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Blood-Group Substances

In the ABO system the genes control the arrangement of sugar residues that determines blood-group specificity.

Winifred M. Watkins

The first divisions of blood into groups were based on differences among the antigenic substances on the surfaces of the red cells of a given species of animal. These antigens are distinguished by means of antibodies in serum that combine with the red cells. Blood-group characters are inherited according to simple Mendelian laws, and the antigens, believed to be products of allelic, or closely linked, genes, are classified together in blood-group systems (*1*). Inherited variations in serum proteins (*2*) and enzyme activities in both serum and red cells (*3*) are other factors now known to differentiate bloods within a species, but the blood-group substances discussed in this article are those related to the groups defined by the red cell antigens.

The first human blood-group system, the ABO system, was discovered by Landsteiner (*4*) as a result of his attempts to determine whether specific serological differences existed between individuals of the same species, and the importance of a knowledge of the ABO groups for the safe practice of blood transfusion was promptly recognized. The second blood-group system of outstanding clinical significance, the *rhesus* (Rh) system, was not discovered until 40 years later (*5*). In the intervening years two other systems, the MN and the P systems, were found from examination of serums from rabbits injected with different samples of human red cells (*6*). The antigens in the MN and P systems are of little clinical importance because the corresponding antibodies occur infrequently in man and seldom produce untoward transfusion reactions. At the time of their discovery, however, the inheritance of very few normal human characters was established, and the M and N blood-group factors were used as research tools in genetics and anthropology. With the outbreak of World War II there was a rapid expansion of blood transfusion services in many countries, and the consequent development of improved blood-grouping techniques, together with the vast increase in the number of blood samples examined, led to the discovery of many new blood-group systems and subdivisions of the existing groups. Since the end of the war studies on the serology and genetics of blood groups have continued to flourish; now some 14 human systems, which include over 60 different blood-group factors, are known (*1*).

Although from the point of view of the clinician it is not necessary to be acquainted with more than the serological relationships of the blood-group antigens and antibodies to carry out successful blood transfusions, it is of great importance to study their chemistry and genetics in order to understand the basis of blood-group specificity. The blood groups are defined by their serological properties; the antigens are identified by means of antibodies which may be derived from the same species as that in which the antigen is demonstrated, or from a different species. In the simplest instances specific combination of antigen and antibody causes red cells to clump or agglutinate; of equal importance in blood-group serology are the so-called "incomplete" or "blocking" antibodies that can be demonstrated only by more refined serological techniques (*1*). Chemical studies of blood-group antigens have not kept pace with the serological and genetical advances, but for the ABO and closely related Lewis systems a pattern of relationships between gene function, chemical structure, and serological specificity is beginning to emerge, and consideration of these systems is the principal topic of this article.

Water-Soluble Blood-Group Substances

Somewhat paradoxically the largest amount of information on the chemical basis of blood-group specificity has come from chemical and immunological examination of substances that are not derived from red cells. In the first three decades after the discovery of the ABO system, attempts to obtain blood-group active materials from red cells met with limited success. A and B substances could not be extracted from the cells with water or salt solutions, but active preparations were obtained by extraction with ethanol; the substances were therefore designated as "alcohol-soluble." The amounts isolated were very small, and reports on their chemistry were conflicting (*7*). Further impetus to chemical studies came from the discovery that, in humans, substances with A- and B-activity are present not only on the red cell, but they also occur in a water-soluble form in the tissue fluids and

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secretions of most individuals (*7*). In addition, shortly after the discovery of the human water-soluble blood-group substances it was found that materials with specificities related to the human A and B substances could be isolated in a water-soluble form from the proteolytic digests of stomach mucosal linings of certain animals (*7*). These water-soluble substances are detected and assayed by their capacity, when mixed with the appropriate antiserum, to combine specifically with the blood-group antibody so that agglutination fails to take place upon subsequent addition of the corresponding red cells. Substances that inhibit blood-group agglutination also occur in certain plants and bacteria, and these have provided another source of material for chemical studies on blood-group specificity (*8*, *9*).

For many years after the discovery of the water-soluble blood-group substances no serious attempts were made to establish the chemical nature of the red cell antigens, but the term "alcohol-soluble" was retained to describe them. The application of newer special procedures for the separation of complex macromolecules has now resulted in the isolation, in water-soluble form, of the A and B antigens themselves (*10*, *11*) from the red cells. However, the term "water-soluble" is nevertheless still largely used to describe those blood-group substances derived from sources other than red cells.

Genetics of ABO and Lewis Systems

Five blood-group specificities, A, B, H, Le^a, and Le^b, are detectable in human secretions by the use of the corresponding antibodies of human or animal origin. The structures responsible for these specificities are believed to arise from the action of four independent, but closely interrelated, gene systems, namely, *ABO*, *Hh*, *Lele*, and *Sese* (*12–14*). The relationships between the gene systems may best be resolved by knowledge of the chemical structures that confer specificity, and by understanding the ways in which these structures are built up under the influence of the genes.

The ABO classification of bloods is based on two red cell antigens, A and B, and the two antibodies to these, respectively, anti-A (α) and anti-B (β); these antibodies always occur in the serum or plasma when the corresponding antigen is absent from the red cell (Table 1). The generally accepted mechanism of inheritance, first advanced by Bernstein (*15*), requires that there be three allelic genes, *A*, *B*, and *O*, any one of which can occupy a given position on a chromosome. The A and B substances are not found in secretions unless the corresponding antigen is present on the surface of the red cells (*16*), but about 20 percent of those with A or B antigens on their red cells fail to secrete the corresponding substances. Schiff and Sasaki (*17*), recognizing that secretion is a dimorphic character, suggested that the secretion of A or B substance is controlled by a pair of allelic genes, now generally called *Se* and *se*, which are inherited independently of the *ABO* genes and do not influence the expression of these genes on the red cell. Gene *Se* in single or double dose results in secretion; *se* in double dose results in nonsecretion.

Table 1. Relation between genotype, antigens on red cells, and antibodies in serum in ABO blood-group system.

Group (phenotype)	Genotype	Antigen on red cell	Antibodies in serum
A	*AA*, *AO*	A	anti-B (β)
B	*BB*, *BO*	B	anti-A (α)
AB	*AB*	AB	
O	*OO*		anti-A (α) and anti-B (β)

The discovery in 1927 (*18*) of agglutinins in certain cattle serums which reacted preferentially with group O cells raised the question whether the *O* gene is recessive, as was believed earlier, or whether it gives rise to a specific product analogous to the products of the *A* and *B* genes. The secretions from group O individuals of the genotypes *SeSe* or *Sese* neutralize the group O cell agglutinins, but saliva from group A, B, and AB secretors also does so. Since individuals of the genotype *AB* cannot have an *O* gene these observations strongly indicated that the substance on group O cells, and in group O secretions, detected by the so-called "anti-O" reagents, was not a direct product of the *O* gene; this substance was therefore renamed H substance (*19*).

According to the views of Watkins and Morgan (*13*), Ceppellini (*14*), and others, the formation of H substance is controlled by a pair of allelic genes *H* and *h* (*20*). The *H* gene in single or double dose gives rise to the H character, and the very rare allele *h*, when present in double dose, results in the absence of this character. The H-active material is probably the substance that, under the influence of the *A* and *B* genes, is mainly converted into A- and B-active substances. The presence of large quantities of H substance in group O individuals can therefore be attributed to the absence of the *A* and *B* genes. Although the *Hh* and *ABO* systems are inheritable independently, the fact that the formation of H substance is a necessary prerequisite for the formation of A and B inevitably means that the two systems are closely related in their phenotypic effects. Further, the secretor genes probably do not directly control the expression of the *A* and *B* genes but act at the stage of the formation of H substance; that is, in secretory cells, although not on the red cells, the presence of two *se* genes is in some way incompatible with the functioning of the *H* gene. The suppression of this one gene results in the absence of H, and hence of A or B substances, or both.

The Lewis system is defined by two allelic genes *Le* and *le*, which are inherited independently of the *ABO*, *Hh*, and *Sese* genes (*1*). The close interrelationship of the ABO and Lewis systems, first observed by Grubb (*21*), is more readily understood now because it is known that, at least insofar as the secreted substances are concerned, the changes produced by the *Le* gene occur on the same macromolecules as those produced by the *Hh* and *ABO* genes. In single or double dose, *Le* gives rise to Le^a-specific structures; *le* in double dose results in their absence. The expression of the *Le* gene is not controlled by the *Sese* genes. The Le^b specificity, once thought to arise from the activity of an allele of the *Le* gene, is now believed to be an interaction product of *H* and *Le* genes (*12–14*).

Six main groups can be distinguished on the basis of ABO and Lewis phenotypes on the red cells and the presence of A-, B-, H-, Le^a-, and Le^b-specific substances in secretions (Table 2). Individuals of group 4 who fail to secrete A, B, H, Le^a, and Le^b substances have in their secretions a substance that is chemically very closely related to the A, B, H, and Le^a sub-

Table 2. Six groups distinguishable on the basis of the ABO and Lewis (Le^a, or Le^b) red-cell phenotype and the A, B, H, Le^a, and Le^b activities present in secretions. The results are expressed as follows: +++, Strong specific activity; +, weak specific activity; —, no activity.

Group	Probable gene combination	Antigens detectable on red cells			Specificities detectable in secretions		
		ABH	Le^a	Le^b	ABH	Le^a	Le^b
1	*ABO,* H, Se, Le*	+++	—	++	+++	+	++
2	*ABO, H, sese, Le*	+++	+++	—	—	+++	—
3	*ABO, H, Se, lele*	+++	—	—	+++	—	—
4	*ABO, H, sese, lele*	+++	—	—	—	—	—
5	*ABO, hh, Le, (Se* or *sese)*	—	+++	—	—	+++	—
6	*ABO, hh, lele (Se or sese)*	—	—	—	—	—	—

*Each individual has two of the *ABO* genes, one received from either parent. In groups 1 to 4 for the red-cell antigens, and groups 1 and 3 for the secreted substances, H activity is strong in individuals of the genotype *OO* and usually much weaker for the other *ABO* genotypes.

stances, and this substance is probably the precursor that in other individuals is converted into the blood-group active materials (*13*). This precursor has no detectable blood-group activity and for convenience is labelled "inactive substance." Groups 5 and 6 correspond to the very rare individuals of the "Bombay" phenotype (*1*) who lack A, B, and H reactivity both in their secretions and on the red cell, but do not show abnormalities in the expression of the *Le* gene.

Chemistry of A, B, H, and Lewis Substances

The most potent sources of blood-group substances among the normal secretions of the body are saliva and gastric juice. Meconium is another rich source (*7*). The substances of human origin that have been studied most are those isolated from ovarian cyst fluids (*22*). These fluids accumulate within the cyst over long periods, and a single cyst sometimes yields several grams of active material (*23*).

Purified blood-group substances from secretions are glycoproteins containing a high percentage of carbohydrate. The average molecular weights of the substances from different individuals range from 3×10^5 to 1×10^6; and probably a preparation having blood-group activity, even from a single individual and a single type of secretion, contains a family of molecules very closely related in general structure and composition. Such substances usually contain about 85 percent of carbohydrate and 15 percent of amino acids. The detailed structure of these molecules is not yet known, but their general properties are consistent with the interpretation that a large number of relatively short oligosaccharide chains are covalently attached at intervals to a peptide backbone.

Preparations of A, B, H, and Le^a substances isolated from ovarian cysts and free from contaminants (*24*), as demonstrated by physical, chemical, and immunological methods, are identical in qualitative composition. Each contains five sugars; a hexose, D-galactose; a methyl pentose, L-fucose; two amino sugars, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine; and the nine-carbon sugar, *N*-acetylneuraminic acid (sialic acid) (Fig. 1, *a–e*); the peptide component in each is composed of the same 15 amino acids. Thus the distinctive serological properties of these substances cannot be traced to differences in qualitative composition. Some differences in quantitative composition are observed, but values for any one component vary slightly among preparations with the same specificity; differences in composition of specimens of different blood-group specificity are therefore significant only if they fall well outside this range. The results of analysis of preparations of A, B, H, and Le^a substances, and a similarly prepared "inactive" glycoprotein from the secretion of a person belonging to group 4 (Table 2), are given in Table 3. Compared with the other substances, Le^a substances usually have a lower fucose content, and A substances a higher *N*-acetylgalactosamine content. The "inactive" glycoprotein has not been subjected to the same rigorous purification procedures and detailed analysis as the active substances, but the results obtained so far indicate that the most outstanding difference in the quantitative composition of this material is its very low fucose content. *N*-Acetylneuraminic acid is the component present in the most variable amounts in preparations with blood-group activity and the complete removal of this sugar does not result in loss of activity (*25*). Many preparations show a reciprocal relation between the contents of fucose and *N*-acetylneuraminic acid comparable with that observed in urinary and other glycoproteins (*26*).

The composition of the peptide component is distinctive in that four amino acids, threonine, serine, proline, and alanine, together make up about two-thirds of the amino acids present (*27*). Sulfur-containing amino acids are virtually absent, and aromatic amino acids are present in only very small amounts. Integrity of the complete macromolecules is essential for maximum serological reactivity, and the role of the peptide component appears to be that of maintaining the correct spacing and orientation of the carbohydrate chains.

Structure and Specificity

Investigations on the structure of the A-, B-, H-, and Le^a-active glycoproteins have been directed more toward finding the underlying basis of blood-group specificity than toward the elucidation of the overall structure of the macromolecules. Immunochemical, enzymatic, and chemical methods have each contributed valuable information about structure. The serological inhibition method, based on the observation of Landsteiner (*28*), that a simple substance with a structure closely related to, or identical with, the immunologically determinant group of an antigen, combines with the antibody and thereby competitively inhibits the reactions between the antigen and its corresponding antibody, gave the first clear indications that the serological specificity of the blood-group substances is associated with the carbohydrate part of the molecules (*29*). An enzymatic inhibition method (*30*), which made use of the well-known fact that many enzymes are inhibited by the products of their own activity, supported and extended the results obtained by the serological inhibition tests.

From these indirect methods it was inferred that at least some of the chains in the A substance have a terminal *N*-acetyl-D-galactosamine residue (Fig. 1*a*), joined in α-glycosidic linkage to the next sugar, and that this

terminal nonreducing sugar unit is an important part of the structure of the A determinant. The B specificity was similarly associated with a terminal nonreducing D-galactose residue (Fig. 1*b*) joined in α-linkage to the next sugar. Both the *N*-acetyl-D-galactosaminyl and D-galactosyl residues are in the pyranose form. These first results therefore pointed to an important conclusion, supported by all subsequent work, that the major difference in A and B specificity can be traced not only to the nature of the terminal nonreducing sugar but, more narrowly, to that of the substituent at carbon atom No. 2 in this sugar molecule. D-Galactose and *N*-acetyl-D-galactosamine are structurally identical except that the hydroxyl group in the former at carbon No. 2 is replaced by an *N*-acetylamino group in the latter. An α-L-fucosyl residue (Fig. 1*c*) is an important part of the H-specific determinant, as judged by inhibition tests. Thus despite the fact that L-fucose, D-galactose, and *N*-acetyl-D-galactosamine are all components of A, B, and H substances, it is possible in each instance to relate specificity to one of these sugars and hence to deduce that the determinant carbohydrate chains vary in the nature of the sugar, or its linkage, or both, at the nonreducing ends of the chains.

Table 3. Analytical figures (percentages) for blood-group specific substances, and an "inactive" glycoprotein, isolated from human ovarian cysts.

Specific substance				"Inactive" glycoprotein
A	B	H	Le^a	
		Nitrogen		
4.9	4.8	5.0	4.9	5.4
		Fucose		
20	18	19	13	1.6
		*Hexosamine**		
32	33	28	29	28
		Reducing sugars †		
51	50	50	56	49

*Expressed as glucosamine after acid hydrolysis.
†Expressed as glucose after acid hydrolysis.

Sometimes A, B, H, and Le^a substances cross-react with horse antibody to the Type XIV pneumococcal polysaccharide (*7*) when in the undegraded state, and they always do so after mild degradation by acid. On the basis of inhibition tests this cross-reactivity was attributed to the presence in the substances of chains ending in 4-*O*-β-D-galactosyl-*N*-acetyl-D-glucosamine units (*31*).

More extensive information about the Le^a determinant was derived from inhibition tests with fucose-containing oligosaccharides isolated from human milk (*32*). Two oligosaccharides containing a branched trisaccharide unit at the nonreducing end, with α-L-fucose and β-D-galactose residues joined by 1→4 and 1→3 linkages, respectively, to an *N*-acetyl-β-D-glucosaminyl unit, strongly inhibited the agglutination of Le^a-positive red cells by a human antiserum to Le^a (*33*). Other closely related compounds, that differed only in the point of attachment of the fucose residue, did not cause inhibition. It was therefore concluded that Le^a activity resided in the spatial arrangement of this branched trisaccharide (Fig. 2*a*). The involvement of L-fucose in two distinct serological specificities, namely H and Le^a, indicated that the nature of the terminal nonreducing sugar cannot, by itself, be responsible for specificity, and that the way in which it is linked to the next sugar, or the nature of this second sugar, or both, must also be important. Another interesting inference from the results with Le^a was that specificity does not necessarily reside in the nature and sequence of sugar units in a straight carbohydrate chain, but that branching sugar residues can contribute to the determi-

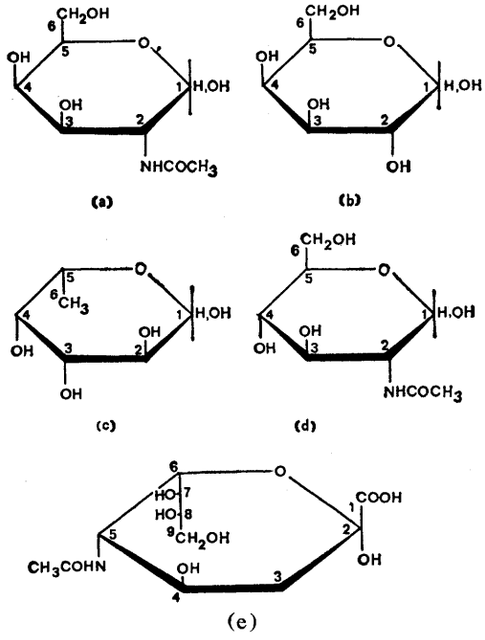


Fig. 1 (above). Monosaccharide constituents of A, B, H, and Le^a substances. (*a*) *N*-acetyl-D-galactosamine; (*b*) D-galactose; (*c*) L-fucose; (*d*) *N*-acetyl-D-glucosamine; (*e*) *N*-acetylneuraminic acid.

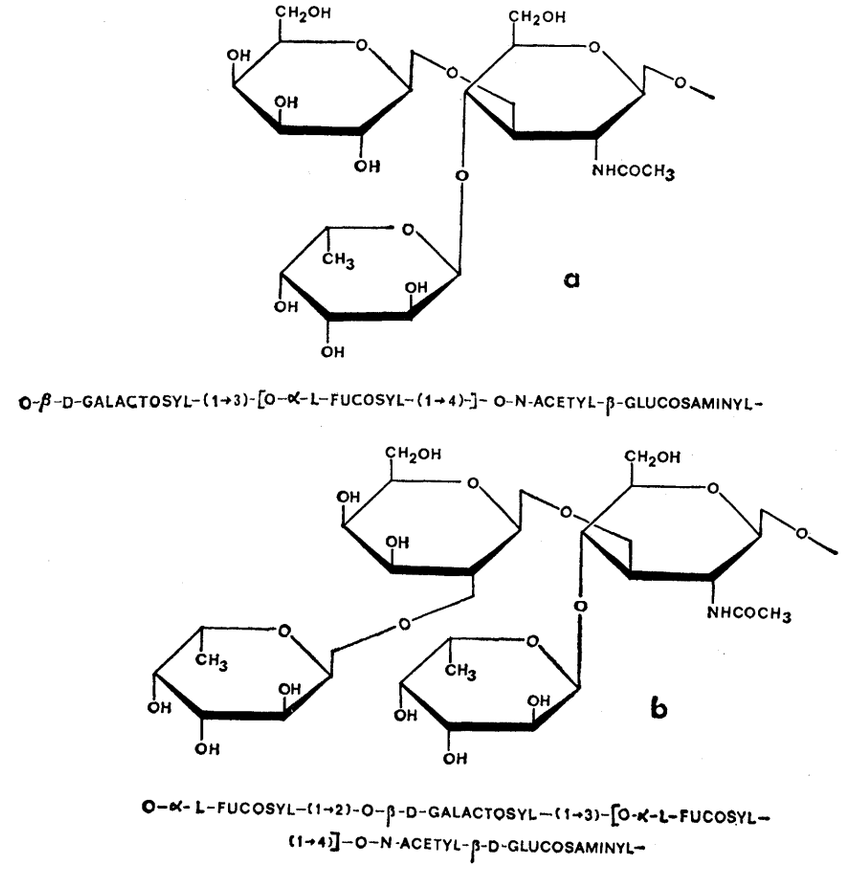


Fig. 2 (right). Structures present in oligosaccharides giving inhibition in (*a*) Le^a hemagglutination tests and (*b*) Le^b hemagglutination tests.

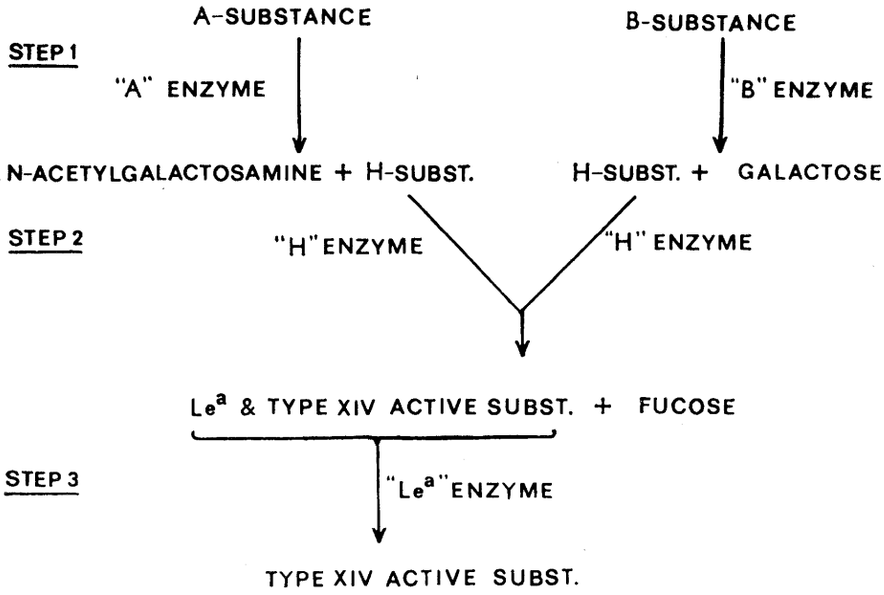
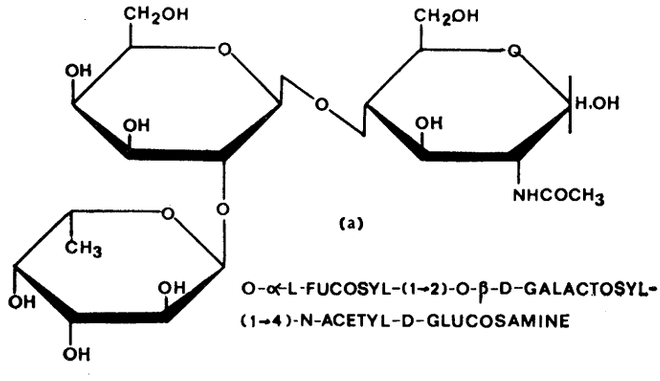


Fig. 3. Specificities revealed by the sequential enzymatic degradation of A and B substances.

nant structure. Further indications of the importance of branching fucose residues came from inhibition experiments with Le[b] (*33*). Oligosaccharides containing two fucose residues attached to adjacent sugars on a backbone chain (Fig. 2*b*) were active inhibitors of Le[b] agglutination, whereas analogs lacking either fucose unit were inactive.

The structure of A, B, H, and Le[a] substances may be further elucidated by the use of certain enzymes that bring about controlled degradation of the molecules. Enzymes from bacteria and protozoa that destroy the serological properties of blood-group substances have been known for many years (*7*), but they have proved surprisingly difficult to purify and characterize in terms of the chemical changes that they catalyze. Several of them are now known to split off the terminal nonreducing sugars from the ends of the carbohydrate chains; that is, they are exoglycosidases. By the action of these enzymes the carbohydrate chains in the blood-group substances can be degraded sequentially and serological specificities uncovered which were not detectable, or only weakly so, in the undegraded substances. The fact that more than one specificity can be present on the same macromolecule was known from precipitation experiments with selected monospecific antiserums (*34*). When, for example, a purified blood-group preparation from an AB subject is precipitated with an antiserum to A, both A and B activities are carried down in the precipitate. Both H and Le[a] structures are demonstrable by similar methods in macromolecules with either A or B activity, or both. Enzymatic degradation demonstrated that, in addition to the various groupings available for reactivity in the undegraded substances, structures corresponding to other specificities



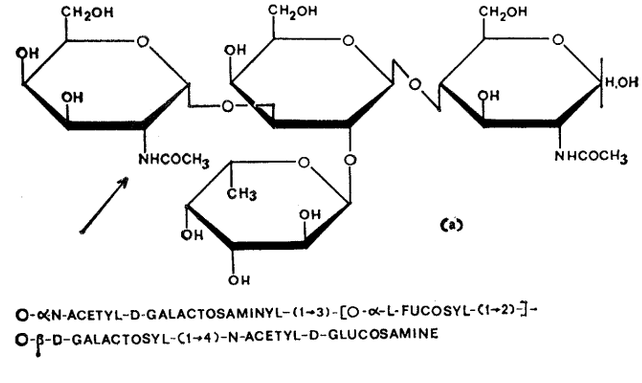


Fig. 4 (left). H-active trisaccharides isolated from (*a*) alkaline degradation and (*b*) acid hydrolysis products of human H substance. Fig. 5 (right). Serologically active tetrasaccharides isolated from alkaline degradation products of (*a*) human A substance and (*b*) human B substance. The arrows indicate the only groupings that differ in the two structures.

are latent in the macromolecules. When A or B substances are treated with purified preparations of certain A- or B-decomposing enzymes they lose *N*-acetylgalactosamine or D-galactose, respectively, and H activity is revealed (*35*). Treatment of the residual H-active substance with a specific H-decomposing enzyme results in the liberation of fucose and the development or enhancement of cross-reactivity with horse antiserum to Type XIV pneumococcus. Moreover, when the original A or B preparation is derived from an individual belonging to group 1 (Table 2), that is, from someone with an *Le* gene, loss of H activity is also accompanied by a development of Le^a activity (*36*). Finally, when the Le^a activity is destroyed by a specific Le^a-decomposing enzyme the residual substance retains its cross-reactivity with Type XIV antiserum (Fig. 3). It appeared therefore that structures related to H, Le^a, and Type XIV pneumococcus determinants are present in A and B substances and that, as the release of a single sugar unit suffices to unmask a new specificity, the structures revealed were initially part of the same carbohydrate chains.

The identification of carbohydrate fragments from the products of partial acid hydrolysis (*37*) and alkaline degradation (*38*) supplied the details of structure necessary to interpret the complex interrelationships of specificities revealed by the enzyme degradation. Fucose and *N*-acetylneuraminic acid are readily cleaved from the blood-group substances by conditions of mild acid hydrolysis and are not found, except in very small amounts, attached to fragments isolated from the products of partial hydrolysis with acid. Since the two sugars most probably occur only as terminal nonreducing end groups, or branching units, the main carbohydrate chains may be reconstructed without knowledge of the points of attachment of the fucose or *N*-acetylneuraminic acid residues. On the basis of the di- and trisaccharides obtained from partial acid-hydrolysis products the structures given in Table 4 were suggested for the two main types of carbohydrate chain in A, B, H, and Le^a substances (*39*). The chains in A substance differ from those in B substance only in the nature of the terminal nonreducing sugar; *N*-acetyl-D-galactosamine in A substance and D-galactose in B substance. Each of these terminal sugars is joined by an α-1→3-linkage to a subterminal galactose residue. All the other linkages in the chains are in the β-form. The chains in H and Le^a substances are identical with those in A and B substances after removal of the terminal nonreducing sugar. The presence of two types of carbohydrate chain in each substance was indicated by the isolation of two serologically active trisaccharides from both single specimens of A and B substances, each pair differing only in the nature of the linkage, (1→3) or (1→4), of the subterminal galactose to the third sugar in the chain, namely *N*-acetylglucosamine (*40*).

Table 4. Partial structures proposed for the carbohydrate chains in blood-group A, B, H, and Le^a substances.

Chain	Structure*
	A-substance
Type 1	α—Gal*N*Ac—(1→3)—β—Gal—(1→3)—β—G*N*Ac—(1→3)—β—Gal—(1→3)—Gal*N*Ac—
Type 2	α—Gal*N*Ac—(1→3)—β—Gal—(1→4)—β—G*N*Ac—(1→3)—β—Gal—(1→3)—Gal*N*Ac—
	B-substance
Type 1	α—Gal—(1→3)—β—Gal—(1→3)—β—G*N*Ac—(1→3)—β—Gal—(1→3)—Gal*N*Ac—
Type 2	α—Gal—(1→3)—β—Gal—(1→4)—β—G*N*Ac—(1→3)—β—Gal—(1→3)—Gal*N*Ac—
	H- and Le^a-substances
Type 1	β—Gal—(1→3)—β—G*N*Ac—(1→3)—β—Gal—(1→3)—Gal*N*Ac—
Type 2	β—Gal—(1→4)—β—G*N*Ac—(1→3)—β—Gal—(1→3)—Gal*N*Ac—

* Abbreviations: Gal*N*Ac, *N*-acetyl-D-galactosaminopyranose, Gal, D-galactopyranose. G*N*Ac, *N*-acetyl-D-glucosaminopyranose.

The point of attachment of some of the fucose units was ascertained by identification of fragments isolated from alkaline degradation products. The identification (Fig. 2*a*) of an active trisaccharide from Le^a substance (*41*) confirmed the prediction from the serological inhibition tests that this unit was responsible for Le^a activity and established that the fucose in the Le^a-active chains is on carbon No. 4 of the subterminal *N*-acetylglucosamine residue. The identification of a serologically active fucose-containing trisaccharide (Fig. 4*a*), obtained from the alkaline degradation products of H substance, and a second active trisaccharide (Fig. 4*b*), isolated in very low yield from an acid-hydrolyzate (*42*), indicated that fucose is linked to carbon No. 2 of the terminal galactose in both of the types of chain in H substance. A highly active, reduced pentasaccharide fragment isolated from products of alkaline degradation of A substance carried out in the presence of borohydride (*43*), and a structurally similar tetrasaccharide (Fig. 5*a*) isolated from the products of degradation with aqueous methanolic triethylamine (*44*), each had a L-fucose residue attached to the carbon No. 2 of the galactosyl unit penultimate to the terminal nonreducing group. A similar tetrasaccharide was isolated from the alkaline degradation products of B substance (Fig. 5*b*) (*44*). On the basis of the structural information gained from serological inhibition tests, enzymatic degradation, and partial hydrolysis, it is thus possible to assign a defined structure to the four blood-group specificities, A, B, H, and Le^a, and to begin to see how these structures are related one to the other.

The points of attachment of the *N*-acetylneuraminic acid residues are not yet determined, but there is no evidence that this sugar plays any part in A, B, H, Le^a, or Le^b specificity.

Genetic Control of Biosynthesis of Blood-Group Substances

The combined serological, genetical, and biochemical results support the idea that the blood-group genes do not each control the complete synthesis of the macromolecules that carry specificity, but impose the specific pattern only at a late stage in synthesis (*13*). The conversion in vitro of an inactive glycoprotein into an active blood-group substance has not yet been achieved. Nevertheless the simplest explanation of the role of the *Le, H, A,* and *B* genes is that they control the formation, or functioning, of specific glycosyl transferase enzymes that add sugar units from a donor substrate to the carbohydrate chains in a preformed glycoprotein molecule. An alternative mechanism by which the sugars are added to oligosaccharides that are subsequently joined to the peptide moiety can also be envisaged, but the argument for the role of the blood-group genes is essentially the same for the two mechanisms, provided growth is from the terminal nonreducing ends of the carbohydrate chains. The alleles of *Le, H, A,* and *B*, namely *le*, *h*, and *O*, are thought

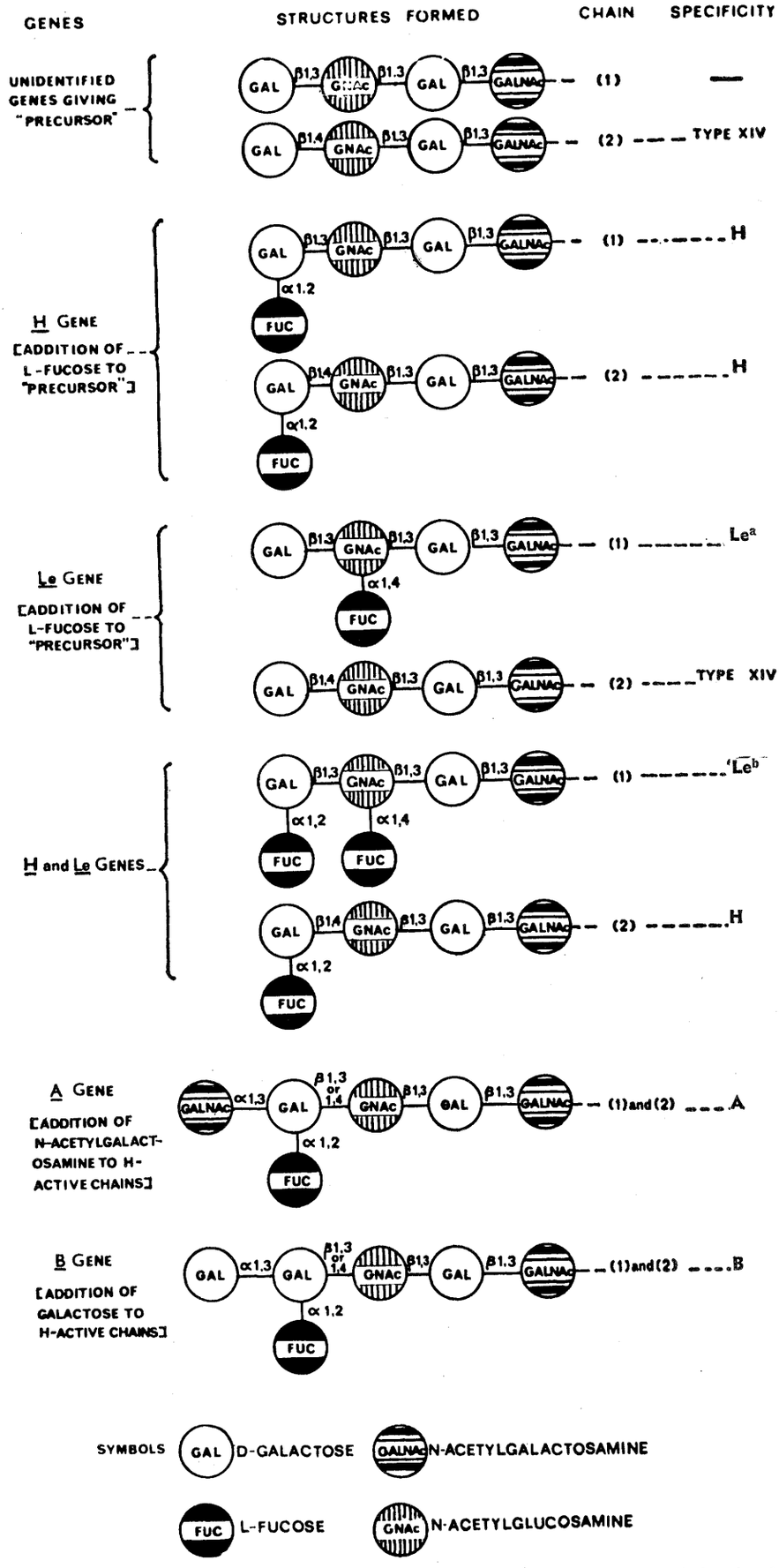


Fig. 6. Representation of the structures proposed for the carbohydrate chains in the precursor glycoprotein and the additions to these chains controlled by the *H*, *Le*, *A*, and *B* genes.

not to control any changes in the glycoproteins and, therefore, from the point of view of the biosynthesis of blood-group active structures, can be considered inactive genes.

The presence in the secretions of certain individuals (group 4, Table 2) of a glycoprotein, chemically similar to A, B, H, and Le^a substances but lacking any of these blood-group activities, supports the idea that, in the absence of the changes controlled by the blood-group genes, it is not the glycoprotein that is missing, but only those groupings that confer serological activity. At least some of the main carbohydrate chains in this glycoprotein are probably complete as far as the terminal nonreducing β-galactosyl units —as indicated in the structures proposed for the chains in H and Le^a substances (Table 4). The type-2 chain ending in an unsubstituted *O*-β-D-galactosyl-(1 $\rightarrow$ 4)-*N*-acetylglucosaminyl unit would confer the cross-reactivity with antiserum to Type XIV pneumococcus observed with these so-called "inactive" preparations. The conversion of the chains in the precursor into Le^a, Le^b, and H, A, and B active groupings could occur as follows (Fig. 6): The transferase controlled by the *H* gene adds L-fucose in α-linkage to the carbon No. 2 position of the terminal galactose in either chain to give an H-active structure. The transferase controlled by the *Le* gene, also a fucosyl transferase, adds L-fucose in α-linkage to the carbon No. 4 position of the subterminal *N*-acetylglucosamine unit in chains of type 1. The subterminal *N*-acetylglucosamine units in chains of type 2 are already substituted at the carbon No. 4 position so that addition of fucose at this position cannot take place. When both *H* and *Le* genes are present, addition of two fucosyl units to adjacent sugars on the chains of type 1 results in a structure different from that produced by either gene alone. This structure is considered to be the Le^b determinant, although it should be emphasized that an oligosaccharide containing the grouping shown in Fig. 2*b* has yet to be isolated and characterized from the degradation products of a blood-group substance.

The presence of the fucosyl unit conferring H specificity on the carbohydrate chains in the precursor substance is believed to be essential for the functioning of the transferases controlled by the *A* and *B* genes. *N*-acetyl-D-galactosamine is added in α-linkage to

carbon No. 3 of the terminal galactosyl units of the H-active structure under the influence of the *A* gene; D-galactose is added, also in α-linkage and to carbon No. 3 of the terminal galactosyl unit, under the influence of the *B* gene. The addition of these terminal nonreducing sugars masks the serological reactivity of the H-active groupings. The substitutions at the ends of the chains controlled by the *A*, *B*, and *H* genes similarly mask the Le^a-active groupings. Under the control of the *Le* gene, fucose is added as a branch to the penultimate *N*-acetylglucosaminyl residue, whereas the sugar additions controlled by the *H*, *A*, and *B* genes are made to the terminal nonreducing galactose residues in the precursor chains; the *Le* gene is therefore not competing for the substrate. The number of Le^a groupings available for reactivity does, however, depend on the activity of the *A*, *B*, and *H* genes, and the strongest Le^a-active substances are found in nonsecretors in whom suppression of the *H* gene by the *sese* genes prevents the formation of H-active structures, and hence of Le^b, A- or B-active structures.

A and B Substances from Red Cells

Extraction of group A and B red cells, or stroma, with organic solvents and purification of the extract by chromatography on cellulose and silicic acid yields active materials that are glycolipids (*10, 11*), that is, compounds containing a carbohydrate moiety joined, through sphingosine, to fatty acids. The most active materials from group A cells so far described had about 50 percent of the activity of a purified glycoprotein preparation of A substance. The activity of some very much less active fractions is increased to this same degree of activity by combination with serologically inactive lipid isolated from the same red cell preparatons. Koscielak (*11*) suggests that the activity of blood-group substances from red cells depends largely on their state of aggregation in aqueous solution. The active glycolipids contain, in addition to fatty acids, sphingosine, and glucose, the same five sugars that are present in the glycoprotein blood-group substances, namely, galactose, glucosamine, galactosamine, fucose, and sialic acid. The analysis of two blood-group A–specific glycolipids is given in Table 5. The preparation insoluble in cold methanol contains mainly lignoceric acid residues, whereas that soluble in methanol contains a higher proportion of short-chain fatty acid residues. The hemagglutination inhibiting activity of the methanol-soluble material is less than 1 percent of that of the methanol-insoluble glycolipid, but its activity is increased to that of the methanol-insoluble glycolipid by combining it with inactive lipid (*11*). Glycolipids specific for blood-group B, essentially similar in composition and properties to the A-specific glycolipids, have been isolated from B cells (*45*).

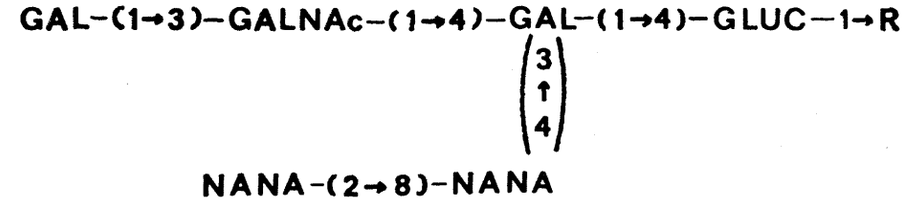


Fig. 7. Structure proposed by Dodd, Bigley, Johnson, and McCluer (*56*) for the immunological determinant of the Rh_o (D) antigen. *Gal*, D-galactose; *Gluc*, D-glucose, *GalNAc*, *N*-acetylgalactosamine; *NANA*, *N*-acetylneuraminic acid.

The red cell A and B substances thus appear to belong to a different molecular species from the A- and B-active glycoproteins from secretions. Nevertheless the possibility remains that the groupings responsible for the specific blood-group properties are identical in the two types of molecule. The structures of the glycolipids have not yet been studied directly, but serological and enzymatic inhibition tests (*46*) indicate that *N*-acetylgalactosamine and D-galactose are the major serological determinant units in the red cell A and B antigens, respectively. Moreover, in precipitin tests by double diffusion in agar gel the secreted and red cell A substances give a reaction of identity with antisera produced in rabbits against either group A red cells or the purified A substance from an ovarian cyst (*46*). On the assumption that the steps controlled by the blood-group genes take place only at the ends of the carbohydrate chains in both the glycoprotein and the glycolipid substances, and that both have the same basic carbohydrate structures, the association of A and B specificities with different classes of macromolecules on the red cells and in secretions is understandable. The reason for this difference is not clear, especially because glycoproteins not dissimilar in general composition to the secreted blood-group A, B, H, and Le^a substances have been isolated from red cells and shown to be associated with M and N specificities (*47, 48*). Another rather baffling finding is that material isolated from group O cells by techniques similar to those used for the isolation of A and B substances yields only inactive glycolipids, and H-active substances have yet to be obtained from red cells (*10, 11*).

Table 5. Analysis of blood-group A active glycolipids (*11*).

Constituent	Solubility of glycolipid in methanol	
	Insoluble	Soluble
Hexosamine (%)	15.8	12.1
Reducing sugar (%)	43.0	41.4
Sialic acid (%)	10.4	10.9
Fucose (%)	1.2	2.3
Nitrogen (%)	2.46	2.48
Sphingosine N (%)	0.81	0.91
Glucosamine: galactosamine	3.0:1	3.1:1
Galactose : glucose	3.1:1	2.8:1
Hemagglutination inhibition*	50	<1

* Expressed as percentage of activity of glycoprotein A substance.

M, N, P_1, and Rh_o (D) Specificities

Evidence that carbohydrate structures are responsible for blood-group specificities other than A, B, H, and Lewis is rapidly increasing. There is now a fairly extensive body of work on the M and N substances from red cells (*9, 47, 48*); and, at least insofar as the specificities detected by human and rabbit antiserums to M and N are concerned, terminal nonreducing *N*-acetylneuraminic acid residues (Fig. 1*e*) are an important part of the serological determinant structures (*49, 48*). The presence of N activity in M preparations derived from cells of the genotype MM, the finding that both the M and N activity are associated with the same macromolecule (*50*), and the exposure of common specificities in M and N preparations by removal of *N*-acetylneuraminic acid (*9, 51*) suggest that sequential, gene-controlled changes in a common precursor sub-

strate may also form the pattern of synthesis for antigens associated with the MN system.

The P_1 antigen of the P blood-group system (*1*) has not yet been isolated from red cells. However, in a substance with a similar specificity obtained from the fluid of the sheep hydatid cyst (*52*), such activity is associated with a carbohydrate structure, and D-galactose in α-linkage is probably the most important part of the serological determinant grouping (*53*). Attempts to determine the nature of the serologically active structures in antigens in the Rh system by the method of hemagglutination inhibition with low-molecular-weight inhibitors have involved so many compounds of diverse chemical nature that interpretation of the results is extremely difficult. Specific inhibition of Rh_0 (D) (*54*) antibody by compounds containing neuraminic acid has been claimed, however, by more than one group of workers (*55*); and recently Dodd, Bigley, Johnson, and McCluer (*56*), on the basis of inhibition by gangliosides isolated from human brain, proposed that the structure given in Fig. 7 represents the serological determinant in the Rh_0 (D) antigen. The similarity of this structure to those proposed for A, B, H, and Le^a specificities makes it an exciting possibility, but Springer reports (*57*) that he has been unable to confirm the inhibition by gangliosides, and therefore further developments must be awaited.

Summary and Conclusions

The picture that emerges from the chemical, serological, and genetical analysis of the ABH and Lewis blood-group substances from human secretions is one in which patterns of synthesis resulting from the sequential action of the products of different blood-group genes on a common precursor glycoprotein lead to the three-dimensional patterns of sugar residues responsible for the serological specificity. The addition of a sugar residue to the end of, or as a branch on, a carbohydrate chain produces a new serological specificity primarily determined by the added sugar, but also dependent on the nature, sequence, and linkage of those sugars already present in the chain. This new specificity may be masked by the addition of another sugar residue. Thus H-specific structures become part of the A- and B-specific groupings when *N*-acetyl-D-galactosamine or D-galactose, respectively, are added to the ends of the chains. It is not yet clear whether the Le^a groupings contribute to the complete A and B determinants in the chains to which fucose is added under the control of the *Le* gene, but the Le^a groupings in the A and B chains are not available for reactivity with Le^a antibodies.

In group AB, when both A and B genes are present, there is, presumably, competition for the carbohydrate chains; but any one chain can only be completed either as an A- or as a B-active structure. Therefore A and B groupings can be present on the same molecule without much apparent interaction. On the other hand it is proposed that, in certain of the carbohydrate chains, the additions of L-fucose controlled by the *H* and *Le* genes take place on adjacent sugars in the same chain, and that the presence of two fucosyl units gives rise to an interaction product, Le^b, which has a serological specificity different from that of the structure produced by either the *H* or *Le* gene acting in the absence of the other gene.

How far the picture for the secreted blood-group substances is applicable to the A and B substances on the red cell is not yet clear. Nevertheless, it is reasonably well established that *N*-acetyl-D-galactosamine and D-galactose, respectively, are important parts of the serological determinants of the glycolipid A and B substances. The difference in A and B specificity, of such great importance in blood grouping and transfusion, thus appears to reside ultimately in a relatively small structural variation, namely, in the nature of the substituent at carbon No. 2 in a sugar with a D-galactose configuration (Figs. 1, *a* and *b*). If the original gene at the ABO locus were one that controlled the formation, or functioning, of an enzyme that transfers a D-galactosyl type of structure in α-(1→3) linkage to a β-linked galactose residue substituted in the 2-position with fucose, then the mutational change which led from *A* to *B*, or vice versa, can be envisaged as a change which permits recognition by the gene products of a hydroxyl group at carbon No. 2 in the galactose configuration in place of an *N*-acetylamino group. Expressed in these terms, the extent of the dissimilarity between the products of the *A* and *B* genes may appear less great, and hence more compatible with the kind of dissimilarity expected in the products of allelic genes, than if the statement that the *A* and *B* genes control the placing of two different sugar residues is taken to mean that configurationally distinct structures are involved.

Inherited variations in protein structures, or other antigenic noncarbohydrate components of the red cell surface, may possibly account for some of the observed blood-group divisions. The pattern of synthesis and genetical control outlined for the ABO and Lewis systems may have wider application since there is evidence that carbohydrate structures are concerned in M, N, P_1, and possibly Rh_0 (D) specificity and that many of the serological observations in the MN, P, and Rh systems can be explained if the specificities arise from the sequential action of genes on a precursor substance (*1, 58*). A large variety of structural patterns can be formed with a relatively limited number of sugar units, and if the role of many of the blood-group genes is the control of the arrangement of these carbohydrate building blocks it is possible to perceive how great diversity of the cell surface can be produced with considerable economy of means.

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Computers and Copyrights

Restrictions on computer use of copyrighted material would protect authors, publishers, and even users.

Curtis G. Benjamin

In the debate over the proposed new copyright law and its possible impact on the future of computer-based information systems, only two points have thus far emerged clearly and incontrovertibly: (i) new legislation is very badly needed; (ii) the new law, though it should adequately protect the owners of copyrights, must not be so stringent as to restrict the development of computerized information systems, particularly in science and applied science. Nevertheless, the Congress may soon deal decisively with this matter in acting upon the new copyright bill which is now before both houses.

To date there has not been enough debate, either public or private, on the problems and issues involved. It may be useful, therefore, to have an analysis of them by a book publisher, though an admittedly biased interest in the matter may be displayed here. But even a publisher can strive for objectivity in considering certain long-range involvements of the future welfare of science information and hence of science itself.

A basic requirement of the new law is to provide for copyright security in a work first produced by means of, or with the aid of, an automated mechanism such as a computer. This requirement has to be dealt with *de novo* because there is nothing in the present copyright law—enacted in 1909 and not overhauled since—that recognizes this kind of production. The pressing need to satisfy this requirement is suggested in a paragraph in the Annual Report (draft copy) of the Register of Copyrights for the Fiscal Year 1965:

> As computer technology develops and becomes more sophisticated, difficult questions of authorship are emerging. In past years the Copyright Office has received an application for registration of a musical composition created by computer. This year copyright was claimed for an abstract drawing, and for compilations of various kinds, which were at least partly the "work" of computers. It is certain that

The author is chairman of the board of the McGraw-Hill Book Company, 330 West 42 Street, New York, New York.