Birefringent fibres of hyaluronic acid

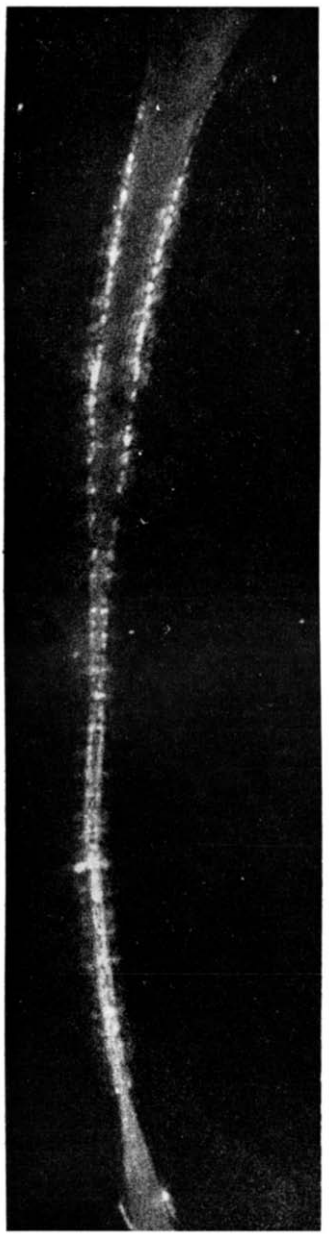

Fig. 1. The development of a fibre of hyaluronic acid. Upper part: Thread of concentrated solution with birefringent borders. Lower part: Condensed fibril showing periodic structure.

Previous streaming birefringence data[1] suggest that large particle size hyaluronates show some particle orientation under flow. Hyaluronate films as well as hydrated fibres obtained by drawing from solution, stretching or rolling have all so far shown an amorphous (non-orientated) structure under the polarizing microscope and in X-ray diffraction studies. Well orientated fibres suitable for further structural studies have, however, now been prepared by the following method from protein-free electrodialysed sodium hyaluronates. This material had an average particle size of about 60,000; for analytical data see SYLVÉN AND MALMGREN[2].

A dilute aqueous solution is allowed to concentrate slowly by evaporation at room temperature in a narrow wedge between a microscope slide and a coverslip. During the drying process birefringent regions appear in the concentrated solution; at a later stage the solution breaks up into fibrils which gradually contract to form fibres showing considerable birefringence in the polarizing microscope. Fibres of varying diameters and lengths up to 3 or 4 mm have been obtained. Pre-existent admixtures of cellulose fibres are not confused with the hyaluronate fibres. The latter readily redissolve after addition of water.

The double refraction was measured with a Sénarmont compensator; the thickness of the fibres was determined with a Smith (Baker) interference microscope of shearing type. The maximum value of the birefringence so calculated was 0.015. The sign was *positive* as in the case of most natural fibres. The magnitude compares favourably with that of cellulose (native ramie 0.068).

The mechanism of fibre formation may be watched as indicated in Fig. 1. A layer of rod-like birefringent material is first formed on the outer surface of the then highly hydrated fibrils (Fig. 1 upper part), which give the impression of thin flattened cylinders. Upon further drying the fibrils contract until they appear filled with birefringent material (Fig. 1, lower part). In most cases an interesting transverse periodicity may be discerned.

A very low concentration of toluidine blue may be introduced into the original hyaluronate solution. The fibres formed from this solution are weakly stained. When examined in polarized light (with no analyser) they show slight dichroism with a maximum absorption when the E-vector is perpendicular to the fibre axis. This dichroism is opposite in sign to that observed with cellulose fibres, dyed with toluidine blue, suggesting that the hyaluronate micelles are probably arranged differently from those in cellulose.

These experiments indicate that hyaluronates can form highly orientated fibres under the influence of very weak forces, which in this case are mainly surface tension. Such "self orientation" phenomena might be expected to occur in biological systems, *i.e.* by concentration of hyaluronate solutions under very still conditions. Similar phenomena may perhaps also play some part in the early organization of fibrous proteins, and during reconstitution experiments of collagenous fibres.

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