zone of left knee joints with 0.5 ml of newly prepared substance. Each application lasted 60 min. Animals were narcotized for this period to keep them quiet. The applied material was 10% solution in vaseline oil of oleic acid cholesterol ester labelled with 1.2 ^3H (specific activity of the preparation was $7.5 \pm 0.5 \times 10^6$ decays/(mg min)). Narcosis was done with 20% sodium hydroxybutyrate using 1.5 g kg^{-1}. In order to prevent the animals from ingesting the preparation by licking it off the skin after the anaesthetic effect ended, the composition tested was washed off with chloroform and water after the application was completed. Three animals were withdrawn from each group after 1, 3, 5, and 7 days, having been subjected to one, three, five and seven daily applications, respectively, as required [12].

The radioactivity of blood, liver, kidneys, the anterolateral part of the left knee joint, and the joint surface of the left femur condyle was determined in these animals by a fluid scintillation technique [13,14].

3. Results and discussion

3.1. In vitro tests

Most researchers [1,15–17] believe that the low friction of living joints results from a specific effect that the molecules of hyaluronic acid (HUA) cause upon the sliding surface, or from the formation on cartilage surface of adsorbed layers of glycoproteins that can separate the rubbing surfaces.

In order to clarify the role of HUA in the lubricating capacity of synovia, the latter was depolymerized with hyaluronidase. This leads to a large (more than an order of magnitude) reduction in viscosity of the initial synovial fluid, Fig. 2(a). The latter viscosity approaches that of water (1 mPa s) [18]. Friction measurements indicate that HUA depolymerization in fact does not vary the friction coefficient in cartilage–glass friction pairs (Fig. 2(b)). This agrees with other researchers' results [15,16], and shows that HUA has a negligible role in reducing cartilage surface friction.

Since the testing technique provides low sliding velocities (0.1 m s^{-1}) and the lubricating effect of synovia is independent of its viscosity, then according to refs. 9, 19 and 20, boundary lubrication must be occurring under these conditions. We believe that ordered layers formed due to liquid crystalline cholesterol compounds, which are likely to exist in the synovial fluid, play an important part in the low friction, perhaps along with glycoproteins.

Further experimental evidence follows.

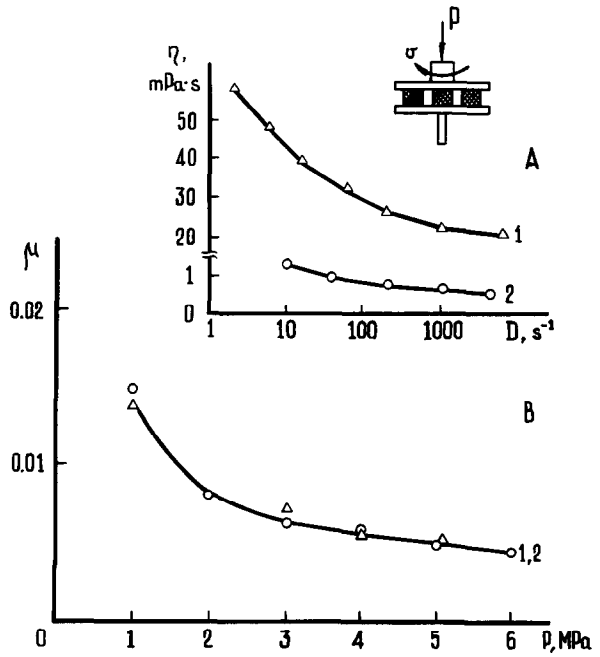

Fig. 2. (a) Viscosity as a function of shear rate and (b) coefficient of friction as a function of load: 1, for natural synovial fluid; 2, for synovial fluid depolymerized with hyaluronidase.

The chemical analysis, performed following the procedure described in ref. 7, used 35 samples of human synovial fluid and 15 samples of animal synovial fluid. The total cholesterol concentration (cholesterol plus its compounds) in the animal synovial fluids tested was 1.0 ± 0.2 mmol l^{-1} (0.04–0.06 wt.%), while for the human samples the values were 3.8 ± 0.4 mmol l^{-1} (0.16–0.20 wt.%). A more detailed investigation showed that the share of cholesterol esters of higher fatty acids in this samples was 25%–35%. By means of chromatographic methods, the composition of cholesterol esters found in synovial fluid was refined. It is shown in Table 1.

We think, that such a qualitative and quantitative composition of cholesterol esters in synovia is necessary to ensure that the material is in the liquid crystalline state in the range of physiological temperatures. This assumption is supported by the following investigation.

In thermopolarization microscopic examinations, dried human and animal synovial fluid contained optically active substances [21]. Under crossed polaroids, points or regions were observed to change their colour and brightness if the preparation was rotated. A detailed examination revealed individual glowing, and therefore optically anisotropic insertions. They were radial ray aggregates identical with those in liquid crystalline substances detected by several researchers in living organisms [22].

When the dried synovia preparation was heated up to 40–43 °C, the inclusions stopped glowing. When the synovia was cooled, the glow returned and continued down to 25 °C, the lowest temperature employed. This behaviour shows that at temperatures from 25 to 43 °C the substance behaves as thermotropic liquid crystalline cholesterol esters are known to do.

The analysis (by thermopolarization microscopic technique) of model systems obtained from water solutions of polymers and liquid crystals showed them to behave similarly to the synovia preparations.

Our results prove the presence of the liquid-crystal-forming cholesterol esters in synovial fluid and suggest that they are in the liquid crystalline state in living

TABLE 1. Composition of cholesterol ester fraction of synovial fluid

Esters	Molecular weight	Component share, per cent by weight
Cholesteryl arachidonate	672	8±4
Cholesteryl linoleate	649	32±5
Cholesteryl oleate	650	24±2
Cholesteryl stearate	636	9±2
Cholesteryl palmitate	624	24±5
Cholesteryl palmitoleate	622	3±2

joints. So the next step was investigation of their influence on the articular cartilage friction.

The cartilage–cartilage pairs tested on the pendulum tribometer (Fig. 3), showed that when a liquid-crystalline substance was added to a pseudosynovial fluid the friction force dropped to that with synovial fluid (curves 5 and 6). The cartilage friction was reduced also by silicone fluid doped with liquid crystals (curve 4). The pendulum damping time also increased (*i.e.* the friction fell) when synovial fluid having a higher content of the total cholesterol was employed (curve 7).

The favourable influence of liquid crystals on the friction of cartilages lubricated with pseudosynovial fluid was observed for the case of cartilage–glass pairs as well, Fig. 4. It should be mentioned that the successive replacement of cartilage samples by new ones gives lower friction coefficients, Fig. 5. This effect was also observed if lubrication was done with synovial fluid, vaseline oil or polymethyl siloxane fluid. When the worn paths were examined with a polarizing microscope they were observed to have optically active inclusions. One can suppose that the replacement of cartilage samples added liquid crystalline substance to the lubricant. This led to build up of more perfect adsorbed lubricating layers on the rubbing surfaces, and thus reduced the friction coefficient.

Scanning electron microscopy of the cartilage surface revealed microgrooves of order 1–10 μm oriented along the direction of preferential motion in the joint [23]. Such a microrelief may be formed by the collagen fibres

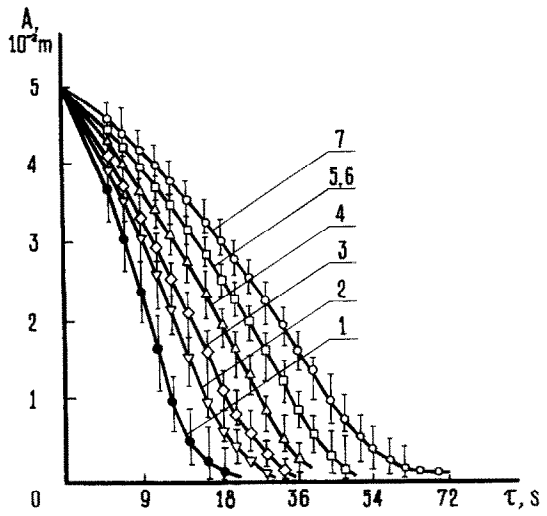

Fig. 3. Pendulum oscillation amplitude (A) *versus* time during cartilage rubbing in the following media: 1, 10% dimethyl sulphoxide solution; 2, polymethyl siloxane fluid (PMS-300); 3, pseudosynovial fluid; 4, PMS-300 plus 2% liquid crystals; 5, pseudosynovial fluid plus 2% of liquid crystals; 6, synovial fluid containing 0.9 mmol l^{-1} cholesterol; 7, synovial fluid with 4.09 mmol l^{-1} cholesterol. Error bars give the span of the measured values.

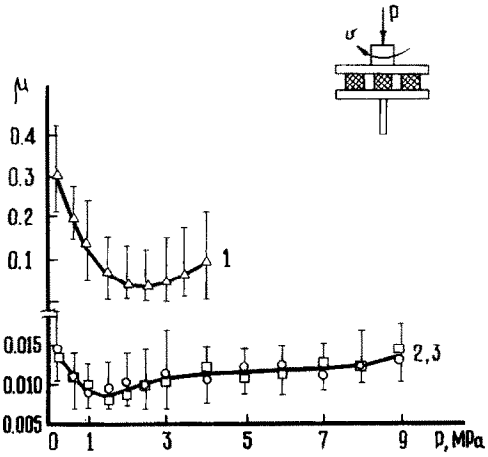

Fig. 4. Coefficient of friction as a function of load (at $v = 0.1$ m s^{-1}) for a cartilage–glass pair in the following media: 1, pseudosynovial fluid; 2, pseudosynovial fluid plus 2% of liquid crystals (○); 3, synovial fluid (□).

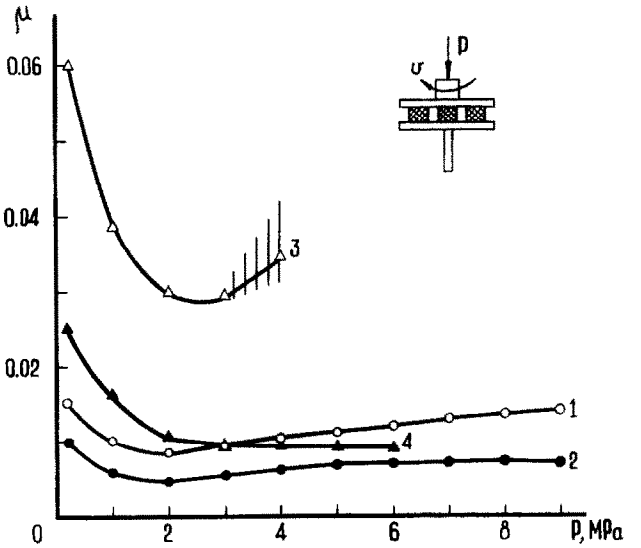

Fig. 5. Coefficient of friction as a function of load (at $v = 0.1$ m s^{-1}) for a cartilage–glass pair in the following media: 1, synovial fluid; 2, the same after the cartilage had been replaced three times; 3, vaseline oil; 4, vaseline oil after the third cartilage replacement.

[24]. The microrelief anisotropy strongly affects the friction coefficient for cartilage–glass pairs lubricated by synovial fluid. The lowest values were registered if motions were along the collagen fibres [23].

Sonin [2] believes that microgrooves on solid surfaces cause planar orientation of the liquid crystal molecules. This structure features minimum shear resistance in the orientational direction. Therefore this fact and the experimental data are evidence that cartilage surface microrelief influences the formation of liquid crystalline lubricating layers with the liquid crystal molecules preferentially oriented in the direction of relative motion of the cartilages.

3.2. In vivo tests

The above results were used to develop experimental samples of pseudosynovial fluids and preparations intended to improve joint lubrication. The results of preclinical investigations on animals support this expectation. When pseudosynovial fluids containing liquid crystalline compounds were injected into arthritic joints of rats, the cartilage surface region remained unchanged and had sharp outline smooth contours and uniform thickness. This indicates effective protection against mechanical degradation. The group of animals which received pseudosynovial fluids containing no liquid crystalline compounds suffered distrophy and mechanical degeneration of the cartilage. The cartilage had an uneven surface with deep, lacerated cavities and nonuniform thickness.

It appears, however, that injecting liquid crystal preparations into the joint capsule is not the only way to introduce liquid crystalline substances into affected joints. Our investigations showed the possibility of introducing liquid crystal preparations by diffusion through the skin (Table 2, cases with animals).

The radioisotope technique established that the preparation accumulated on the femoral condyle surface. With normal joints, the amount depended directly on the duration of the experiments and hence, on the number of applications that were made on the joint region. Simultaneously, the preparation was detected in the blood flow, liver, kidneys and joint capsula.

In the case of adjuvant arthritis, radioactivity appeared in the tissues sooner and in larger quantities than when the joints were normal. However, it stayed higher only in joint tissue. Probably, this is associated with a higher permeability of the vessel bed.

Preparations containing liquid crystals were tested clinically on a group of volunteers. The patients' condition improved after five to seven applications made with the test preparation onto osteoarthritic knee joints. The preparations were useful in reducing joint pain and increasing the flexor angle. They were more effective than the most widely used therapeutic agents.

4. Conclusion

Liquid crystalline substances exist in living tissue and play a great role in metabolism [25]. If liquid crystal structures are altered in biological tissues and liquids, various illnesses result (sickle-cell anemia, atherosclerosis, etc.) [6].

Our investigation has established the presence of liquid-crystal-forming cholesterol compounds in human and animal synovial fluids. The aggregates glowed under crossed polaroids in dried synovia samples in thermopolarization microscopy because they rotated the polarization plane of linearly polarized light. Thus they must be structurally ordered at temperatures not exceeding 45 °C. The higher the concentration of cholesterol compounds in synovial fluid, the better it lubricates. Pseudosynovial fluids containing liquid crystals when used in cartilage–cartilage and cartilage–glass pairs behave like synovial fluid. These facts and the dependence of friction on surface microrelief anisotropy [23] lead to the inference that liquid crystalline compounds become oriented in the direction of preferential motions of cartilages in a joint, and low friction results.

Modern medicine has no effective means of protecting joint cartilage against mechanical degeneration in the case of osteoarthritis (OA). Therefore the development of preparations containing liquid crystalline compounds that can improve joint lubrication by either being injected intra-articularly or diffusing through the skin is of great importance.

TABLE 2. Results of radioisotope investigation of cholesterol esters (labelled 1,2 ^{3}H) diffusivity through rats' skin

Duration of experiments		Radioactivity of preparation (decays min^{-1} per 1 mg tissue)				
		Joint cartilage of femur condyle	Joint capsula	Liver	Kidney	Blood
1	1st group[a]	3.4±1	4.6±1	5.9±1	3.7±0.4	559±150
	2nd group[b]	20±15	37±20	35±17	34±13	972±420
3	1st group	4.2±3	3.9±2	7.6±0.5	3.0±0.4	621±216
	2nd group	32±17	46±21	53±9	32±8	287±135
3	1st group	7.5±4	9.0±3	25±2	26±14	722±365
	2nd group	33±17	28±1	80±0.4	43±17	294±147
7	1st group	14±6	5.0±3	25±5	71±40	1267±620
	2nd group	5.5±3	29±13	36±2	25±7	294±147

[a]For physiologically normal conditions.
[b]For adjuvant arthritis.

Such preparations may be an adequate model of the natural joint lubricant, with rheological and tribological properties and structures which are characteristic of synovial fluid. They contain liquid crystalline components found in natural synovia that may be responsible for its antifrictional properties. Owing to the liquid crystalline components, the preparations inhibit wear and degradation of OA cartilages, increase their resistance to loads and improve joints' functioning.

We think the role of the liquid crystalline phase in synovial fluid, and also the relationships between intra-articular transport of cholesterol liquid crystalline compounds and their therapeutic effect, deserve close attention by specialists from different fields. Further investigations in this area may open new ways of designing artificial lubricants for joints, and of understanding the mechanism by which different diseases affect joint function.

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Appendix: Nomenclature

η	dynamic viscosity (mPa s)
D	shear rate (s^{-1})
μ	coefficient of friction
P	loading (MPa)
A	oscillation amplitude (m)
τ	time (s)
v	sliding velocity (m s^{-1})