The preparation and analysis of a blood group
A-active mucin glycoprotein

Tricks of the trade in glycoscience

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Many aspects of glycosylation are conserved among animals, and it can be advantageous and sometimes critical to identify a readily available and abundant source of carbohydrate material that harbours a hard-to-characterize antigen or ligand of interest. The Biochemical Journal Classic paper by Morgan and King is a well-written account of serviceable methods for the extraction and quantification of a carbohydrate antigen. These methods were highly influential in subsequent studies of the blood group antigens. Some of these tricks of the trade still have a place in modern glycobiology.

Not all highly cited papers deliver a critical breakthrough in their fields. Sometimes a well-written account of some careful work of the more 'incremental' kind can be cited time and again for nearly half a century, for the sake of the methods used rather than the results obtained.

In 1943, Walter T.J. Morgan and his research student H.K. King submitted to the Biochemical Journal an account of their work in isolating and characterizing a component of hog gastric mucin with considerable blood group A activity, as measured by isoagglutination inhibition and erythrocyte lysis assays.

Two purification methods are described, both of which avoid extreme conditions and so yield a more active product than had previously been achieved. These two methods, and erythrocyte agglutination and lysis assays, are clearly described, and it is for these methodological advances that the paper was valued over the succeeding 40 years. The haemagglutination method is referred to repeatedly in papers discussing the characterization of blood group activity in diverse preparations, from sputum glycoproteins to Egyptian remains.

When the paper was written, no-one knew exactly what the A antigen was, although it was clear that it could be found at cell surfaces or in soluble form and that carbohydrates were involved in addition to amino acids or lipid. Morgan and King refer to their preparation simply as 'the A-substance'. This substance gave an opalescent viscous solution, readily degraded under heat or alkaline conditions. The degraded material lost its ability to inhibit isoagglutination, but had somewhat enhanced ability to inhibit cell lysis.
At the time, blood group substances could not be isolated from erythrocytes in sufficiently high yields for chemical characterization so this porcine material proved useful as a benchmark in the work of other researchers, such as in the study of Bray et al.\textsuperscript{2} that first saw fucose (and mannose) in the A-substance and the development of chromatographic methods for monosaccharide analysis, that confirmed the presence of fucose, but not that of mannose. Porcine material contained only blood groups A and O; however, and it was not many years before the fluid from human ovarian cysts became the starting material of choice for the characterization of human blood group materials\textsuperscript{4}.

The Morgan and King paper was the seventh in a series with the overall title ‘Studies in Immunochemistry’, consisting of 23 papers in the Biochemical Journal on the subject of carbohydrate antigens. Indeed both authors made substantial contributions to the Biochemical Journal and the Biochemical Society, and Walter Morgan was made an Honorary Member in 1969. Some of the papers, such as the first in the series\textsuperscript{5}, deal with \textit{Shigella} polysaccharides, but most are concerned with the isolation, purification and characterization of blood group substances, and so make good use of the Morgan and King methods. Papers in the series describing the preparation of human blood group substances A\textsuperscript{4} and B\textsuperscript{9} from ovarian cyst fluid are good examples.

The ABO blood-group system was identified in humans by Landsteiner\textsuperscript{10,11} and, although the genetics and many immunological characteristics of the system were understood by the time of the 1943 paper, it was only in the 1960s that the carbohydrate structures that constitute the ABO(H) blood group antigens were confidently determined by the groups of Morgan and Watkins and Elvin A. Kabat (as reviewed in\textsuperscript{12,13}). Winifred Watkins\textsuperscript{14}, writing in 1966, presented the specific A, B and H structures (Figure 2) having been very recently confirmed.

One of the earliest papers to cite Morgan and King was by Kabat and Bezer\textsuperscript{15} who used the hog gastric mucosal A-substance to develop a precipitation method for the quantification of blood group substances. The two techniques of haemagglutination (in the Morgan group) and precipitation (in the Kabat group) proved complementary in the structural elucidation of the ABH and the Lewis\textsuperscript{ab} blood group antigens\textsuperscript{16}. The names of Kabat, Morgan and Watkins are those cited by recent commentators\textsuperscript{17} in the context of structural studies of the ABH epitopes; the friendly rivalry between the two laboratories provided scientific strength as each group provided evidence confirming and extending the other’s findings.

According to the biographical account by Watkins\textsuperscript{18} written in 1999 (from which we obtained the likeness shown in Figure 1), Walter T.J. Morgan was born in 1900 and outlived the 20th Century. His early to mid-career was influenced by both World Wars; the Second World War in particular provided a spur to research to improve compatibility of blood transfusions. Walter Morgan joined the Lister Institute as a PhD student and stayed there for 50 years, including a short ‘retirement’ break, ending as the last Director when the institute closed down its research premises in 1975. The Lister Institute, established in 1891, was an internationally important organization that undertook both basic medical research
and the production of vaccine, antisera and antitoxins. It was central to the development of biochemistry in Britain, and held in extremely high regard. The organization still exists, providing prestigious fellowships to young researchers. During his time at the Lister Institute, in 1949 Walter Morgan was elected a Fellow of the Royal Society and in 1959 he was made a CBE. At the age of 75, when at the instigation of one of us (T.F.) Winifred Watkins’ laboratory moved to the MRC Clinical Research Centre at Northwick Park, Walter moved there, finally ending his work at the bench in 1989. A most interesting biographical memoir of Walter was published by the Royal Society19.

In the 70 years since Morgan and King published their method for the isolation of blood group substance from hog gastric mucosa, their methods have been in continuous use. They have been updated, of course, to take account of new reagents and methods, but are still essentially the same today. By coincidence, a very recently published article introduced the neoglycolipid (NGL) technology for the design of oligosaccharide probes for detection and structural characterization of ligands of carbohydrate-recognizing proteins, and in 2002 this became the basis of the first microarray system for sequence-defined oligosaccharides, intended to encompass entire glycomes. e.mail: b.mulloy@imperial.ac.uk

Glycobiology technologies have changed in many ways. For example, quantitative haemagglutination and precipitation techniques have given way to modern carbohydrate microarray technologies as used for elucidating the F77 antigen20; however, the identification of an abundant source of a compound of interest and development of a simple and gentle method for its isolation remain tricks of the trade in glycobiology that have stood the test of time. ■

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