Relations Between the ABO, Secretor/ Non-Secretor, and Lewis Systems with Particular Reference to the Lewis System

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THE PHENOTYPIC and genotypic interpretations of the blood groups have gradually proved extremely complicated. If the *description could be simplified* or if the different findings could be *classified better*, much would be gained.

It must always be borne in mind that despite this complexity, the blood groups are among the simplest biological phenomenon in man, that despite the numerous phenotypic variations they can be collected within fixed limits. It has been clearly demonstrated by more than 100,000 investigations that they are transmitted by simple heredity, although of course a large number of *curiosities* have been encountered within such a vast collection of material. Most of these curiosities serve merely to remind us of the multiplicity of nature, but *exceptional variants* give clues to the unravelling of the maze of heredity.

The following presentation is not a complete description of the systems, and therefore the material is somewhat inhomogeneous—other facts might have been used in the description.

The ABO, Lewis, and secretor/non-secretor systems are now considered an entity in which all antigens are chemically related, but in which independent systems of genes determine the phenotype.

While the heredity of the ABO system and the secretor/non-secretor system must be considered as established, the heredity and the entire structure of the Lewis system is still a matter of discussion.

The phenotypes within these systems comprise reactions with:

(1) antigens attached to the red cells or to other cells;

(2) antigens dissolved in plasma, saliva, and other secretions.

The main antigens are:

(a) The antigens of the ABO system; A (variants to be mentioned later), B, and H. H is an antigen closely related to the others, whose occurrence is, however, unrelated to the genes of the ABO system.

(b) The antigens of the Lewis system, Le^a, Le^b, Le^{b_2}, Magard factor, and Le^x. These antigens have been studied particularly in the red cells and in their water soluble form in saliva and other secretions.

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The presence or absence of the water soluble antigens A, B, or H in saliva and certain other secretions divide humanity into two groups: secretors and non-secretors.

Less work has been done concerning the presence of the antigens in cells other than the red blood cells, but A, B, and H antigens are present in an alcohol soluble form in most cells (24). During the past few years, our knowledge regarding the antigen content in the cell has been extended by the use of antibodies with fluorescent stains, Glynn and Holborrow (18).

Still less is known about the content of antigens in the plasma and serum, although the presence of the antigens of the Lewis system in these body fluids is of decisive importance.

Before proceeding, it would be well to recapitulate some of the most important facts concerning the Lewis system.

THE LEWIS SYSTEM

A. PHENOTYPE

Red cells:

In 1946 Mourant (34) found an agglutinin (anti-Le^a) in the serum from a woman, Mrs. Lewis. This serum agglutinated about 24 per cent of the persons studied [Le(a+)], regardless of their ABO group.

In 1948 Andresen (4) demonstrated that this blood group property was presumably inherited as a recessive character and that its distribution differed in babies and adults.

In 1947 Andresen (5) found an agglutinin, anti-Le^b, reacting with most blood cells, which did not react with the Le^a agglutinin [Le(a−b+)], about 10 per cent of the samples failed to react with either of these sera [Le(a−b−)]. However, anti-Le^b gave a weaker reaction with A_1 cells, so that the percentage of Le(a−b−) was higher within group A_1.

In 1948 Grubb (19) showed that saliva from most people (about 90 %) contains a substance (Le^a substance) characterized by binding anti-Le^a and thus preventing the agglutinin from reacting with blood cells of group Le(a+).

At the same time, Grubb found that all persons of blood group Le(a+) were non-secretors of ABH substance, whereas their saliva contained large quantities of Le^a substance.

Lastly, Grubb demonstrated that saliva from all persons, including those of group Le(a−b−), who were secretors of ABH substance, also contained a substance, *Le^b*, which prevented anti-Le^b from agglutinating Le(a−b+) cells.

In 1949 Andresen and Jordal (7) discovered an agglutinin (anti-Le^x), which agglutinated all blood cells except those, which failed to react with both anti-Le^a and anti-Le^b. This gives blood group Le(a−b−)X−. Anti-Le^x nearly always co-exists with anti-Le^a, and only a few sera containing anti-Le^a are completely devoid of anti-Le^x. This is the reason why it is so often impossible to obtain completely negative reactions with anti-Le^a, except with blood cells of group Le(a−b−)X−. Many workers assume that anti-Le^x is a mixture of anti-Le^a and anti-Le^b.

In 1951 Grubb (20) elaborated his studies, showing that in all persons whose saliva did not contain Le^a substance, the blood cells were group Le(a−b−) and that this group must be identical with that, which Andresen, Andersen, Jordal, and Henningsen (8) had called Le(a−b−)X−.

In 1950 Brendemoen (13) discovered another agglutinin reacting with blood cells of group Le(a−b+), but which is not identical with anti-Le^b (Andresen) and which was later designated anti-Le^{b_2}. The assumption is that it reacts with another receptor and is not inhibited by Le^b substance in the saliva from persons of group Le(a−b−), who are secretors. On the other hand it is inhibited by saliva from some group Le(a+b−) individuals. Another peculiarity is that this receptor is not weaker in group A_1 persons.

Since these two antibodies have not been clearly distinguished, some confusion has resulted.

In 1952 Jordal and Lyndrup (27a) demonstrated that all newborn infants (cord bloods) are group Le(a−b−), but that 90 per cent show group Le(X+), i.e. the same distribution of Le(X+) as in adults.

In 1955 it was shown by Sneath and Sneath (40) that blood cells of group Le(a−b+) suspended in plasma from group Le(a+b−) persons will absorb Le^a substance, so that they test Le(a+b+).

In 1956 Mäkelä and Mäkelä (35) in a similar way demonstrated that the Le^b substance from plasma may be absorbed and will convert Le(b−) cells to Le(b+). However, this conversion did not take place, if Le(b−) cells were suspended in a fluid containing Le^b substance from saliva.

In 1958 J. Andersen (2) discovered an agglutinin, which reacted with a receptor (the Magard receptor) present only in the red cells of group A, Le(a−b−) persons, who are secretors.

Since this antibody appears to be combined with a very weak α_1, it is difficult to decide from the experiments whether the receptor is present also in saliva.

If the receptor is present in saliva, it is present in all group A secretors.

In 1960 it was shown by Levine and Celano (28) that it is also not possible to convert Le(a−) cells to Le(a+) by Le^a substance from saliva. On the other hand, this conversion could be accomplished by using tanned red cells of group Le(a−) placed in fluid containing Le^a from saliva. By means of these converted red cells Levine and Celano could immunize rabbits, which then formed anti-Le^a antibodies.

B. HEREDITY

On the basis of family studies of red cells tested with anti-Le^a and anti-Le^b, the theory advanced by Andresen (5), that Le(a+) is transmitted as a recessive character, was the first to be generally accepted.

According to this theory each person's Lewis group depends upon the two allelic genes Le^a and Le^b, Mourant's original Lewis group Le(a+b−) having genotype $Le^a\ Le^a$, and group Le(a−b+) genotype $Le^a\ Le^b$ or $Le^b\ Le^b$. The occurrence of phenotype Le(a−b−), however, could not be explained by this theory. Andresen *et al.* (8) therefore, extended the genetic theory—after having

demonstrated the character Le(X+), by introducing a gene *Le*^{x} which was dominant and governed not only Le(X+), but also the occurrence of receptors Le^{a} and Le^{b} in red cells. Consequently group Le(a−b−)X− must have genotype *Le*^{x} *Le*^{x}. The pair of alleles *Le*^{x} and *Le*^{x} was independent of the Lewis genes as well as of the genes of the ABO system.

All family series published so far are formally in agreement with the theory, especially after it was shown by Ceppellini (15) and J. Andersen (3) that matings of the combination Le(a+b−) × Le(a+b−) may give rise to children of group Le(a−b−), who are non-secretors.

Grubb's studies on the occurrence of Le^{a} and Le^{b} substance in saliva, however, militated against the theory, as it could not explain the presence of Le^{b} substance in Le(a−b−) secretions. In particular, Grubb (20) found that genes *Le*^{a} and *Le*^{b} could not possibly be allelic and therefore put forward a theory based on a dominant allele, *Le*^{a}, which determined the presence of Le^{a} substance in the secretions, and an allelic gene l indicating the alternate of the Le^{a} allele.

To be able to explain the occurrence of Mourant's Lewis groups and the relation between the secretor/non-secretor characters, Grubb (like Andresen and later Cepellini) had to operate with two genetically independent gene systems, one of which corresponded to the secretor/non-secretor genes.

Many workers have tried to explain the heredity of the Lewis system on the assumption of three alleles. As already stressed by Andresen *et al.* (8) 1950, this is not possible, if also the relation to the secretor/non-secretor system is to be explained.

Wiener (46) tried to set up a theory operating with three alleles, considering only the phenotypes of the red cells. This theory corresponded to the percentage distribution of the groups and to the results of the family series available at that time. Wiener's theory was overthrown by Ceppellini's and Andersen's finding that mating of two Le(a+) persons might produce children of group Le(a−), as expected by Grubb and by Andresen. Sneath and Sheath's demonstration that mating Le(a−b−) × Le(a+b−) persons might give rise to Le(a−b+) children would also be at variance with Wiener's theory.

Since Ceppellini could not accept all of Grubb's results in regard to the presence of receptor Le^{b} in saliva, he tried to modify Grubb's theory. Ceppellini (15) stressed the fact that saliva from group Le(a−b−) secretors did not inhibit anti-Le^{b} of the type Brendemoen (= Sneath's anti-Le^{b_2}), and thought therefore, that Grubb's anti-Le^{b} reacted partially as an anti-H. This opinion has been clearly contradicted by Grubb. Incidentally, it has been unambiguously demonstrated by Ceppellini as well as by Jordal, and by Sneath and Sneath that saliva from Le(a−b−) secretors contains a substance, which inhibits anti-Le^{b} of the type demonstrated by Andresen.

In point of fact, Ceppellini's, Grubb's and Andresen's theories are identical, the only difference relating to the explanation of the Le^{b} receptors and substances.

This difference, however, is of decisive significance in relation to the biochemical interaction in the formation of the various receptor substances. This question will, therefore, be considered in more detail later.

Grubb (20) assumed, even at an earlier date, that the presence of the blood cell characters Le(a+b−) and Le(a−b+) were due to the blood cells taking up Le^a and Le^b substance from the plasma, group Le(a−b−) being due to the absence (or deficiency) of the substances in the plasma. He considered that the Le(a−b−)X− group described by Andresen *et al.* was identical with the group lacking Le^a substance in the saliva. If so, Grubb's *Le*^{a} gene and Andresen *et al.*'s *Le*^{x} must be the same and the two genetic theories identical. In order to unite the two theories it is necessary to establish that Andresen and Jordal's anti-Le^x is in fact a specific agglutinin and not a mixture of anti-Le^a and anti-Le^b.

(1) *A priori*, it is unlikely that anti-Le^a and anti-Le^b will occur in the same person.
   (a) Anti-Le^a has been found only in persons, who are Le(a−b−) and secretors of ABH substance, Jordal (27), Miller *et al.* (30). Typical anti-Le^x always co-exists with anti-Le^a, most anti-Le^a containing a major or minor quantity of anti-Le^x.
   (b) Anti-Le^b (Andresen) has been demonstrated only in non-secretors of group Le(a−b−). Brendemoen (13) demonstrated anti-Le^b in non-secretors of group Le(a+b−), but this was anti-Le^{b_2}.

(2) Jordal's (26, 27a) investigations revealed that blood cells of newborn infants are divisible, by means of anti-Le^x, into the two groups Le(a−b−)X+ and Le(a−b−)X−, corresponding exactly to this classification in adults. In others words, this classification can be made despite the fact that the blood cells of newborn infants fail to react with either anti-Le^a, anti-Le^b, or anti-Le^{b_2}.

Consequently, *anti-Le*^{x} *must be considered as a specific agglutinin*, which has a corresponding specific receptor X closely related to the Le^a substance. The presence of receptor X in the blood cells is always accompanied by the presence of Le^a substance in the secretions. The presence of the X receptor is entirely independent of the subject's secretor/non-secretor status. There is no correlation between the X receptor and Le^b substance.

These were the most important experimental results obtained by using typical anti-Le^a, anti-Le^x, anti-Le^b, and anti-Le^{b_2} sera.

If an attempt is made to classify the various phenotypic findings into not one, but *two independent systems*, a number of the difficulties are overcome. The reason why such a clear division has been avoided so far is that, although independent, these two systems are based largely upon the presence or absence of the same chemical substance: the Le^a substance.

In the following, it is of decisive importance to interpret Le^x [Andresen and Jordal (7)] as a specific receptor and to consider this receptor and the occurrence of Le^a in secretions as a manifestation of the effect of the same gene Le (= *Le*^{a}: Grubb = *Le*^{x}: Andresen and Jordal).

The first system, which might be called the Lewis substance system, has two phenotypes. One is characterized by the presence of Le^a substance in the secretions and by the red cells reacting with anti-Le^x. The other lacks Le^a substance in the secretions, and the red cells are not agglutinated by anti-Le^x. (From the

absence of Le^a substance it also follows that the red cells must be of phenotype Le(a−b−), *vide infra.*)

The second system comprises, as far as the red cells are concerned, the original Lewis system with groups Le(a+b−) [= Mourant's Lewis group], Le(a−b+), and Le(a−b−). The Le(b+) group may be demonstrated with anti-Le^b (Andresen) as well as anti-Le^{b_2} (Brendemoen). This, the original Lewis system, is thus, as shown by Grubb (20), a specific phenotypic manifestation on the part of the blood cells, of the already known secretor/non-secretor system, the blood cells taking up from the plasma some of the substances, which are formed in the glands or other cells.

All the phenotypic factors relating to the secretor/non-secretor system are explicable on the basis of the conversions caused by the dominant *Se* gene in the glands.

Se gives rise to conversion of the ABH blood group substances present in the glands and many other cells to a water soluble form. In the course of this process new water soluble substances are formed, especially Le^b, Le^{b_2}, and Magard substance.

These activities are more complicated than was perhaps assumed initially. It is worth emphasizing that originally the secretor/non-secretor system was characterized exclusively by the secretion or non-secretion of water soluble ABH substances. Studies of numerous families confirm heredity determined by two alleles, the dominant *Se* and the recessive *se*. Since the original Lewis system is a manifestation of this inherited system, its heredity is explained by the same genes, and *Se* is therefore = Le^b (Andresen) and *se* = Le^a (Andresen).

The only difficulty in understanding the heredity and phenotypes of the original Lewis system is now easily solved, the blood group character Le(a+) in non-secretors being present only in persons, who are able to form Lewis substances at all (i.e., those who have the *Le* gene = Le^a (Grubb) = *X* (Andresen and Jordal). Blood group Le(a−b−) must then comprise secretors as well as non-secretors in the same ratio as in the general population (table 1).

Before discussing the formation of the various blood group substances, let us

TABLE 1. THE GENOTYPE AND PHENOTYPE OF THE SALIVA AND THE RED CELLS IN THE LEWIS SUBSTANCE SYSTEM AND IN THE ORIGINAL LEWIS SYSTEM

Lewis substance system				
Genotype	Phenotype			
Le^x-red cells Le^a-substance in saliva		Genotype	Orig. Lewis system phenotype	ABH secretor + non-secretor −
Le Le *Le le*	+	*Se Se* *Se se*	Le(a−b+)	+
		se se	Le(a+b−)	−
le le	−	*Se Se* *Se se*	Le(a−b−)	+
		se se	Le(a−b−)	−

have a further look at the H-antigen. By way of introduction, it may be emphasized that the H antigen present in the red cells is completely independent of the subject's secretor/non-secretor status and is an alcohol soluble form of H. In addition to this alcohol soluble form of the H antigen, there is, as already mentioned, a water soluble form in saliva and other secretions. The occurrence of the antigen depends upon gene *Se* (38). Whether the different H receptors are identical is not known. As early as 1948 Morgan and Watkins (see 44) pointed out that agglutinins reacting with group O blood cells could be divided into two categories. One was called anti-H, since the agglutination was completely inhibited by the water soluble H antigen; the other was called anti-O. As demonstrated by Sanger (37), anti-H occurs only in serum from persons of group Le(a+b−), i.e. non-secretors, while anti-O may occur in secretors as well as non-secretors. This finding might possibly be interpreted to the effect that blood cells of group O have two receptors, whereas the water soluble antigen has only one. In the following, the H antigen of the blood cells will be designated $H^{1\text{-}2}$ and the water soluble one H^2.

A particularly strong anti-H occurs in persons of the so-called Bombay-group "O" whose blood cells are characterized by the absence of the A, B, and H substances (they also lack the Le^b group). With one exception reported by Simmons (39) all are Le(a+). Anti-H is as strong as iso-anti-A and B and also reacts at 37°. Ceppellini called attention to the very close relationship between the occurrence of water soluble H antigen and the occurrence of Le^b (Andresen) substance.

The heredity of the Bombay group was first explained theoretically by Ceppellini (14) on the assumption of an inhibitory gene. This theory has subsequently been elaborated by Levine's family studies. In one family there was an AB child, although its mother apparently was group O. Levine demonstrated that the mother was "O", and the father was group A. Levine explained that the mother must be genotype *xx BO*. The mother had the phenotype "O", because the phenotype B and O (reaction with anti-H) only will be developed with genotype *XX* or *Xx*. [Watkins has later proposed *H* and *h* (42)].

Closely related to the H antigen are the numerous variations of blood group A. I shall not go into these peculiarities which—though some must be designated as curiosities—may acquire great significance, e.g. in medico-legal decisions [van Loghem (29)]. Only the variant first designated A_x by Gammelgaard (17) and later A_m by W. Weiner (45), will be mentioned. What characterizes this group is an ample content of A substance in the saliva, while the A character is practically undemonstrable in the blood cells. Weiner's family studies explained the heredity by assuming the existence of a dominant gene, *Y*, necessary for the transfer of the A character to the blood cells. The genotypes of A_m must be *yy* and in this case the A substance cannot be transferred to the blood cells. A similar variant of group B was found by Armstrong, Gray, Race, Sanger, and Thompson (10): The blood group was determined as O, while the saliva contained ample B substance and the serum no anti-B.

Levine's investigations into the Bombay group and Weiner's explanation of

the A_m group have extended the picture representing the heredity of the ABO system. Race and Sanger (36) have brought this view up to date (1958), and later Watkins and Morgan (44) have dealt with the question from a biochemical point of view in their paper "Possible Genetical Pathways for the Biosynthesis of Blood Group Mucopolysaccharides" (1959). Thereby, these authors have opened up a possibility for biochemical-serological consideration of the phenotypic factors with a view to the heredity.

The groups of atoms which have the specific immune properties constitute only a small proportion of the macromolecules which make up the blood group substance. Various receptors may be attached to one of these molecules or aggregates of these molecules. Many findings indicate that the same secretion may contain uniform macromolecules with different combinations of the specific groups (31, 42). Watkins and Morgan (44) were the first to try to apply this knowledge in a schematic illustration of the interaction between these chemical substances and the blood group genes. The blood group substance is assumed to be formed when enzymes, corresponding to the blood group genes, act upon a human non-antigenic ground substance (precursor substance) or compounds derived from it.

I shall now try to describe Watkins and Morgan's theory and Ceppellini's (16) extension of this theory to comprise also the group characters of the red cells (similar to Watkins). However, I have modified the schematic presentation to be able to apply it also to the views propounded in this paper.

The precursor substance is assumed to be made of a number of uniform macromolecules in a fairly loose chain, here designated as: — — —, while the derived specific compounds are designated: A A A or Le^a Le^a Le^a or A A H or AH AH, etc.

Since the biochemical properties have been elucidated in any detail only in the case of the water soluble blood group antigens, Watkins and Morgan's scheme comprises only these antigens. They use Ceppellini's (15) theory concerning the Lewis system. Thus, in addition to the *A*, *B* and *O* genes of the ABO system, they include the *L*, *l*, and genes as interpreted by Ceppellini. In consequence, Le^b in Watkins and Morgan's scheme indicates a receptor binding anti-Le^b (Brendemoen) and thus not identical with the receptor, which binds anti-Le^b (Andresen). Therefore, Watkins and Morgan's Le^b will be designated here as Le^{b_2}.

Table 2 illustrates the processes, which Watkins and Morgan believe are required to explain the formation of the water soluble antigens from the precursor substance. Watkins and Morgan, however, put forward two suggestions to solve the problem. The former comprises only processes 1–6 according to which H could be formed only by Le^a Le^a Le^a + S' = H(+Le^{b_2}). In order to satisfy the demands made by the known genetic series, it is necessary, however, to assume also the possibility of the process — — — +S = H H H. Gene S' must then be able to transform both Le^a and precursor substance to H receptor.

The substances, which one might expect to find in the secretions according to

TABLE 2. WATKINS AND MORGAN: "POSSIBLE GENETICAL PATHWAYS—" (44). THE FIRST PART OF FIG. 1 (p. 109) AND 2 (p. 110) IN A SLIGHTLY MODIFIED FORM. NOS. 1–6 REPRESENT FIG. 1, NOS, 1, 2, 3, 5, 6 AND 7 FIG. 2. THE TABLE SHOWS THE DIFFERENT INTERACTIONS BETWEEN SUBSTANCE AND GENES

No.	Substance	+	Gene	=	Converted to substance	+	Unconverted substance
1	— — —		*L′*		Le^a Le^a Le^a		
2	Le^a Le^a Le^a		*S′*		H H Le^{b_2} Le^{b_2}		Le^a
3	— — —		*l′*				— — —
4	— — —		*S′* or *s′*				— — —
5	Le^a Le^a Le^a		*s′*				Le^a Le^a Le^a
6	H H H		*A* (*B O*)		A A A		H H
7	— — —		*S′*		H H H		

Precursor substance: — — —
Le^a substance: Le^a Le^a Le^a
ABO genes: *A B O*
Lewis system genes: *Le′* and *l′* (Ceppellini)
Secretor/non-secretor genes: *S′* and *s′*

H substance: H H H
A substance: A A A

TABLE 3. WATKINS AND MORGAN: "POSSIBLE GENETICAL PATHWAYS —" (44). SECOND PART OF FIG. 1 AND FIG. 2 IN A SLIGHTLY MODIFIED FORM, INCLUDING THE SUBSTANCES WHICH ACCORDING TO TABLE 1 WILL BE FOUND IN THE SECRETIONS FROM THE VARIOUS GENOTYPES. A COLUMN SHOWING THE PHENOTYPES OF THE BLOOD CELLS IS ADDED (ONLY A, THE SAME FOR B AND O). 2 CORRESPONDS TO WATKINS AND MORGAN'S THEORY 1, 2A TO THEORY 2

No.	Genotype AA or AO	Secretor + non-secretor −	Substances in the secretions	Blood group (P.H.A.)
1	*L′L′ S′S′* or *S′s′* or *L′l′ S′S′* or *S′s′*	+	A H Le^a Le^{b_2}	A Le(a−b+)
2	*l′l′ S′S′* or *S′s′*	−	"inactive substance Fl."? — — — —	A Le(a−b−)
2a	*l′l′ S′S′* or *S′s′*	+	A H Le^a Le^b	A Le(a−b−)
3	*L′L′ s′s′* or *L′l′ s′s′*	−	Le^a Le^a Le^a	A Le(a+b−)
4	*l′l′ s′s′*	−	"inactive substance Fl."? — — —	A Le(a−b−)

this theory are listed in table 3, the occurrence being given separately for each of the possible genotypes. Table 3 corresponds exactly to Watkins and Morgan's scheme, except that it includes also the blood groups, which will be found in each case. — — — has not been definitely demonstrated in saliva, but in an ovarian cyst, Watkins and Morgan found a substance, "inactive substance Fl", which must be assumed to be identical or closely related to the precursor substance.

TABLE 4. CEPPELLINI'S "SCHEME OF THE METABOLIC PATTERNS WHICH LEAD TO THE SYNTHESIS OF ABH ANTIGENS OF THE RED CELLS" (16) IN A SLIGHTLY MODIFIED FORM

Substance	+	Gene	Converted to Substance	+	Gene	Converted to Substance
— — —		*X* Levine	H H H		*B*	B B B group B
— — —		*X*	H H H		*O*	H H H group O
— — —		*x x*	— — —		*B*	— — — group "O"

Watkins has tried to apply these theories (42) also to the group properties of the red cells, but emphasizes: While it seems fairly certain that the actual group specific structures on the red cell antigens will be chemically identical with those on the water-soluble substances, it is not known whether these specific structures are part of the same type of mucopolysaccharide molecules as are found in secretions. Here Watkins stresses the significance of H substance in the red cells and the importance of the *X* gene (Levine) for which the designation *H* gene has been proposed.

Although the biochemical features are not known in detail, Ceppellini (16) too has tried to extend Watkins and Morgan's considerations also the group characters of the red cells on the basis of serological and genetic views. Since Ceppellini also included the Bombay group, he introduced the *X* gene (Levine). In fact, Ceppellini's elucidation in respect to the secretions corresponds exactly to Watkins and Morgan's, and there is no reason to go into it in more detail, although here too he has introduced the *X* gene as a complementary gene in connection with *S* in order to account for "O".

Table 4 gives Ceppellini's scheme of the red cell types. What is particularly notable is that as far as the blood cells are concerned he believes that *X* acts upon — — —, converting it into H H H. According to this theory, therefore, H may be formed in three ways: *X* is able to transform precursor substance, and *S'* will be able to transform both Le^a and precursor substance to H receptor. Since in Ceppellini's opinion all the Lewis groups in the blood cells have been formed in glands and other cells and have been transmitted through plasma to the red cells, the *Le* gene is not included in his account of the red cells.

In order to set up the theory advanced in the present paper in a corresponding schematic form, I have included all known blood group expressions and the presence of the alcohol soluble ABH receptors in the glands and other cells. Like Ceppellini's, the present theory is based exclusively upon serological and genetic results, but it is supported by the possibilities opened by Watkins and Morgan's biochemical results. The simplest assumption seems to be that all the group specific structures are derived from the same precursor substance and that the difference between the water soluble and alcohol soluble blood group substances is due to a binding of the specific structures to different molecules. Thus, the precursor substance must be assumed to be a macromolecule, large it is true, but not as large as the molecules of the water soluble blood group substances.

Of the precursor substance we know nothing apart from the fact that under the influence of the blood group genes it may be transformed to serologically active specific structures demonstrable by specific agglutinins and other antibodies. The "inactive substance F1" mentioned by Morgan (32) is presumably a binding of precursor substance and the mucopolysaccharides formed by the glandular cells.

The following five, genetically independent groups of genes are included in the ABO, Lewis substance, secretor/non-secretor system: *X x* (Levine), *A B O* (Bernstein), *Y y* (Weiner), *Se se* (Shiff), and *Le le* (Grubb, Andresen).

The series of processes required before all group substances have been formed must be divided into three groups: (1) initial conversions which must be assumed to proceed in the same way in haematopoietic tissues and other cells, especially in glands; (2) processes which take place only in the haematopoietic tissues, and lastly; (3) processes which take place in the glands and other cells in order to form the water soluble, secretable group antigens.

The point on which this theory differs from the others is the completely independent placement of the antigens of the Lewis system, arising through the action of the *Le* gene upon the precursor substance. All the other group substances are derived in some way or other from the conversion by the *X* gene of the precursor substances to H antigen.

Table 5 illustrates the course of the initial processes in all cells which can form group specific antigens.

(1) Gene $X = H$ (Morgan) influences the precursor substance, resulting in the formation of $H^{1\text{-}2}H^{1\text{-}2}H^{1\text{-}2}$ substance.
(2) Lacking *X* (genotype *xx*), no $H^{1\text{-}2}$ will be formed.
(3) Gene *Le* acts upon the precursor substance, forming Le^x substance.
(4) Lacking *Le* [genotype(*lele*)], no Le substance will be formed.
(5) Next, due to the action of genes *A* or *B* of the ABO system a major or

TABLE 5. THE VARIOUS INTERACTIONS BETWEEN PRECURSOR SUBSTANCE AND ITS DERIVATIVES AND THE DIFFERENT GENES, INCLUDING THE PROCESSES WHICH TAKE PLACE BOTH IN THE HAEMATOPOIETIC TISSUE, THE GLANDS, AND CERTAIN OTHER CELLS. THE PROCESSES RESULT IN THE PRESENCE OF SUPPOSED ETHANOL SOLUBLE ANTIGENS IN THESE CELLS

No.	Substance	+	Gene	=	Converted to substance	Rest of unconverted substance
1	— — —		*X* Levine		$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$	
2	— — —		*x x*			— — —
3	— — —		*Le*		$\underline{Le^x}\ \underline{Le^x}\ \underline{Le^x}$	
4	— — —		*le le*			— — —
5	$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$		*A* (*B*)		$\underline{A}\ \underline{A}\ \underline{A}$	$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$
6	$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$		*O*			$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$

TABLE 6. THE PROCESS DEMONSTRATED BY WEINER IN WHICH GENE Y TRANSFERS THE A ANTIGEN TO THE RED CELLS. IT MUST BE ASSUMED THAT GENES CORRESPONDING TO THE B, H, AND Le^X ANTIGENS EXIST

Substance found in the haematopoietic cells	+	Genotype	Antigen acquired by the red cells
A A A		*YY* or *Yy*	blood group A
A A A		*yy*	no reaction with anti-A

TABLE 7. HOW—INDEPENDENTLY OF THE BLOOD GROUP GENES—Le^X IS CONVERTED TO WATER SOLUBLE Le^a SUBSTANCE IN THE GLANDS AND SECRETED

Substance	By the secretion of the glands converted to
Le^X Le^X Le^X	Le^a substance = Le^X − M

M = muchopolysaccharide macromolecules from the cells.

minor portion of H^{1-2} will be converted into A or B, while *OO* will preserve all H^{1-2} substance formed. Of the named blood group substances at least A and B are alcohol soluble.

The subsequent processes differ in the different cells.

Table 6 is meant to illustrate the process whereby the formed antigens are transferred to the red cells. This is known only for the A antigen in which the presence of the *Y* gene (Weiner) is necessary for the red cells to develop the A character. It is reasonable to assume that corresponding genes are required for the transmission of the other group properties. At any rate, as already mentioned, a person has been found, whose saliva contained ample B substance, while his red cells did not react with B-agglutinin.

The formation of the water soluble blood group substance is more complicated and must be assumed to form a link in the formation of glandular secretion. All the above-mentioned secretions contain varying quantities of mucopolysaccharides (now designated as M), and it must be considered likely that the water soluble blood group substances are formed by a binding of the named H^2, A, B, Le^b, Magard and Le^x substances to M as a link in cellular function. There is an essential difference between the ability of the A, B, H^{1-2} receptors and of the Le^x substance to form these compounds. While Le^x can enter into the metabolism of the cells, Le-M = Le^a substance being formed, the other receptors cannot enter into such a metabolism until they have been converted by the action of the *Se* gene.

The formation of Le^a substance is illustrated in table 7. The assumption affords a natural explanation of why Le^a substance can always be found as soon as *Le* is present. Provided that ample Le^a substance is formed (partly in *se se* individuals and partly in babies in whom the secretor ability is not yet fully developed), it passes on to the blood and is bound to the blood cells (it must be considered doubtful whether this is simple physical binding).

The next sphere comprises the formation of the other water soluble antigens designated as: A, B, H^2, Le^b, Le^{b_2}, and the Magard factor. The formation of these antigens is more complicated than one might perhaps have expected. Gene *Se* reacts only with A B H^{1-2}, but the ultimate result depends upon the presence or

TABLE 8. THE INTERACTION BETWEEN GENE *Se* AND *se* AND THE FORMED BLOOD GROUP ANTIGENS IN PERSONS OF GROUP Le(a−b−) (GENOTYPE: *le le*). MACROMOLECULE OF MUCOPOLYSACCHARIDE M COMBINED WITH THE RECEPTORS, M−

Antigen substance	+	Gene	=	Antigen cells metabolism gives the water soluble antigen M−	Secreted in	
					Secretion M−	Plasma M−
A A A		*Se*		A A A + Magard receptor	A + Magard-receptor	Magard-receptor
B B B		*Se*		B B B + ?	B + ?	
$H^{1-2}H^{1-2}H^{1-2}$		*Se*		H^2 H^2 H^2 + Le^b	H^2 + Le^b	
A A A						
B B B		*se se*		non-secretor of A, B, H, Le^b and Le^a		
$H^{1-2}H^{1-2}H^{1-2}$				"inactive substance Fl"?		

absence of Le^x. It must be assumed that *Se* does not react directly with Le^x, since Le^b has not been demonstrated in group "O", which may occur in the presence of the *Se* gene. That a close relationship exists between Le^x and *Se* is apparent from the fact that the amount of Le^a substance, which forms is inversely proportional to the manifestation of *Se*. This phenomenon is particularly apparent in the gradual decrease of Le(a+) reactions in infants as they develop from 1 to 6 months of age.

Since the formation of A, B, and H^2 is independent of Le^x, the simplest procedure would be to consider first the conversions, which take place in the glands of Le(a-b-) persons, who are secretors. These processes are illustrated in table 8. It will be seen that in these cases water soluble antigens other than A, B, and H^2 are formed. This accords with the theories of Watkins and Morgan, and of Ceppellini. The present explanation of the Magard factor was indicated also by Ceppellini, and the formation of Le^b gives a natural explanation of the close relation between the occurrence of the Le^b receptor (Andresen) and the H^2 antigen as suggested several times by Ceppellini.

Tables 8 and 9 show in each individual case whether the water soluble antigens pass into the plasma. I feel that there may be reason to stress that no known relationship exists between the formation of water soluble antigens and their transfer to the plasma. Le^b does not pass into the blood, but the Magard factor does (table 8).

Before leaving genotype *le le*, I should like to emphasize that according to Race and Sanger the amount of water soluble ABH antigen in the saliva is particularly ample in this type of person, if he is a secretor.

Table 9 shows the interaction in cells, which contain both M and Le^a substances (genotype: *Le Le* or *Le le*). The ABH antigen converted by *Se* can be bound to M as well as to Le^a substance. By combining Le^a substance with Le^b and Magard receptors, the Le^a receptor will be converted to Le^{b_2} receptor, and the Magard

TABLE 9. THE INTERACTION IN THE GLANDS AND OTHER CELLS BETWEEN GENE *Se* AND *se se* IN PERSONS OF GENOTYPE *Le le* OR *Le Le*, GROUP Le(a−b+) AND Le(a+b−)

Antigen substance +	Gene	= Antigen cells metabolism gives the water soluble antigen M−	Secreted in	
			Secretion M−	Plasma M−
$\underline{H^{1\text{-}2}Le^X}$	*Se*	H^2 H^2 $\underline{Le^b\ Le^{b_2}}$	H^2 + $\underline{Le^b\ Le^{b_2}}$	$\underline{Le^b\ Le^{b_2}}$
$\underline{A\ Le^X}$	*Se*	A $\underline{Le^{b_2}}$	A $\underline{Le^{b_2}}$	$\underline{Le^{b_2}}$
$\underline{H^{1\text{-}2}Le^X}$	*se se*	the cells convert all $\underline{Le^X}$ to $Le^a = Le^X - M$	Le^a	Le^a
$\underline{A\ Le^X}$				

receptor will be lost: $Le^b + Le^a\ (Le^X - M) = Le^b - Le^{b_2} - M$, and Magard receptor + $Le^a\ (Le^X - M) = Le^{b_2} - M$.

Since a single macromolecule M can bind several receptor groups, many combinations may occur, e.g.

$\begin{matrix} A \\ H^2 \end{matrix} - M - Le^{b_2}$ $\quad H^2 - M - Le^{b_2} - Le^b$ $\quad H^2 - M \begin{matrix} - Le^{b_2} \\ - Le^a \end{matrix} - Le^b$ etc.

A number of these combinations are known [Watkins (42)], and the explanation is entirely in keeping with Ceppellini's interpretation of the relation between H^2 and Le^b. The theory explains why the quantity of Le^a substance varies in inverse proportion to the manifestation of the *Se* gene, and why Le^b must vary with the amount of $H^{1\text{-}2}$.

SUMMARY

After introductory remarks on the ABHO, Lewis, and secretor/non-secretor system, the author discusses the most important serological findings which have been considered links in the Lewis system.

The description comprises the Le^a, Le^b, Le^{b_2}, X, and Magard receptors, especially the relation of these blood cell properties to the secretor/non-secretor system. It is emphasized that receptors Le^b and Le^{b_2} are serologically different receptors. Grubb's demonstration of the independent role of Le^a substances in the secretions is stressed.

The complicated theories on the inheritance of the Lewis system are reviewed. The importance of the H receptor is mentioned. It is stressed that in this respect also two properties have to be considered, viz. those which react with the so-called anti-O, and those which react with anti-H (H^1 and H^2).

After trying to prove the existence of receptor X (Andresen and Jordal), the heredity is further elucidated. The blood cell properties, which have been included in the Lewis system [the original Lewis system (Andresen)] are phenotypic properties governed by the secretor gene *Se*.

The presence of the Le^a substance in the organism is an independent, inherited

property determined by gene *Le* (Grubb). It is suggested that this system be designated *the Lewis substance system.* Since the Le^a substance plays an important role in the original Lewis system as well as in the Lewis substance system, the phenotypic conditions will always be conditioned by the combined effect of the genes of both systems.

After reviewing Watkins and Morgan's "Possible Genetical Pathways for the Biosynthesis of Blood Group Mucopolysaccharides" and Watkins' and Ceppellini's considerations regarding the application of certain theories to the group properties of the red cells, these ideas are applied to the theory advanced in the present paper.

It is assumed that all the group properties (receptors) of the named systems are derived from one precursor substance converted under the influence of the genes governing the various systems. Formation of the Le^x substances is an independent aspect governed by gene *Le*. On the other hand, the formation of $H^{1\text{-}2}$, A, and B substance (alcohol soluble as well as water soluble) and of Le^b, Le^{b_2}, as well as the Magard substance will pass through a more complicated process under the action of genes *X*, Levine (= H, Morgan, *A*, *B*, and Y (W. Weiner), and *Se*.

The formation proper of the water soluble blood group substances must be assumed to be the result of the cellular metabolism, since the serologically active substances derived from the precursor substance are bound to mucopolysaccharide macromolecules (M), and it is emphasized that one macromolecule M can bind a number of different receptors.

Receptors A, B, H, and Le^b, and the Magard receptor can also be bound to an already formed Le^x – M (Le^a substance). When Le^b, or the Magard receptor, is bound to Le^a substance, the Le^a receptor is lost, being converted into Le^{b_2} receptor, and the Magard receptor is completely lost.

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