THE RH BLOOD GROUP SYSTEM

M. Hauge, M. D. *

Even though the Rhesus system is more than 25 years old, we are still very far from having a satisfactory genetic model which can fit all of the observations made during this period. It is impossible to draw inferences of the supposed genetic basis of the Rh system with the same confidence as we can when dealing with the genetic basis of the ABO system. However it is extremely interesting to notice how the latest observations in the genetics of the Rh system are closely related to the most modern ideas on the spatial and structural basis of what we usually call genes and are fully compatible with the most recent theories based on studies in microbial genetics.

The main emphasis in this brief survey of the Rh system will be on the genetic implications of the serological work done during the last 25 years.

The Rh blood group system owes its name to the means by which it was discovered. Landsteiner, after discovering the MN and P systems, continued the systematic search for new antigens by trying to provoke the production of new antibodies through injections in various animals. As an extension of this work (now done in ccoperation with Wiener) he used red cells from Rhesus monkeys, which when injected into rabbits and guinea pigs produced a new antibody.

It is historically interesting to notice the far reaching effect of what looks like simple neglect. Levine, who had for a long time worked with Landsteiner, was the first to observe and explain the potential deleterious effect of one of the antigens, later called an Rh antigen, or rather of Rh differences between two individuals. In his paper, «An unusual case of intragroup agglutination», (intragroup referring to the compatibility as far as the ABO groups were concerned), he gave the correct explanation of the cause of this type of hemolytic disease of the newborn, and the severe reaction resulting from an incompatible transfusion. Certain that he had encountered a new antigen outside of the ABO, MN and P systems known at that time, he even made a small investigation into the population fre-

* University Institute of Human Genetics, Copenhagen, Denmark.

quency of this antigen, and tried to immunize rabbits. He gave no name to the new antigen, but had he done so, another term would undoubtedly have been used, and no name would exist which often deceptively gives the impression of a specific relation to monkeys.

Landsteiner and Wiener in the following year sent out their paper concerning the new antibody against Rhesus monkey red cells, which was shown to divide mankind into two groups: those reacting, the Rh positives, and those not reacting to this new antibody, the Rh negatives.

The identification of this antibody with the one found by Levine was later confirmed, and in 1941 Levine and his co-workers were able to solve to a great extent the puzzling problem of erythroblastosis fetalis by showing that it was in many cases due to Rh incompatibility. This disease had been the cause of much speculation; often familial, it nevertheless did not follow Mendel's laws, so that the geneticist had been unable to give any staisfactory explanation of its etiology.

At about the same time Wiener showed that a number of transfusion reactions, which could not be caused by ABO or MN incompatibility, were obviously due to an Rh-difference between the donor and the patient. The detection of the incomplete antibodies ought to be mentioned briefly. In a number of cases of erythroblastosis fetalis, at first no anti-Rh could be detected in those mothers even though the constellation of the genotypes in the father, fetus and mother was such that Rh incompatibility seemed to be the true explanation for the hemolytic disease in the newborn. Detection of incomplete antibodies solved this problem and opened the way to the discovery of new blood group systems. These events represent the peak of clinically useful discoveries connected with the Rh system; few details of this nature were added later.

As a sign of how few clinical changes have been made since then, the terms coined at that time are still in use in most clinics. The first symbols introduced were very simple: the antibody was called anti-Rh, Rh+ and Rh— described the reactions to the new antibody, and the genes were thought of as Rh and rh, the first being dominant.

What happened over the years must be seen in the light of contemporary historical events; the Second World War was raging, and possibilities of meetings, formal or informal, or of exchange of material and results were limited. Events after the war have shown

that many difficulties, which disturbed the rapid evolution of the Rh system, were to a great extent due to differences in personality.

In England, Race entered the field of Rh serology and genetics with all his skill and enthusiasm, and in the United States, Wiener continued his studies, and developments were rapid. However, there was limited direct contact and collaboration and soon it seemed as if there were serious disagreement between Wiener and Race and their respective schools. It was evident that they had two different viewpoints as to the genetic basis of the Rh system, but to geneticists, and to Race, it was clear that neither of them could at that time be proved to be the correct one. Wiener supported the theory that there is one Rh-locus with a series of alleles. Each allele may be the determinator of one or more blood factors, equaling what we would call antigens, each of the factors being detectable by a specific antibody. This implies that one gene could have two or more different, observable and specific effects. The theory worked out by Race and Fisher in Great Britain was based on the theory of one gene per detectable, specific antigen, and three closely linked loci were the minimum required to explain the findings, each locus having two or more alleles at its disposal. Wiener's most outstanding criticism of this hypothesis was that crossing-over had not been seen between these loci and that it was thus not reasonable to speak of more than one locus.

In this article the Fisher-Race ideas are followed, first of all, because the notation is much simpler; the ceaseless stream of discoveries has made few changes of notation necessary, although naturally additional symbols have been needed to take care of new facts. Wiener's notations have had to be changed repeatedly; Roman, as well as Italic letters had already been used to such an extent that Germanic letters had to be introduced as well, with the ensuing difficulties with the printers. An even more cogent argument is that the analogy with modern genetic facts and thinking is more obvious with Race's theories, although this does not mean that the theories put forward here are the right ones.

The core of the discrepancy between the so-called Wiener theory and the Fisher-Race theory, is not in a discussion of *facts,* but of interpretation.

The Fisher-Race theory was launched by Fisher at a very early stage when few antibodies belonging to the Rh system were known, few family studies had been carried out, and the range of variation

observed was still very limited. Fisher noticed that two of the four antibodies discovered by British workers at that time defined antigens which were obviously determined by allelic genes, and he suggested that not one, but three closely linked Rh-loci exist. In the Fisher-Race notation they would be: the C-c, D-d and the E-e loci. According to this theory it may be said that the Rh blood group system is of a polymeric character. Fisher anticipated the finding of two more antibodies which would substantiate his theories; one of these was actually found by Mourant. It was claimed that the other, the anti-d, was found once or twice in America in the late forties; although it is still doubtful, perhaps to-day even more than before, it neither invalidates the theory nor the use of the symbols as proposed by Fisher. We speak about the phenotype and the gene O even if we know that no antigen O and no true anti-O antibody exists.

We have in the main five distinct antibodies:

anti-C		anti-E
anti-c	anti-D	anti-e

The fifth is rare, but this is a technical problem.

The reactions may be summarized as in Table 1.

TABLE 1

	genotypes		
	Cc	CC	cc
anti-C	+	+	—
anti-c	+	—	+
	Ee	EE	ee
anti-E	+	+	—
anti-e	+	—	+

If family investigations are made with only two of the paired antibodies, they obey Mendel's first law completely. The C-c locus seems to be an entity with two allelic genes without dominance or recessiveness. The same applies to the E-e locus.

As far as anti-D is concerned, only two reactions are possible:

	genotypes		
	DD	or	dd
	Dd		
anti-D	+		—

This looks as though the D gene is a regular dominant, expressed also in the heterozygous state.

The next step is to see, if these three sets of allelic genes follow Mendel's second law. If so we should, in population studies, find the expected frequency of concurrence of, for instance CDe, equal to the product of the frequencies of the C gene, the D gene and the e gene. This is not the case. The deviations from expected values are very highly significant.

If family studies are examined, it will be found that parents having, for instance, the reactions C+c+D—E+e+, and C—c+D—E—e+, respectively, do not give to their children the combination of genes expected if Mendel's second law applied. If they have these combinations of reactions, they would have the genotype combinations CcddEe and ccddee, respectively.

The first parent, say the father, would, if Mendel's second law applied, from sperms of the following types CdE, Cde, cdE, cde, and the eggs would contain one combination only, cde. Four types of children should be seen. However only two types of children are observed from these marriages. Two types of mating are possible, because the alignment of genes in each of the homologous chromosomes in the father can be:

1. Cde/cdE, and the mother cde/cde, which gives Cde/cde and cdE/cde children, the two other types are missing. Or the alignment may be:

2. CdE/cde and the mother again cde/cde giving CdE/cde and cde/cde children.

This demonstrates the phenomenon of linkage clearly; the genes are handed on to the next generation in blocks; and if the theory of linkage is correct, it must be a very close one. Crossing over has never been verified, but if you try to estimate the number of conditions which have to be fulfilled before we can expect it to be demonstrated clearly, you will find that the chances are few, and if the crossing-over frequency is very low, you cannot expect it to happen very often. I will just mention a few of the technical problems: only if the crossing-over happened in a mother of a number of children could we accept the evidence; the father does not count. All the five antibodies would have to be used, extensive studies of the parents and perhaps of even more relatives of the mother in question would have to be carried out to establish the alignment of the genes on

her two homologous Rh-chromosomes, and even so only *some* combinations of gene complexes would allow any conclusions. Only about one thousand families have been studied thoroughly, or rather the cases have been published, with all five antibodies up to now.

Exceptions to the regular inheritance of the alleles of each of these three loci are very few, and the majority of them have been shown to have illegitimacy as their most probable cause.

The phenotypes of the Rh system consist of combinations of the observed serological reactions of the red cells to specific antibodies; and a rich variety of notations has been used to indicate the results of the tests. Some researchers use plus and minus, with or without the symbol denoting the supposed antigen. This is a good and honest method, because nothing is inferred, it is a description of observations. This makes it the notation preferred by many serologists dealing with paternity cases; but even they have to go a bit further, they can say nothing about paternity without thinking of the genes. Therefore, the second level of notation is not far from what they must have in their minds. This is the so-called «most probable genotypes», inferred from the phenotypes.

When we see the reactions C—c+D+E—e+ this may mean either cDe/cde or cDe/cDe.

The problem of which of these two is the most probable can only be decided when we know enough about the *population* frequencies of the various complexes. In Europe cDe/cde is about 30 times more frequent than the homozygous cDe/cDe; the cDe complex rarely occurring. In other populations, these two possibilities would be perhaps equally frequent.

The use of the most probable genotypes indicates that one has chosen between two, three or in some cases, four possible genotypes, and has used some previous knowledge of gene or rather complex frequencies.

This points out again the value of family studies, which would in many cases permit the deduction of the true phenotypes. It is obvious that our inability to disclose the d-antigen (if it exists) is among the most important obstacles in the deduction of genotypes.

Finally, when using these symbols and trying to indicate the probable nature of the complexes in an individual, some short symbols are often used:

CDe:R_1	cDe:R_o	CDE:R_z
cDE:R_2	Cde:R′	CdE:R_y
cde:r	cdE:R″	

These are all combinations anticipated by Fisher and later observed.

The second stage of development brought a few new problems to the clinicians. A donor having no D-antigen, which is equivalent to the original Rh antigen should be called Rh-negative as he is genotypically dd. He may, constitutionally, however, also have a C or an E, which might act as a stimulus to a patient without these antigens, who is the pure Rh-negative constitution, cde/cde. Therefore Rh negative occasionally needs qualifying; either by stating the probable genotype, indicating the danger, or as is more often preferred, a distinction may be drawn between the function of a person as a donor and as a recipient. If Cde/cde, he may be called Rh positive as a donor, and Rh negative as a recipient.

It might be mentioned that Fisher, at this stage, by considering the frequencies of the complexes, and predicting what would result if the theory of the three closely linked loci were correct, was led to the supposition that the order of the loci was DCE. Later developments have supported the belief that the relationship between D and Cc on the side and Cc and Ee on the other is closer than between D and Ee.

The next stage of definite genetic interest was reached when a new antibody, anti-f, was discovered, which led Race to believe that a fourth locus existed.

It was obvious (from hundreds of tests) that f could not be an allele of any of the three old loci. Although he had some reservations, Race launched the theory that a new locus was disclosed, with the f gene and a supposed allele F, making out four loci on the Rh chromosome, all determining Rh antigens. Arduous work resulted in a change of attitude, however. Positive reactions with anti-f were only (with a few puzzling exceptions) found in individuals having both the c and the e antigen, and in the cis-position in the *same* chromosome. The exceptions seem so few that it must almost inevitably be made a rule that this antibody is an anti-ce antibody which may be valuable in distinguishing between ce in the cis-position and in the transposition. For instance CDe/cDE does not react, whereas the uncommon type CDE/cDe does give a positive reaction. A few exceptions exist.

This gave support to the theory that C-c was closer to E-e than to D-d. It was one of the first hints of the necessity of introducing the term «compound antigens.»

The number of loci was thus kept at three; with at least two alleles at D and E, but three at the C locus, making 78 different Rh genotypes. The third allele at the C locus is C^w, frequently occurring, but only diagnosed by the specific antibody. Most anti-C sera also react with C^w, and so are really anti-CC^w.

The next level is the present phase. The immense flood of discoveries has mostly been based on new antibodies which determine distinct antigens, each inherited as part of the Rh system. These are not explicable as simple products of an allelic gene at any of three so-called established loci. A number of the observations have not yet given any particularly useful genetic information, perhaps because their place and real nature are not fully understood.

The latest additions to genetic knowledge include the breaking down of the D-antigen discovered by finding a number of healthy people, without anemia, who had what seemed to be normal reactions to anti-D antibodies, and therefore supposedly a normal D-antigen, but who at the same time possessed anti-D in their blood, active against most other «normal» D-antigens. Cross matching of red cells from one person with sera from another within this group has yielded very peculiar results which are for the time being interpreted as indication of a highly complex D-antigen with four or more subcomponents.

A new antibody, termed anti-V, was found to react only with red cells containing the compound antigen ce^s where e^s is an allele at the E locus.

The antigen G seems to be present normally in all cells containing C and/or D, and the so-called anti-CD sera are supposed to be either anti-C+G, -D+G, or -C+D+G. This explains why a woman with a cde/cde genotype without a history of previous transfusions can produce an apparent anti-CD even when the fetus lacked D. As had been shown later, it must have been an antibody of the anti-C+G type. Anti-G may be isolated from the mixtures.

Finally, we have to deal with some very rare but illustrative Rh groups; those showing lack of representatives from one, two or all three so-called allelic series:

The first was the D-- complex, which has been observed in a number of people who are supposedly homozygotes. Only by family studies can heterozygotes be detected. Probably in all cases these are born to consanguineous parents. More than 25% (nearer 50%) of the sibs of the propositi were D- -/D- - individuals, which would indicate that it is not a recessive condition. In passing it should be mentioned that these people have *very* strong D-antigens and naturally run a high risk of becoming immunized.

Turning to the details of the genetic basis of the Rh system it appears first of all as if it is no longer worthwhile to argue whether the three sites are to be placed within or outside the boundaries of one gene when no one is capable of defining what the exact boundaries of a gene are.

The splitting up and subdivision of what were supposed to be single loci has been going on for quite some time, based on observations which tended to show that recombinations and mutations took place *within* genes. This has been demonstrated quite clearly in bacteria and viruses as well as in some higher organisms. Perhaps more illuminating was the breakdown of one locus called the dumpy locus in Drosophila. Originally, three allelic genes were supposed to be available for this locus:

l being a recessive lethal,
o giving a reduction in wing size,
v producing some prominences on the thorax.

After observing more than half a million flies it appeared that there could be at least seven mutational subloci: l-ol-olv-o-lv-ov-v, i.e. four capable of producing the character l, four producing o and four producing v.

Crossing-over between l and v was about 1 per cent. A given type of effect is not restricted to a given sublocus, and vice-versa. This is in some respects similar to the present observations in the Rh system, but more studies are needed before the genetic picture of the Rh system is complete.