THE CHEMICAL BASIS OF SEROLOGICAL SPECIFICITY WITHIN THE ABO BLOOD GROUP SYSTEM.

1. ISOLATION AND PROPERTIES OF THE GROUP SUBSTANCES.

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In 1901 Landsteiner observed that when the erythrocytes of certain individuals were mixed with the sera of others that agglutination or clumping of the red cells frequently, but not invariably, took place. He considered that the agglutination was the result of the interaction of antigens or agglutinogens on the red cell surface with their specific antibodies or agglutinins present in the agglutinating serum and on the basis of his observations concluded that there existed two antigens, arbitrarily designated A and B, the presence or absence of which on the erythrocytes gave rise to three types of blood A, B and O. A year later, Decastello and Sturli recognised a fourth and rarer group, the erythrocytes of which possessed both antigens simultaneously. Bloods of this type were classified as group AB.

The existence of the A and B antigens was readily substantiated by the occurrence in the serum of anti-A (α) and anti-B (β) agglutinins when the red cells did not carry the corresponding antigen. Antibodies which preferentially agglutinate group O erythrocytes occur only very rarely in man but as the investigation of the ABO blood group system continued anti-O agglutinins were found in a number of sources, such as the sera of rabbits immunised with group O erythrocytes, the normal serum of the eel Anguilla anguilla* and extracts of the seeds of certain plants. The reciprocal relationship between the antigens on the erythrocytes and the antibodies in the serum is shown in Table 1.

<table>
<thead>
<tr>
<th>Antigen on Erythrocytes</th>
<th>Blood Group</th>
<th>Antibody in Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>anti-B (α)</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>anti-A (β)</td>
</tr>
<tr>
<td>A and B</td>
<td>AB</td>
<td>none</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>anti-A and anti-B</td>
</tr>
</tbody>
</table>

Subsequent genetical investigation showed that the blood group characters are inherited and that they behave according to Mendelian laws. The theory of inheritance now generally accepted is that of Bernstein where the blood group of an individual depends on the presence of any two of three allelomorphic genes, A, B and O, the gene O being recessive to the A and B genes.

It is now known that the blood group characters of erythrocytes are due to the presence on their surfaces of substances which are the products—

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of the activity of the blood group genes. In the case of the ABO blood groups about 80 per cent. of individuals have been found to secrete their group substances in a water-soluble form and their isolation from secretions has shown that the specific substances are macromolecular complexes of polypeptide and polysaccharide. These complexes are known as mucoids or mucopolysaccharides. The saliva of secretors, which contains the active mucoid, possesses the capacity to inhibit the agglutination of red cells by the appropriate antiserum, e.g., the saliva of a group A "secretor" will inhibit the agglutination of group A erythrocytes by a serum.

**H-Substance.** While the saliva of group O "secretors" inhibits the agglutination of group O erythrocytes by anti-O agglutinins, it has also been observed that the saliva of secretors of genotype A,B, who, according to Bernstein's theory, could not possess the O gene, also contains a substance which inhibits anti-O sera. It is, therefore, now considered that the O-like substance of saliva is not a product of the O gene. Morgan and Watkins suggested that the mucoid material secreted by group O individuals be termed H substance, to distinguish it as a heterogenetic or basic substance common to the majority of red cells, tissue fluids and secretions irrespective of the ABO group of the individual. The serologically active material secreted by group O persons is, therefore, now referred to as H substance, not as O substance.

**Isolation of the ABH substances.**

The exact nature of the blood group substances on the red cell surface is not known but the specific substances appear to occur in the form of lipoprotein complexes, insoluble in water but soluble in ethanol. Earlier attempts to isolate the group substances were made by the extraction of erythrocytes with alcohol and other organic solvents but due to the special difficulties associated with their isolation from this source the preparations obtained were of low serological activity and were not completely purified. The results, however, established that the group substances were predominantly carbohydrate in nature.

Later studies showed that the blood group substances occurred in a large variety of tissues, the major sources in the body being the glandular mucosal cells which secrete gastric juice, bile, saliva, etc., and serologically active materials have been isolated from saliva, from gastric mucosa of hog, human, horse and bovine origin, from meconium (the first stool of the newborn) and from the contents of ovarian cysts. The presence of the group substances in pseudomucinous ovarian cyst fluids of secretors was detected by Morgan and van Heyningen, and these cysts have proved to be the best source of human blood group substances. Certain ovarian cysts contain paramucin, a rigid jelly-like material, and it was not possible until recently to examine the contents of these cysts, when a method of liquefying the gel structure under very mild conditions was found. Subsequent purification of the paramucin showed that it can also be a rich source of the blood group substances.

**Methods used in the isolation of the blood group substances.**

Many methods have been devised for the purification of the blood group substances but it has generally been found that the best procedure is the deproteinisation of the freeze-dried secretion (e.g., saliva, cyst fluid,