I. Research in Britain on Connective Tissues

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From the end of the last century strong schools have been built up in Britain with interests in the structures of natural products and in intermediary metabolism. Purdie and Irvine developed methylation procedures to a fine art in the elucidation of the structures of carbohydrates, and later in the hands of Hirst and Howarth these methods became the foundation of the attack on the structures of polysaccharides. From 1920 important intracellular polysaccharides were investigated and some plant gums and polyuronides were described in essentially the terms we recognise nowadays. In various guises the methylation methods are still of value in structural investigations.

The powerful groups of carbohydrate chemists which arose following the success of these investigations soon turned their attentions to the structures of the more complex amino sugar-containing molecules and it was in Birmingham that the final proof of structure and unequivocal synthesis of glucosamine and galactosamine were achieved. Many other indispensable reference compounds in the monosaccharide series were forthcoming during these years.

Work on what are now known as glycosaminoglycans proceeded from the middle 1930's in the hands of Morgan and his collaborators at the Lister Institute, which resulted in the foundation of our present understanding of blood group substances and in an appreciation of the importance in immunology of glycoproteins. Also dating from this time are the Elson-Morgan and Morgan-Elson tests, which are arguably the most important analytical colour reactions to have been used in this field. They are still at the heart of many biological and chemical investigations, — an astonishing record for colorimetric methods, lasting over 40 years, (i.e. almost from the very outset of the era of colorimetry). Modern research into the structures and composition of connective tissues may be said to have begun with the invention in Britain of the chromatographic methods associated with Martin, Synge, Consden, Gordon and James. They were applied to small amounts of tissue extracts with the discovery of many new materials and structures. About this time the work of Neuberger on the biosynthesis of collagen began, as did also his interest in the structures of glycoproteins, which led to the recognition of the asparagine-glucosamine link, the first covalent bridge to be discovered between sugars and proteins. Astbury, in Leeds in the 1930's brilliantly pioneered the application of X-rays to the structure of proteins, particularly including collagen. Randall and the King's College group followed this up, as did
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Crick (of DNA fame) with great effect.

In the 1950's new groups sprang up, some based on previous contributors to the field and others arising in environments in which connective tissue work was new. In general biochemistry departments were not well represented at this stage. Most workers were either in applied research, i.e. associated with glue, gelatine and leather industries, or with medical units, in which an interest in the so-called collagen diseases was a new bandwagon. The name of Partridge, who has contributed much to the modern picture of elastin, particularly with his discovery of desmosine and isodesmosine, is associated with the former kind of environment, (i.e. meat research). Helen Muir, working at the National Institute of Medical Research, Mill Hill, and later in St. Mary's Medical School, followed lines which led to the recognition of chondroitin sulphate as bound through serine to a protein core, and later to the discovery of the aggregation of proteoglycan with hyaluronate. My own interests (centred on the isolation and characterization of chondroitin sulphates, etc. from tissues, see below) were also dictated by the demands of a medically orientated department (chemical pathology at Manchester University). David Jackson worked on extractable collagens in a rheumatism unit, and much other fine work was contributed from the Eye Hospitals, Orthopaedic Hospitals, Institutes of Dermatology, and Bone Disease Centres. However, research on enzymes involved in degradation of glycosaminoglycans tended to be an academic exercise initially, and Dodgson in Cardiff built on early work with the sulphatases to produce a very active group which works on connective tissues in general.

Nowadays, the number of groups has tended to decrease slightly, due to financial stringencies, and a new pattern is discernible. There has been an amalgamation of specialist units working either in the protein fibre areas or the glycosaminoglycan field into groups with interests in connective tissues as a whole. Such teams are present in several institutes in London, Cambridge, Manchester, Cardiff, and Oxford. Specialized groups are still to be found, for instance, working on GAG physical chemistry in Lancaster, fibrous proteins in Bristol, and on dolichol sugar compounds in glycoprotein synthesis in Nottingham.

As well as the more concentrated groups, there are still many individual scientists and medically qualified people with direct and sometimes full-time interests in this field. It was thought essential in the 1960's to bring together all the interested people into clubs. The first to be formed was the Mucopolysaccharide Club, founded in Taplow in 1967, and shortly afterwards the Collagen Club, also founded in Taplow. In partnership with the older French Club, the Mucopolysaccharide Club took the initiative in promoting European collaboration between connective tissue groups, and the first meeting of the Federation of European Connective Tissue Clubs took place in Cambridge in 1968\(^3\). This movement has grown until upwards of 1,000 people in Europe are likely to find something of interest in the meetings, which are held every two years.

Nowadays, the Mucopolysaccharide and Collagen Clubs work more and more closely together, with many joint meetings. Prior to the existence of these Clubs, the Bone
and Tooth Society was in vigorous life, dating back to 1950. This probably makes it the oldest connective tissue club in the world, and it too was responsible for organising collaboration between national Bone and Tooth Societies on an international scale. It seems inevitable that all three British groups will coalesce, and then we can expect a very lively and rather large connective tissue society, with more than 500 members.

Reference

II. Critical Electrolyte Concentration Phenomena in Connective Tissue Research

In 1953, as a post graduate in the Department of Chemical Pathology, Manchester University, I was given the problem of analysing arterial tissue for "mucopolysaccharides" as a part of an investigation of atherosclerosis. At that time, the structures of the mucopolysaccharides were uncertain, and no systematic methods were available for their analysis. I was to try out various techniques. Paper chromatography was very popular, although very little used on polymeric materials. Kerby had achieved separations of impure chondroitin sulphates, and this was followed up. The commoner solvent systems did not move the polysaccharides from the origin, and on the principle that the more soluble the compounds were in the solvent, the faster they would move, amines were incorporated into the solvent systems. The best amines should thus have a large organic portion, and on the shelf was a preparation of I.C.A. cetrimide (impure cetyltrimethyl ammonium). With this in the solvent the results were puzzling. It was impossible to stain the polysaccharide with toluidine blue, although no difficulty had been encountered with other solvent systems. Other results suggested that the cetrimide was not moving with the solvent front, but some distance behind it, and there would be a time during which the polymer was extracted with solvent without amine. I therefore added cetrimide to a solution of chondroitin sulphate before applying it to the paper and was rewarded by the sight of a flocculant white precipitate. This complex was very insoluble, and could be obtained from solutions of chondroitin at concentrations of less than 1 ppm. This offered a basis for recovering polysaccharides for analysis, and one important difficulty would be overcome.13

It was soon found that the precipitates were soluble in salt solutions. The polyanions could then be recovered as inorganic salts by precipitation with ethanol, or by absorbing or extracting the cetrimide in organic solvents such as chloroform, and then dialyzing away the salt. We then had a practical method for rapidly isolating very small amounts of chondroitin sulphate, etc.22