ACTA CHEMICA SCANDINAVICA 14 (1960) 1499–1502

Effect or Urea and Sodium Chloride on the Colloid Osmotic Pressure of Hyaluronate

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Colloid osmotic pressure of hyaluronate was measured in solutions containing varying amounts of sodium chloride or urea.

The effect of salt strength was considered physiologically nonsignificant.

In 6.5 M urea the colloid osmotic pressure of 0.25 % solution of hyaluronate increased 2.5-fold. The apparent molecular weight decreased from 110×10^3 to 46×10^3. On the basis of sedimentation and viscosity data molecular weights of 950×10^3 (original) and 600×10^3 (in 6.5 M urea) were calculated.

The purpose of these measurements was to determine whether physiologically possible changes in the sodium chloride content of extracellular fluid could affect the colloid osmotic pressure of hyaluronate and whether the hydrogen bonds were significant. Because the use of urea revealed an apparent dissociating effect, the sedimentation and viscosity data were determined.

EXPERIMENTAL

The osmometer of Bull [1] (immersed in a water bath at $+25 \pm 0.01$°C) was used and the colloid osmotic pressure could be measured with an accuracy of 0.01 mm H_2O.

The material was prepared from human umbilical cords by extraction with sodium chloride and fractional precipitation with »Cetavlon» according to the principle of Scott [2]. It was ascertained by column chromatography [3] that it did not contain chondrosamine and it was homogeneous in the ultracentrifuge (Fig. 3). The sample contained 3.58 % N and 16.7 % ash. The sample was kept in a desiccator above conc. sulphuric acid. For the measurements 0.5 % or 0.25 % solutions were prepared in 0.0067 M phosphate buffer (pH 7.3) containing 1 mg/100 ml sodium azide as preservative.

The salt effect was studied all the time with the same cellulose membrane bag and same 0.5 % hyaluronate solution (37 ml) but the outer fluid (68 ml, containing increasing amounts of sodium chloride in the phosphate buffer) was changed.

The effect of urea was studied similarly, but with 0.25 % solution of hyaluronate. The fluids contained 0.25 M sodium chloride.

The sedimentation studies were carried out with a »Spinco» analytical ultracentrifuge using wedge cells. The calculations were made according to Ogston [4].

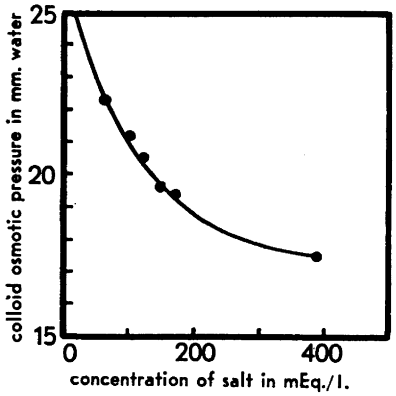

Fig. 1. Colloid osmotic pressure of 0.5 % hyaluronate solution plotted against sodium chloride concentration. (At the zero concentration of salt the colloid osmotic pressure was 66 mm H_2O).

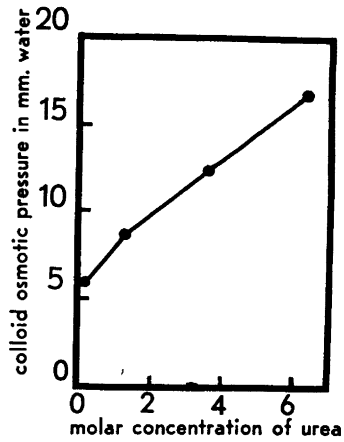

Fig. 2. Colloid osmotic pressure of 0.25 % hyaluronate plotted against urea concentration.

RESULTS

The effect of salt concentration on the colloid osmotic pressure is shown in Fig. 1 and of urea in Fig. 2. At the 140 mequiv./l level of sodium chloride a 10 mequiv./l increase in the salt concentration causes a decrease in the colloid osmotic pressure of only 0.4 mm H_2O at 0.5 % concentration of hyaluronate, which roughly corresponds to that present in tissues. Fig. 3 shows the sedimentation pattern and Table 1 contains the viscosity and sedimentation data with some relevant calculations.

DISCUSSION

The relations between hyaluronate and salt observed earlier [6] seem to be best explained by the different diffusion rates of the electrolytes in solu-

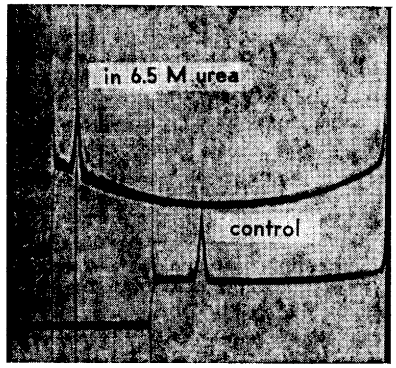

Fig. 3. Sedimentation pattern of hyaluronate (52 min. after reaching the speed of 59 780 rpm., c = 0.25 %, angle 75°). The control cell has leaked.

Table 1. Viscosity and sedimentation data of hyaluronate in urea.

Solvent	0.25 M NaCl in 1/300 M pH 7.3 phosphate buffer	6.5 M urea and 0.25 M NaCl in 1/300 M pH 7.3 phosphate buffer
Solvent density at 20°C	1.0100	1.1045
Solvent viscosity at 20°C (in poises)	0.0107	0.0158
Data for hyaluronate solution:		
Intrinsic viscosity (c = g/100 ml)	8.4	9.3
Sedimentation coefficients (s_0 in Svedberg units)		
uncorrected	4.95	2.19
corrected to $s_{w,20}$	5.65	4.14
$d(1/s)/dc$, $c \rightarrow 0$;		
from uncorrected s-values	2.09	3.04
from corrected s-values	1.97	1.69
Calculated molecular dimensions[4]*:*		
(assumptions[5]: $\overline{V} = 0.69$, $\xi/k = 0.37$)		
J from corrected s_0-values	5	10
V from corrected s_0-values	135	70
M from uncorrected s_0-values	920×10^3	670×10^3
from corrected s_0-values	950×10^3	600×10^3
from osmotic pressure (c = 0.25 %)	110×10^3	46×10^3

tions containing hyaluronate. The significance of the Donnan effect on the colloid osmotic pressure of the extracellular fluid is slight.

Bull and Currie [7] studied the effect of urea on the molecular weight of β-lactoglobulin and Gutter, Sober and Peterson [8] the effect on hemoglobin. In both cases a degradation of the protein molecule was observed. The experiments reported above give an analogous result.

There exist controversial opinions on the necessity of a density correction in the ultracentrifugation due to the presence of urea [8,9]. If in the calculations uncorrected s_0-values are used with the density and viscosity of the urea solution, this difficulty is avoided. In this case the corrected s_0-values with density and viscosity of the phosphate-NaCl-solution gave almost identical results.

There is a large difference between the molecular weights obtained from the osmotic pressure and from the sedimentation data. Blumberg and Ogston [10] pointed out that the osmotic pressure of hyaluronate tends to be unduly high because of the high entropy of dilution consequent to the large volume of the particles (*cf.* discussion by Jensen [11] and by Bull [1]). If true, this concept has a physiological implication in the maintenance of the tissue turgor in spite of the low concentration of the polysaccharide.

Acknowledgement. We thank Professor A. G. Ogston, F.R.S., for advice and acknowledge the financial support from the *Jenny and Antti Wihuri Foundation* and the *Sigrid Jusélius Foundation.*

REFERENCES

1. Bull, H. B. *Physical Biochemistry*, 2nd ed., John Wiley & Sons, 1951.
2. Scott, J. E. *Biochem. J.* **62** (1956) 31P.
3. Gardell, S. *Acta Chem. Scand.* **7** (1953) 207.
4. Ogston, A. G. *Trans. Faraday Soc.* **49** (1953) 1481.
5. Ogston, A. G. and Stanier, J. E. *Biochem. J.* **46** (1950) 364.
6. Kulonen, E. *Acta Physiol. Scand.* **27** (1952) 82.
7. Bull, H. B. and Currie, B. T. *J. Am. Chem. Soc.* **68** (1946) 742.
8. Gutter, F. J., Sober, H. A. and Peterson, E. A. *Arch. Biochem. Biophys.* **62** (1956) 427.
9. Schachman, H. K. and Lauffer, M. A. *J. Am. Chem. Soc.* **72** (1950) 4266.
10. Blumberg, B. S. and Ogston, A. G. in *Chemistry and Biology of Mucopolysaccharides*, Ciba Foundation Symposia, Churchill, London 1958.
11. Jensen, C. E. *Studier over Hyaluronsyre*, Thesis, Nyt Nordisk Forlag Arnold Busck, København 1957.

Received March 30, 1960.