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Personal Recollections ☆

A.G. Ogston *

8 Dewsbury Terrace, York YO1 1HA, UK

Abstract

I hope that this article will provide a short review of my involvement with physical and biological aspects of 'excluded volume' or 'molecular crowding'. The list of references is not exhaustive, but is meant primarily to acknowledge my debt to my many colleagues [1].

1. Beginnings

I was a young physical chemist, newly interested in biology, and on the lookout for a chance to work on some biological problem. My chance came in 1938, as an invitation to collaborate with the great endocrinologist, Solly Zuckerman [1], on the oestrous swelling of the sexual skin of rhesus monkeys. World War II put an end to that work without much achieved in the way of results. But I had become interested in the 'mucin' that seemed to play a part in the swelling of the extracellular matrix. Karl Meyer [2] had characterized the mucin as a glucosaminoglycan (then called hyaluronic acid = HA) and shown its wide distribution in the extracellular spaces of connective tissues. Of these, synovial fluid seemed the most convenient source. So I started to work with that in 1946.

For some five years my main collaborator was a recent Oxford graduate, Jean Stanier [3], supported by the Medical Research Council — in spite of my vagueness about 'what I hoped to discover'. We were lucky at the start to find a means of separating HA from other components of synovial fluid by the mildest possible treatment. This relied on the HA being retained by a very fine fritted glass filter (Jena, grade 5) which passed the other components of the fluid.

We [3] had at our disposal a number of physicochemical instruments or methods with which we spent probably too long a time establishing the molecular characteristics of HA. (These were: Svedberg oil turbine ultracentrifuge [4]; Gouy diffusiometer [5]; rotating cylinder Couette viscometer [6], modified for measurement of flow-birefringence [6]; small home-made elastoviscometer; later, Spinco Model E ultracentrifuge.) Partly because of retention of HA on the filter, I had started with, and clung too long to, the idea of the HA molecule being a highly elongated stiff rod. This could have agreed moderately well with the sedimentation diffusion data, but was quite inconsistent with the low values of birefringence. This and simultaneous work with John Fessler [7] on synthetic sarcosine polymers and with

☆ Additional footnotes provide by T.C. Laurent, Department of Medicinal Chemistry, University of Uppsala Biomedical Center, S-75124 Uppsala, Sweden.

* Corresponding author.

[1] Professor Ogston has also given a more extended account of his research in a lecture with the title "An Open-ended Tale" at the Australian Biochemical Society. This was published in Search, 1 (1970) 60–63.

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Frank Woods [8] on dextrans showed that we must adopt the Flory–Huggins [9] random coil model.

This model takes each chain to be flexible, its segments distributed randomly within a spherical domain. Dynamically and thermodynamically, at extremely low concentration, each chain within its domain acts as a single statistical unit. At high enough concentration the chains overlap and/or interpenetrate to form a continuous network of chain segments. Dynamic and thermodynamic properties become dependent only on the network [2]. On this basis the molecular weight of HA came out at about 10^7 and the specific domain volume as about 2×10^3 ml g^{-1} [10].

There's a moral to be drawn. If an expert is called in to help with a complex problem, he's all too likely to do, not what is required, but what he knows how.

2. Physiological questions

Lacking the means to do direct measurements on live connective tissues we can only make informed guesses based upon what we can measure.

Stabilization of connective tissue: I regard connective tissue as a fine network of HA mechanically stabilized by entanglement or attachment to a relatively coarse network of collagen fibres. Statically [10,11] the HA provides an osmotically active solute to maintain loading of the tissue with solvent [3]. Dynamically, comparison of solvent conductance with the equivalent sedimentation velocity shows that HA can account for the greatest part of the resistance offered to flow through the tissue consequent on change of posture or local [10] distortion.

Lubrication of joints: MacConaill in 1932 [12] suggested that anatomical joints, by the form of the cartilages, behave as 'slipper bearings'. These have the characteristics that both load bearing capacity and drag are proportional to the rate of shear and the viscosity of the lubricant. Synovial fluid, because of the inverse variation of its viscosity with rate of shear, would seem to make an ideal lubricant allowing load bearing to be independent of speed.

Protection of cartilage: An elastoviscous fluid should have the property of stiffening under sudden stress. We constructed a small elastoviscometer [13]. It was great fun to play with but it indicated elastic forces much lower than could be significant. We made another rather simple device for measuring the effects of SF in keeping apart glass surfaces under load [13]. The indication was that this might be important, if collagen surfaces behave like glass.

3. Interactions in macromolecular systems [4]

Further developments began with the application of the idea of 'excluded volume', in the first place to a steric model of spheres (e.g. BSA) excluded from a network of thin fibers (e.g. HA) [14]. With use of adaptation of P.J. Flory's 'dilute solution', theory [9] we showed that the model describes qualitatively and quantitatively various related properties of polymer systems; for example, the excess of osmotic pressures of mixed HA/BSA over the summed contributions of the solutes measured separately and the corresponding degree of exclusion [10,11].

In another application, light-scattering [15] of mixed solutions of HA/BSA was measured, keeping the relatively high concentration of BSA constant while varying that of HA. In effect this measures the

[2] The early work on the characterization of HA was summarized by B.S. Blumberg and A.G. Ogston in the Ciba Foundation Symposium on "The Chemistry and Biology of Mucopolysaccharides" (1958) pp. 22–37.

[3] An important seminar on "Physical Chemistry of Connective Tissue" was held in Stowe, Vermont, in 1965 and was published in Federation Proceedings, May–June 1966. At this symposium Ogston explained to the physiologists the ambiguity of the term 'water binding'.

[4] Ogston approached the problem of macromolecular interaction in two ways. First he looked upon it in a mechanistic way and calculated from first principles the excluded volume for a spherical particle with a given diameter in a random suspension of thin fibers (ref. [14]). Together with Charles Phelps he made the first experimental analysis of the phenomenon in a macromolecular system (A.G. Ogston and C.F. Phelps, The partition of solutes between buffer solutions and solutions containing hyaluronic acid. Biochem. J., 78 (1960) 827–833). Secondly, he approached the problem from a thermodynamic point of view: a paper which is often referred to is A.G. Ogston, "Some thermodynamic relationships in ternary systems with special reference to the properties of systems containing hyaluronic acid and protein", Arch. Biophys. Biochem., Suppl. 1 (1962) 39–51.

excluded volume of BSA by HA at zero concentration of the HA.

A useful technique, first applied to HA but later extended to other chain polymers, was that of equilibrium sedimentation [16]. Under suitable conditions (high initial concentration, long cell height, small cell thickness, relatively high rotor speed) the gradient of concentration can be determined, thence the second virial coefficient, over a wide range of concentration, with much less effort and time than would be needed for the corresponding measurements of osmotic pressure.

With the last-mentioned facility, we could investigate the osmotic properties of gels, such as Sephadex [17]. Thus, the degrees of shrinkage of a gel bead exposed to polymer solution can be measured optically. If the polymer molecule is large enough to be totally excluded from the gel, the virial coefficients of the gel and polymer solution can be directly compared. If the polymer is not totally excluded, expression of its interaction with the gel must be included [18,19].

With values of the virial coefficients of BSA, dextran fractions [4] and polyethylene oxide samples [2] we were able to make use of 10 different pairs to investigate the occurrence of 'incompatible phase separation', i.e. the separation of a single mixed solution into two of differing composition [10]. Since the occurrence of this depends on the values of the separate and cross coefficients, it provides yet another test of consistency.

Finally, a stochastic treatment was devised [20] to account for the retardation of compact molecules through solutions of chain polymers [21].

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