DESCRIPTION OF THE HAEMOLYTIC SYSTEM FOR TESTING THE BLOOD GROUPS IN SMALL RUMINANTS

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Abstract

The paper analyses the biochemical, structural and physiological peculiarities of the haemolytic system *(antigen–antibody–complement) which underlines the technique used in the serology of blood groups* – haemolysis – *for detecting the blood group factors in sheep and goats. The evolution of the whole haemolytic process in ovicaprinae is presented (sensitiveness of erythrocytes, their coupling with antibodies, forming operation of antigen-antibody complex, activity phases of the complement fractions and* $C_1^{`}$ *-esterase, alteration of erythrocyte membrane, haemoglobin remission).*

Key words: antigen, antibody, complement, blood group, ovicaprinae.

INTRODUCTION

The techniques used in the blood group serology in animals are based on the reciprocal reaction capacity of antigens with antibodies when they are in immediate contact. The combination of these two immunobiochemical entities engenders a complex (*antigen-antibody complex*) with diverse properties of those of the component elements.

In the blood group serology in animals there are two phenomenon categories at the level of antigen-antibody complex: 1) aggregation phenomena by *haemagglutination* and 2) phenomena of structural and functional cellular alteration by *haemolysis* reaction. In this last phenomenon, besides antigens and antibodies, the presence of a third component of haemolytic system – *complement* – is necessary.

In small ruminants (sheep and goats), the immunogenetic test for detecting the blood group factors is haemolysis.

MATERIAL AND METHODS

In such experimental works, the blood of different animal species (sheep, goat, cow, rabbit, guinea pig etc.) is used. The blood is drawn on sodium citrate (anticoagulant).

The classical method of haemolytic test for immunogenetic studies is based on the antigen-antibody immunoserological reaction principle. Thus, the reactant elements are as follows: red cells of sheep or goats are used as *antigens*; the erythrocyte suspensions are prepared from the fresh drawn blood according to the standard method. As *antibodies*, the monospecific reagent sera are used; these anti-sera can be obtained either by isoimmunisation method or by heteroimmunisation method, depending on the animal species used as donor in the *donor-receiver couples* in the immunization process. In order to normally develop the antigen-antibody reaction, the *complement* of rabbit or guinea pig, absorbed on sheep or goat red cells, is added to these component elements. At the same time, two serological controls (one of the physiological serum and another of the complement) are made to avoid pseudo reactions. All immunoserogical reactions take place in the micro-titrater Takátsy under thermostatic conditions of 25-26^{0}C.

The aspect of cellular or membrane ultrastructure can be pointed out by electronic microscopy and the aspects of biochemical structures can be achieved by electrophoresis, chromatography etc.

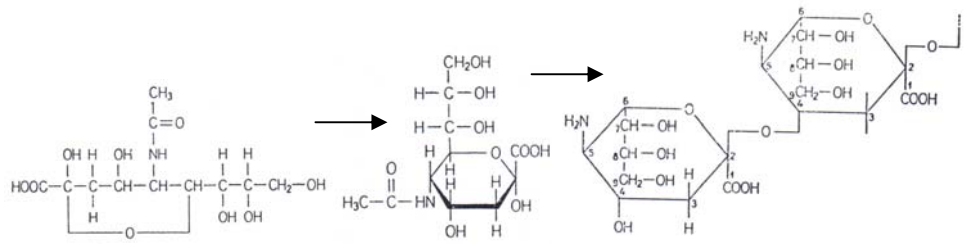
METHODOLOGY AND DISCUSSIONS

The essence of the haemolytic process in the blood group serology in sheep and goats is based on the structural and electrochemical

structure between antigen and antibody and the specificity of enzymatic reactivity of the complement on the antigen-antibody complement.

Antigens

The *antigens* of haemolytic system are represented by erythrocytes that own a mosaic of glycopeptides, lipoproteins and mucous-polysaccharides structures endowed with antigenic specificities on the external surface of their membranes [3, 4, 7]. It seems that the antigenic specificity of the macromolecular structures of erythrocyte membrane is conferred by the sialic acid [fig. 1b] which is constituted, in its turn, of the neuraminic acid residues [5, 8]. The neuraminic acid is a more complex aminoglucide composed of a pyruvic acid residue and another of mannose amine [fig. 1a]. Therefore, the sialic acids are homogeneous mucous polysaccharides made of N-acetylated neuraminic acid residues associated by 2,4- β-glycoside bindings [fig. 1c]. These glycoproteins are named sialoglycoproteins because the sialic acid is present in their structure; the sialoglycoproteins are constituents of the cellular membranes and have an important role for membrane integrity. The sialoglycoproteins have molecular masses of approximately 30000 daltons. Depending on the biochemical composition, isomeric structure and stereochemical configuration, there are three types of sialoglycoproteins: glycophorine A, glycophorine B and glycophorine C. Approximately 131 amino-acids enter in their structure having the N-terminal extremity orientated to the exterior of erythrocyte membrane and the C-terminal extremity in cytoplasm [3, 6, 7, 8]. These substances can be destroyed by neuraminidase.



a
Neuraminic acid

b
Sialic acid

c
Fragment of sialoprotein

Fig. 1 – Biochemical structures of glycoproteins on the surface of red cells

The antigenic specificity conferred by sialoproteins can be increased by the presence of some complex lipids (named sphingolipids) [fig. 2] which are associated with glycoproteins in the structure of red corpuscle membranes [fig. 3], together determining the specificity of blood group.

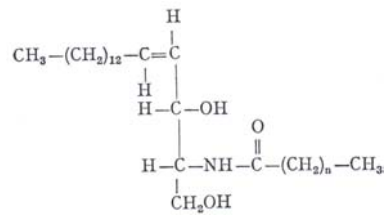


Fig. 2 – Biochemical structure of sphingolipid (ceramide)

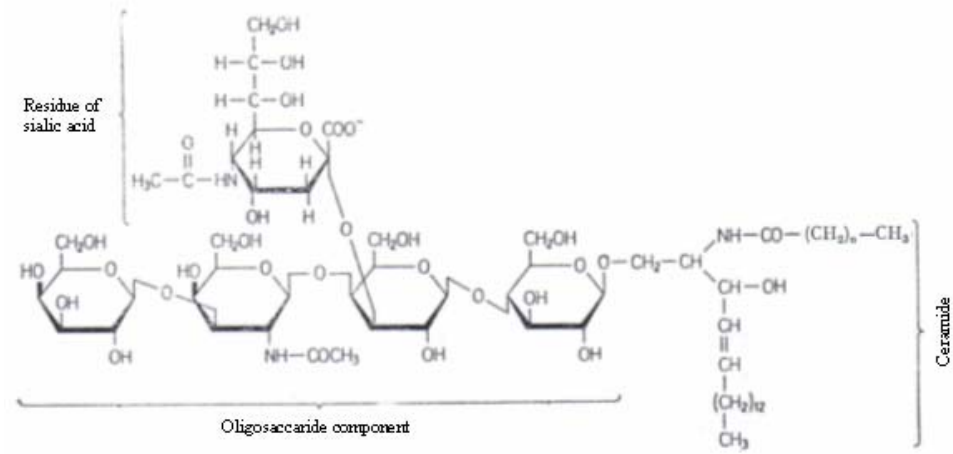


Fig. 3 – Saccharide-lipid-protein complex on the red corpuscle membrane

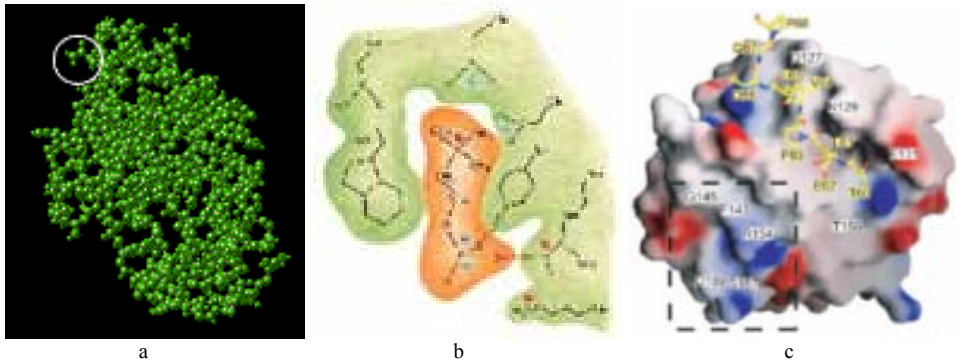
In generic terms, the antigens are constituted of two fragments:

- a protein which confers antigenic valence to antigen molecule, having a high enough molecular mass;

- a coupled grouping which confers strictness specificity to antigenic molecule and which electively joins with the adequate antibody. Separated from the protein macromolecule, the coupled grouping is able to specifically join with the antibody, but it is almost missed of antigenic valence [3, 4, 7].

The antigenity of erythrocyte antigens is conferred not so by their chemical composition (being very similar in most species), but especially by the presence of some isomeric and stereochemical structures of the erythrocyte membrane [fig. 4a] [5, 6, 7].

The biochemical structures on or in the red corpuscle walls, which confer antigenity, are also named *haptenes* or *antigenic determinants* [fig. 4b], representing the antigen fragments on which the antibodies are fixed; the haptenes are represented by the coupled groupings of the antigen molecule. The configuration of these biostructures determines their specific interaction with the antibody molecules, the haptene finding a complementary structure on the antibody molecule. More haptenes can be fixed on a cellular membrane, either the same haptene in more places, or different haptens on whole erythrocyte surface. Consequently, the antigen molecule contains more combination zones (*antigenic determinants*), where the antibody molecules are bound; therefore, the antigen is "polyvalent" and the antigenic determinants are represented by different chemical groupings (-NH_2, -OH, -SH etc.) or cyclic amino-acid radicals (Phe, Tir, Trp) [fig. 4c] [3, 4, 5, 7].



a
Stereochemical structure

b
Haptene

c
Molecular structure of haptene

Fig. 4 – Antigen structure

The differences among the antigenic determinants concerning their arrangement in or on the cellular membrane, isomeric structure and spatial conformation will lead up to as many differentiations among the glycoprotein, lipoprotein or mucous-polysaccharide molecules; these aspects determine a certain antigenic individuality, a certain specificity for each substance, conferring a metabolic individuality to each red cell and, consequently, to each organism. Thus, the surface of red cell of one individual has a certain antigenic constellation. The vast antigenic diversity is pointed out by the agency of the specific antibodies.

Antibodies

The *antibodies* of the haemolytic system are represented by γ-globulins with decreased electrophoresis mobility at pH=8.6 and γ-microglobulins of Bence-Jones type [fig. 5a]. Because the antibodies are implied in the immune response they are also named immunoglobulins (*Ig*) [1, 2, 4].

All immunoglobulins are composed of four chains: two longer chains named *heavy chains* (HC) and two shorter chains named *light chains* (LC). The heavy chains are constitutes of approximately 446 amino-acids and the light chains are formed of 214 amino-acids. Disulphide bindings (S-S) join the heavy chains between them and the heavy chains with the light chains [fig. 5b, c]. The molecular mass of the L chains is 23000 daltons and the one of the H chains is 55000 daltons, so that whole molecular mass of an antibody molecule is approximately 150000 daltons. The two chain classes are combined in such a way as to result a characteristic configuration of the antibody molecule, having a forked shape (Y), which suit to the functional valences. Each branch of Y structure is named *Fab* fragment and it is endowed with a combination zone for antigen. The caudal zone of Y structure is named *Fc*, being responsible of affixation of the antibody molecule on the red cell surface with antigenic properties [fig. 7] [1, 2, 7].

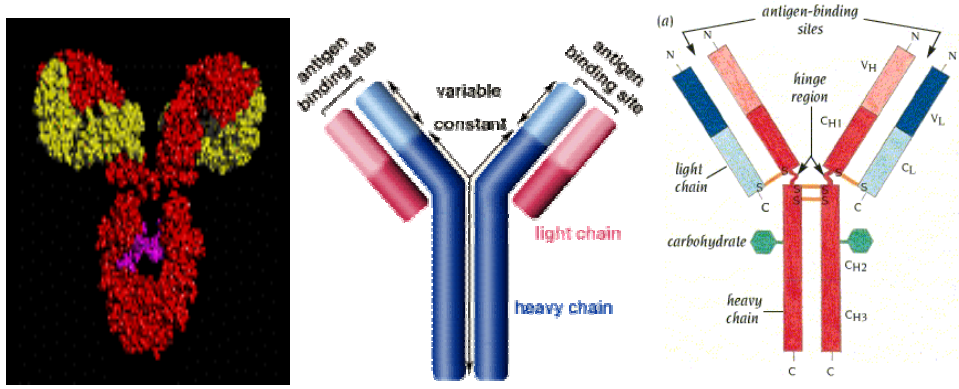
The sequence of amino-acids pointed out that about a half of each light chain and about three quarters of each heavy chain keep the same structure of the COOH extremity in all immunoglobulins. That is why these regions were named *constant regions* (C). The amino extremity (NH_2) of each heavy chain and of each light chain differs from an antigen class to another one owing to a certain number of substitutions. These regions were named *variable regions* (V). In certain zones of these regions, an emphasised variability appears, so that these zones were named *hypervariable regions* too; they occupy the positions 26-32, 48-55 and 90-95 in the light chains and the positions 31-37, 51-68, 84-91 and 101-110 in the heavy chains [fig. 5b, c]. However, trifling variations appear in the constant regions too, but only as regards the amino-acids succession. These variations are named *allotypes*. If the V region determines the antibody specificity, the constant region is implied in the antibody attaching on the red cell surface [2, 7].

The two chain types form pleat series named *fields*. In the constant region of heavy chains there are three fields: C_H3; C_H2 şi C_H1; each field is composed of 108 amino acids. In the constant region of light chains there is only a similar structure to the one of the heavy chain fields [7].

The variable region has capacity to specifically bind the antigens. The constant fields intervene in other immune processes. It seems that they join with the complement generating a reaction series that lead to the red cell destruction.

At the end of each branch of the bifurcation fork (Y) there is the binding site of antigen which consists in a hollow resulted by folding of the N-terminal regions of the co-operant light and heavy chains. So, each *Ig* molecule has two identical binding sites for an antigen [7]. Therefore, unlike antigens, the antibodies are “bivalent”, their molecule containing two combination sites at which level the antigens are bound. These sites are characterised by a certain spatial organization (*stereospecificity*) which is complementary to the antigenic determinants [fig. 5a]. The *Fab* fragments confer to antibody molecule the property to combine with the respective antigen; in fact, the two *Fab* molecular fragments determine the “bivalence” of the

antibody molecule, therefore the capacity to be bond with the two antigen molecules [fig. 7]. The variability of primary structure from the level of the *Fab* N-terminal segments appears to be the structural base of the antibody specificity [1, 7].



a
Stereochemical structure

b
Immunoglobulin chains

c
Chemical bindings

Fig. 5 – Antibody structure

The antibody biosynthesis is started by an antigen. The antibody owns the capacity to specifically react with the antigen which caused their forming operation. So, the antibody synthesis is determined by an antigenic stimulus and constitutes the immune response within the defence reaction of organism against some strange agents. The biochemical process of antibody producing is named *immunisation* and the animal which biosynthesized antibody becomes *immunised*. The *immune serum* or a*ntiserum* represents the blood serum of an animal which was immunised [8].

The two combination types from the same antibody molecule (Ac) have the same specificity for a certain antigen (Ag) and take part in achievement of reaction between these structures, a *complex antigen-antibody* (Ag-Ac) resulting. The antigen-antibody reaction is stereospecific and the combination between Ac and Ag is based on existence of a *structural and electrochemical complementarity* between the Ac site and antigenic determinants [fig. 7].

Both polypeptides chains (heavy and light) from both regions (constant and variable) are synthesized in the organism under a strictly genetic control. On that account the antibodies have a high specificity being coupled only with the antigen which determined their appearance. The forming operation of an antibody implies the integration of the two regions (C and V). Because there is a vast diversity of variable regions and a small number of constant regions, it can be assumed that the two regions are separately synthesized, being united by a certain mechanism in the somatic cells. Therefore, the constant and variable regions both of heavy chains and of light chains are controlled by two genes.- one for the constant regions and an other for the variable regions.

In immunogenetics, the alleles are named *allotypical variants*. The genes V^H and C^H, (that control the synthesis of variable and constant regions of heavy chains), on the one hand, and the genes V^L and C^L (that control the synthesis of variable and constant regions of light chains), on the other hand, are linked to direct the synthesis of a single polypeptide [3, 8].

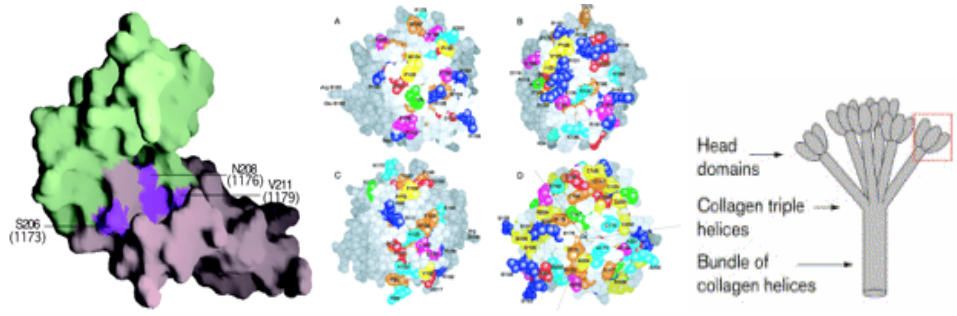
To immunoserologically typify the sheep and goats, both isoantibodies (antibodies proceed from immunization of sheep or goats) and heteroantibodies (antibodies proceed from immunization of animals belonging to other species in which the sheep or goat erythrocytes were injected) are used.

In the haemolysis reaction, a molecule category, represented by complement, intervenes for amplification and completion of the effect of antibodies on the antigenic red cells.

Complement

The *complement* or *alexine* is a thermolabile substance being present in blood serum and achieves the lysis of red cells of sheep and goat that were sensitized by an immune serum. In the laboratory, the most frequent source of complement is the fresh blood serum of rabbit or guinea pig. The complement is that integrant part of haemolytic system which makes the red cell on which the antibodies were fixed to be destroyed. The haemolysis reaction does not occur without the presence of complement.

The complement system includes a complex activity series implied in immunological mechanisms of the haemolytic process. The complement is found dissolved in plasma in variable quantities and it is composed of several globulin and lipoprotein fractions which can react with the complex antigen-antibody. Its biochemical composition presents two structural groups: a grouping named "*haptophor*" which achieves the binding with erythrocyte antigens and the second grouping named "*toxophor*" which accomplishes the proper lysis processes [fig. 6a] [3, 4]. The complement, symbolised by C`, is composed from four various factors: $C_1^{`}$, $C_2^{`}$, $C_3^{`}$ and $C_4^{`}$. It is possible that, besides the four factors of complement, there can be found another four factors ($C_5^{`}$, $C_6^{`}$, $C_7^{`}$ and $C_8^{`}$), but their structure and immunological function are not yet well-known [fig. 6b]. There was demonstrated that the $C_1^{`}$ factor has a complex enough structure, being composed from three fractions: $C_{1q}^{`}$, $C_{1r}^{`}$ and $C_{1s}^{`}$ [fig. 6c] [4, 7].



a — Stereochemical structure

b — Complement factors

c — Fraction $C_{1q}^{`}$

Fig. 6 – Complement structure

Antigen-Antibody Interaction

The action of diverse factors of complement is achieved in more phases. First of all, the sensitized erythrocytes of sheep or goat react with anti-red cell antibodies of sheep, respectively goat: E + A =EA (sensitized erythrocytes). Then the factors of complement intervene in a certain succession in the haemolysis reaction. The first fraction which reacts is $C_1^{`}$, after that the fraction $C_4^{`}$ and then the fraction $C_2^{`}$ intervene and the fraction $C_3^{`}$ appears towards the end which finally releases the haemolysis.

By identification of polymorphism of serumal esterases [2, 3] and of more complex structure of the $C_1^{`}$ factor, the action mechanism of the complement is much more

complex in the haemolysis phenomenon. Another fraction of the $C_1^{`}$ factor was discovered, being noted $C_{1a}^{`}$. In fact, this fraction is an isoenzymatic form of esterase, the $C_1^{`}$-esterase, substance that represents only a portion of the $C_1^{`}$ macroglobulin. Practically, the $C_1^{`}$-esterase represents the active haemolytic fraction, its role being, on the one hand, the hydrolysis of some esters with limited number of amino-acids and, on the other hand, inactivating the components $C_4^{`}$ and $C_2^{`}$ when they are in solution.

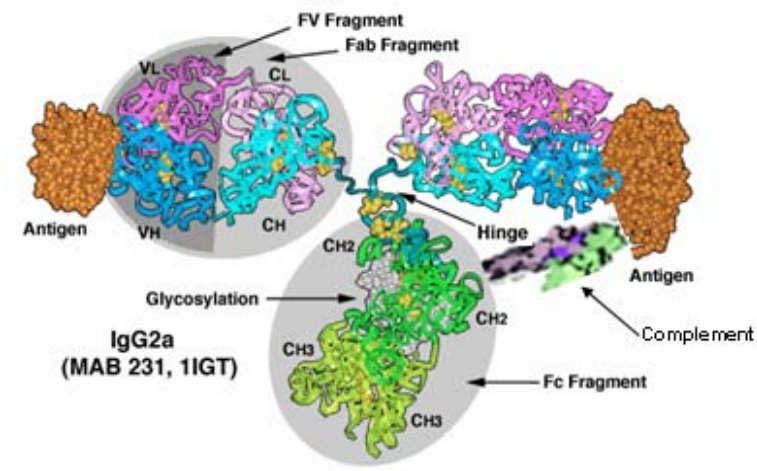


Fig. 7 – Antigen-Antibody-Complement complex in the haemolysis process

As a result of involvement of the $C_1^{`}$-esterase in the haemolytic phenomenon, its successive phases should be the following: the sensitized erythrocytes of sheep or goat join with antibody achieving the immunogenetic couple erythrocyte-antibody (EA), at which the fraction $C_1^{`}$ of complement is attached, resulting the complex EA$C_1^{`}$. To fix the fraction $C_4^{`}$ at this complex the presence of active form of the $C_1^{`}$-esterase is necessary to achieve the intermediate complex EA$C_1^{`}$ $C_4^{`}$. But the $C_1^{`}$-esterase can not carry on the enzymatic activity if it has not the fraction $C_2^{`}$ as substratum. In the presence of magnesium ions, another intermediate complex is achieved: EA$C_1^{`}$ $C_4^{`}$ $C_2^{`}$. This intermediate complex has a very short existence because the $C_1^{`}$-esterase lyses the fraction $C_2^{`}$ in the moment in which the fraction $C_3^{`}$ is also attached, fraction which, practically, determines alteration of erythrocyte membrane and, at last, causes the erythrocyte lysis and the haemoglobin remission in solution [3, 4, 7].

To avoid the crossed reactions in the haemolysis reaction the absorption of serum of rabbit or guinea pig on sheep or goat erythrocyte is obligatory.

CONCLUSIONS

1. The *haemolytic system* (antigen–antibody–complement) underlines the technique used in the serology of blood groups – *haemolysis* – for detecting the blood group factors in sheep and goats.

2. The antigens of the immunoserological system in ovicaprinae are represented by erythrocytes. The antigenic valences are represented by the mosaic of glycopeptides,

lipoproteins and mucous-polysaccharides structures (especially of sialoglycoproteins) met on or in the erythrocyte membrane.

3. The antibodies are immunobiochemical replies of specific erythrocyte antigens; they are composed of γ-globulins arranged in *heavy chains* (HC) and *light chains* (LC) and present a stereospecific forked configuration (Y) having combination sites with antigens (*variable regions*) and complement (*constant region*). The biosynthesis of protein chains is genetically controlled.

4. The complement is composed of several thermolabile globulin and lipoprotein fractions which cause the erythrocyte lysis of sheep and goat sensitized by an immune serum, presenting two structural groups: "*haptophor*" (which makes the binding with erythrocyte antigens) and "*toxophor*" (which accomplishes the proper lysis processes). The complement is composed of several fractions having enzymatic action on the antigen-antibody complex.

5. The whole haemolytic process in ovicaprinae takes place in more phases: sensitiveness of erythrocytes, their coupling with antibodies, forming operation of antigen-antibody complex, activity phases of the complement fractions, alteration of erythrocyte membrane, haemoglobin remission.

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