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Scott J E & Dorling J. Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions. *Histochemie* **5**:221-33, 1965. [Medical Research Council Rheumatism Research Unit. Canadian Red Cross Memorial Hospital. Taplow. Maidenhead, Berkshire. England]

The application of the "critical electrolyte concentration" concept to the differentiation of acidic glycosaminoglycans (mucopolysaccharides) is described. Alcian blue 8 GX stains with increasing selectivity as increasing amounts of magnesium chloride are incorporated into the dye solution. Experiments showed that binding of dye to carboxylate or phosphate groups ceased at low electrolyte concentrations (<0.3M) whereas dye continued to be held by sulphate ester groups at concentrations 5 to 10 times as high. The conditions in which this principle can be used in a practical technique are described. [The *SCI*® indicates that this paper has been cited in more-than 895 publications.]

Foundations of Chemical Morphology

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In 1953 I was pitchforked, unprepared and unaware, into research on "mucopolysaccharides" in arteriosclerosis by an equally uninformed supervisor in the Manchester Medical School. Suck-it-and-see analytical methods, requiring vast amounts of material, were applied to substances of unknown structure. Although paper chromatography, then a dazzling new technique, promised resolving power and sensitivity, insights into the behaviour of polymers in systems designed for small molecules (sugars, amino acids, etc.) were few. My early results had only the charm of novelty. I tried to understand them, modifying the paper, and experimenting with detection reagents, particularly toluidine blue and Alcian blue, which stained chondroitin sulphate, etc. The "best" solvent systems contained amines, and I finally tried cetyltrimethyl ammonium bromide (Cetavlon). The detection methods then no longer worked. A control experiment, mixing Cetavlon with polymer in solution, produced a marvellous precipitate. Within the day I had found that the reaction was given only by polyanions, even at very high dilution, opening up new possibilities of isolation and purification. Inorganic salts flocculated the precipitates for easy centrif ugation, but some (from pectin, etc.) dissolved in the salt solution. I found that *all* such complexes were soluble (or did not precipitate) in salt solutions; that the phenomenon was critically dependent on the salt concentration (the CSC, later changed to CEC, "critical electrolyte concentration"); and that the CEC was characteristic of the chemical structure, particularly the type of polyanionic charge (carboxylate, phosphate ester, or sulphate ester). Paper chromatography was forgotten. A new supervisor (John Kench, later professor in Durban, South Africa) gave me carte blanche to pursue my fractionation scheme through to a PhD thesis and the A.V. Hill Prize. These CEC methods worked from submicrogram levels up to the industrial scale.[1] I proposed patenting heparin isolation, but my professor refused, on pain of my dismissal. Much of the world's heparin has since been made on CEC principles. It is difficult to reckon the loss to the department and the university (and myself!).

I presented the theoretical bases of CEC phenomena to a Biochemical Society meeting in November 1960.1 spoke last, and only the chairman, secretary, and a foreign student, who I am convinced was lost, were left in the hall. My treatment predicted relationships (later validated) between CECs and M_rs of polyanion fractions—and also that any precipitating cation could be used in CEC systems. Coloured cations (Alcian blue particularly) differentially stained polyanions on chromatograms, eiectrophoretic strips—and in tissues, giving direct comparisons between chemical fractionation and ultrastructural localisation. Biochemists and biologists then enjoyed talking the same language, based on a very simple technique that both could use. The infant subject of chemical morphology was thus established. Alcian blue-CEC methodologies were applied by microscopists (anatomists, pathologists, etc.) when tissue polyanions (nucleic acids, acidic polysaccharides, mucins, etc.) from every kind of organism were to be specifically localised and classified in a rational, simple way.

Histochemical "fractionations" (i.e., differential stainings) are 1,000 times more sensitive than their chemical counterparts. A further dramatic increase in sensitivity came with the synthesis of CEC reagents (Cupromeronic blue, etc.) designed for electron microscopy,[2] ultimately achieving "fractionation" of single molecules in the tissues, permitting elucidation of supramolecular organisation in extracellular matrices.[3]

For this paper and related work I was awarded the Biochemical Society's Gold Medal (1973) and the Robert Feulgen Prize (1988) by the Gesellschaft für Histochemie. Jack Dorling retired as senior technician in histology in 1978.

1. Scott J E. Aliphatic ammonium salts in the assay of acidic polysaccharides from tissues. (Glick D, ed.) *Methods of biochemical analysis.* New York: Interscience, 1960. Vol. 8. p. 145-97. (Cited 795 times.)

2.----------. Proteoglycan histochemistry—a valuable tool for connective tissue biochemists. *Collagen Relat. Res.* 5:71-81. 1985.

3.----------. Supramolecular organization of extracellular matrix glycosaminoglycans, in vitro and in the tissues. *FASEB J.* 6:2639-45. 1992.

Received April 2. 1993