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DEXTRAN SULPHATE: THE RELATION OF MOLECULAR FEATURES TO BIOLOGICAL PROPERTIES

BY

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In a previous paper (Ricketts, 1952a) a series of dextran sulphate preparations varying widely in molecular weight and sulphur content was described. The biological properties of these compounds were examined by Walton (1951, 1952), who found that molecular weight was the principal feature determining toxicity, the smaller molecules being the least toxic. At molecular weights (M_N) of the order of 7,000 a blood anticoagulant activity of about 15 international heparin units per mg. was attained when the degree of sulphation exceeded an average of 1.3 sulphate groups per glucose unit. It is in this region, where therapeutically useful anticoagulant activity is to be found, that a more detailed exploration of the relation between molecular features and biological properties than was provided by earlier work is necessary. The fractional precipitation of dextran sulphate from solution brings about, to some extent, a separation of molecular sizes. Exploration of the biological properties of such fractions provides an indication of the optimum molecular weight distribution in dextran sulphate intended for clinical use as an anticoagulant.

MATERIALS AND METHODS

Sulphur content was determined as previously described (Ricketts, 1952a). Intrinsic viscosity was measured in a No. 1 B.S.S. viscometer at 37° C. Over the range of concentration 1 to 4% in a 0.9% sodium chloride solution the plot η sp.*/c against c was linear, the intercept being the intrinsic viscosity.

Anticoagulant activity was measured as follows: sodium dextran sulphate 60 to 120 μg. in 0.1 ml. saline mixed with oxalated horse plasma (1 ml. 0.1M-potassium oxalate per 9 ml. blood) was incubated for 30 minutes at 37° C. Thrombokinase solution, 0.1 ml. (Difco, diluted 1:10 to 1:400 as required to give convenient clotting times), was added, followed by 0.1 ml. 0.025M-calcium chloride solution. Tubes were tilted at minute intervals and clotting was recorded up to 20 minutes. If clotting occurred at the same time in two tubes they were taken to contain the same number of units of anticoagulant activity. Intermediate values were obtained by interpolation. Accuracy is limited, because the observations are at minute intervals, to about ±15%.

Samples of dextran sulphate powder contained 8 to 15% moisture, which was determined for each sample by drying to constant weight *in vacuo* over phosphorus pentoxide at 60° C. The figures of Table I are corrected for this moisture content.

The precipitation of fibrinogen and other proteins from blood plasma is a limiting factor in the use of sulphated polysaccharides as clinical anticoagulants (Walton, 1952). This had previously been found to occur with dextran sulphate having a molecular weight in excess of about 20,000. Its occurrence in occasional samples of lower molecular weight suggested that such samples were unusually polydisperse. This possibility was investigated by fractional precipitation of one such sample with ethanol.

The fractionation was carried out as follows: ethanol was added to 15.4 g. of sodium dextran sulphate in 77 ml. 0.72% sodium chloride buffered with sodium bicarbonate at *p*H 7.3, to incipient turbidity at 30° C.; 108 ml. was required. The mixture was warmed until it became homogeneous at 33° C., and allowed to cool slowly. The precipitated syrup was separated and triturated in ethanol to a powder, fraction 1. Successive fractions were obtained similarly after the addition of further amounts, 2.5 to 25 ml., of ethanol.

RESULTS

Table I summarizes the properties of the initial sodium dextran sulphate and the seven fractions derived from it. Consideration of this table shows that 77% of the initial material is accounted for in these fractions. There was reason to suppose that the larger molecules would appear in the earlier fractions, and the intrinsic viscosity measurements show that this occurred. If, as seems likely, intrinsic viscosity is an additive property in this system, nearly the whole (80.2%) of the intrinsic viscosity can be accounted for. Material remaining in the solution at the final ethanol concentration, 52% v/v, was most probably of very short chain length and correspondingly low intrinsic viscosity. There is remarkably little variation in the sulphur content of the fractions until fraction 7, which has appreciably less

*η sp. is specific viscosity.

TABLE I
FRACTIONATION OF DEXTRAN SULPHATE

Fraction No.	% by Weight	Intrinsic Viscosity	%S in Na Salt	Activity (Units per mg.)	Fibrinogen Precipitation	Deposit in RE Cells
Original material	—	0·044	17·9	16·5	+	+
1	14·69	0·070	19·4	20·7	+++	+
2	11·44	0·058	19·3	20·2	++	+
3	10·25	0·054	20·6	20·7	++	+
4	11·23	0·044	19·4	17·7	+	+
5	15·44	0·035	19·6	14·2	0	0
6	9·83	0·026	19·3	11·9	0	0
7	3·95	—	14·9	4·6	0	0

sulphur. In all, 82.9% of the original sulphur is accounted for.

Comparisons of the original dextran sulphate with the International Standard Heparin preparation have indicated an activity close to 15 u./mg., or 16.5 u./mg. dry basis. This figure was assumed for the purpose of comparing the fractions with the original material. The sum of the activities of the fractions accounts for 80% of the original activity. From these measurements anticoagulant activity is seen to decrease slightly with decreasing intrinsic viscosity in the range 0.054 to 0.026.

The occurrence or otherwise of precipitation of fibrinogen *in vitro* by these fractions was investigated under physiological conditions of temperature, *p*H, and ionic strength by the technique previously described (Walton, 1952). Plus signs in Table I denote the extent of the precipitation observed.

It has previously been shown that similar precipitation occurs *in vivo*. The insoluble dextran sulphate protein complex formed in the blood stream is removed by the phagocytic activity of reticulo-endothelial cells in the liver, spleen, and bone marrow, and may be detected histologically in sections stained with toluidine blue (Walton, 1951).

It will be seen from the table that fibrinogen precipitation persists down to fraction 4 and that in this respect there was satisfactory correlation of the *in vitro* and *in vivo* tests for its occurrence.

DISCUSSION

Considering firstly the factors which affect fractionation, the solubility of a molecular species of dextran sulphate in ethanol-water solution is determined by the concentration of each component, temperature, ionic strength, and *p*H. Theoretically, dextran sulphate molecules may vary in length, and possibly in degree of branching, as well as in average degree of sulphation and uniformity of distribution of sulphate groups. Some limitation is, however, imposed by the relatively small number of glucose units in each molecule and by the comparatively high degree of sulphation. Provided sufficient electrolyte be present to avoid anomalies in viscosity, and to give a linear relation of η sp./c with c, the intrinsic viscosity so obtained is probably a measure of the length of the molecule. Although slightly lower intrinsic viscosities may be obtained in molar sodium chloride, there is probably some value in figures obtained in 0.9% sodium chloride, since activity measurements and other biological tests are made close to this ionic strength. Use of the original material as a standard of comparison for anticoagulant activity helps to overcome uncertainties about the validity of comparing different substances in a biological assay. Although the accuracy of the activity assays is not high, the progressive fall of anticoagulant activity with intrinsic viscosity is undoubtedly real.

For a clinical anticoagulant, the highest activity consistent with the absence of fibrinogen precipitation and its associated pathological effects is required. The indications are that this would be provided by preparations similar in molecular composition to fractions 5 and 6. When these fractions were injected into rabbits the expected elevation of blood clotting time occurred. In view of the results with sulphuric esters of oligosaccharides in the maltose series, which displayed anticoagulant activity *in vitro* but were relatively ineffective *in vivo* (Ricketts, 1952b), it would be of interest to explore the properties of even smaller dextran sulphate molecules, and this is being done.

SUMMARY

1. Earlier work indicated that the smaller molecules of dextran sulphate might be useful anticoagulants. This paper describes more precisely the gradation of biological properties with molecular size.

2. Sodium dextran sulphate having about two sulphate groups per glucose unit was subjected to fractional precipitation from ethanol-water solution. A fraction containing 19.6% S and having an intrinsic viscosity of 0.035 showed an anticoagulant activity of 14.2 units per mg., and was free from undesirable properties.

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