

## On the Molecular Weight of Potassium Hyaluronate from the Vitreous Body of Cattle

J. A. CHRISTIANSEN and C. E. JENSEN

*Københavns Universitets Fysisk-kemiske Institut, Copenhagen, Denmark*

It has been reported by Blix and Snellman<sup>1</sup>, Meyer and Palmer<sup>2</sup>, and very recently by Laurent<sup>3</sup> that hyaluronate from the vitreous humor has a much lower viscosity than hyaluronate from human umbilical cord, and consequently a lower molecular weight.

Using streaming birefringence measurements, Blix and Snellman<sup>1</sup> and Brunish and Rowen<sup>4</sup> estimated a molecular weight of vitreous hyaluronate at less than  $250 \times 10^3$ , and about  $500 \times 10^3$ , respectively, while Laurent<sup>3</sup> found  $340-500 \times 10^3$  by the light scattering technique.

In this institute we have determined the molecular weight of a preparation of vitreous hyaluronate by means of the osmometrical technique described by the present authors<sup>5</sup>.

The cattle eyes were enucleated just after killing, divided into two parts by an equatorial cut, cleaned from surrounding, especially retinal tissues, minced with a homogenizing tube, and filtered through eight layers of gauze. Potassium hyaluronate was isolated according to Jensen<sup>6</sup>.

The nitrogen contents determined after Kjeldahl, and the sulphur contents determined after Bürger's<sup>7</sup> method modified by Zimmermann<sup>8</sup> were 3.10 % and less than 0.1 %, respectively. The relative viscosity of the preparation dissolved in McIlvaine buffer (pH = 7) was measured at 3.8 in a Dalgaard-Mikkelsen-Kvorning microviscometer. The result of the osmometrical measurement is in Fig. 1. For details of this technique confer Christiansen and Jensen<sup>5</sup>.

The value obtained,  $270 \times 10^3$ , seems small compared with some of the figures cited above, *e. g.* those obtained by Laurent; however, we may consider our statement to be realistic on account of the supposed polydispersity of hyaluronate from the vitreous body. The molecular weights obtained with the aid of light scattering are weight averages like those obtained from ultracentrifugation, while

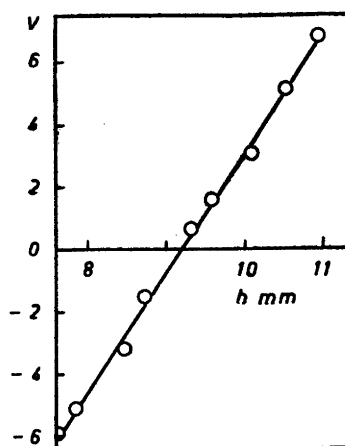


Fig. 1. The graph from which the molecular weight is calculated. The inner liquid contained 10.12 mg in 1 ml of the buffer solution which was used as the outer liquid.

those obtained from osmometrical measurements are number averages. Consequently the light scattering values tend to be high relatively to the osmometrical values if the solution contains a small fraction of molecules much larger than the average.

Laurent himself points out that the value obtained by him is probably too high because of the polydispersity of the employed solution, judged from the scattering envelope. Earlier investigators (Blix and Snellman<sup>1</sup>, Brunish and Rowen<sup>4</sup>) have also reported solutions of *corpus vitreum* hyaluronate to be polydisperse.

1. Blix, G. and Snellman, O. *Arkiv Kemi, Mineral. Geol.* 19A (1945) No. 32.
2. Meyer, K. and Palmer, J. W. *J. Biol. Chem.* 114 (1936) 689.
3. Laurent, T. C. *J. Biol. Chem.* 216 (1955) 262.
4. Brunish, R. and Rowen, J. W. *Federation Proc.* 13 (1954) 187.
5. Christiansen, J. A. and Jensen, C. E. *Acta Chem. Scand.* 7 (1953) 1247.
6. Jensen, C. E. *Acta Chem. Scand.* 7 (1953) 603.
7. Bürger, K. *Angew. Chem.* 54 (1941) 479.
8. Zimmermann, W. *Mikrochemie ver. Microchim. Acta* 31 (1943) 15.

Received October 10, 1955.