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ON THE Hr FACTOR AND THE Rh GENETIC THEORY

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IN the recent discussions on the genetics of the Rh multiple alleles, no provision is, as yet, made for the role of a gene determining the Hr factor.[1,2] This agglutinable property was described very early in the course of the studies on the pathogenesis of erythroblastosis fetalis.[3] It was advisedly designated by Levine[4] as Hr (reversal of the letters Rh) because of peculiar relationship to a special variety of anti-Rh sera, now designated anti-Rh′. This is indicated in Table 1, which gives, at the same time, the four sub-types of Rh and their frequencies resulting from the reactions of anti-Rh_0 and anti-Rh′ sera.

From the beginning of the studies on erythroblastosis fetalis, Levine has held to the view that the relationship of the anti-Hr and anti-Rh′ sera is analogous to that of anti-M and anti-N sera. In other words, only three types of reactions are observed, and in both systems bloods failing to react with both anti-sera were never found. It was only after hundreds of bloods were tested that the term Hr and anti-Hr were designated. These results were not published more fully because it was clear that the first anti-Hr serum was of weak activity and gave too many negative reactions.

Subsequently, Race and Taylor described a similar

[1] R. R. Race, G. L. Taylor, K. E. Boorman and B. E. Dodd, *Nature*, 152: 563, 1943.

[2] A. S. Wiener, SCIENCE, 100: 595, 1944.

[3] P. Levine, L. Burnham, E. M. Katzin and P. Vogel, *Am. Jour. Obstet. and Gynec.*, 49: 925, 1941.

[4] P. Levine, Yearbook of Path. and Immunol., 508, 1941.

TABLE 1

BASED ON TESTS WITH 334 RANDOM BLOODS (WHITE) CARRIED OUT IN APRIL, MAY AND JUNE, 1941*

Terminology of Wiener and Landsteiner	Mrs. M.F. Anti-Rh_0	Mrs. M.S. Anti-Rh′	Mrs. K.F. Anti-Hr	Incidence of type (per cent.)
Rh_1	+	+	0 or ±	71
Rh_2	+	0	+	14
Rh′	0	+	0 or ±	2
Rh–	0	0	+	13

* At the written request of Dr. Wiener, the scheme of the reactions indicated was made available to him for inclusion in the third edition of his book, "Blood Groups and Transfusion," pp. 253–254 (C. C Thomas).

serum (called St) which contained more potent agglutinins.[5] These workers made the significant observations that individuals whose blood is Hr negative are homozygous for the Rh factor. At this point of their studies, only anti-Rh_0 sera were at their disposal so that they were not aware of the fact that all bloods of the subtype Rh_2, whether homozygous or heterozygous, react strongly with anti-Hr sera.[6] As pointed out by the writer,[7] individuals of the genotype Rh_1Rh_2, although heterozygous and reacting with anti-Hr sera, possess two dominant genes, Rh_1 and Rh_2, each determining the presence of a dominant agglutinable blood factor capable of inducing isoimmunization of the Rh negative mother. Accordingly, the genetically heterozygous Rh_1Rh_2 individual may be considered clinically as homozygous, since in matings with Rh negative women, all offspring must be Rh positive and therefore susceptible to erythroblastosis fetalis. These findings, published in 1943, are presented in Table 2, in which the gene determining the rare property Rh′ is not included.

TABLE 2

Phenotype	Genotype	Reaction with		
		Anti-Rh_0	Anti-Rh′	Anti-Hr
Rh_1	Rh_1Rh_1	+	+	0
	Rh_1Rh_2	+	+	±
	Rh_1rh	+	+	±
Rh_2	Rh_2Rh_2	+	0	+
	Rh_2rh	+	0	+
Rh–	rhrh	0	0	+

Nevertheless, the contribution of Race and Taylor is important because the genotype Rh_1Rh_1 is far more frequent than the genotype Rh_1Rh_2. With this observation, the analogy to the M and N relationship was still closer, since the homozygous M (MM) can not be differentiated from the heterozygous M (MN) with the aid of anti-M sera.[8] This differentiation can be made only with anti-N sera. Similarly, individuals of the genotype Rh_1Rh_1 can be recognized by the failure of their blood to react with potent anti-Hr sera.

The scheme given in Table 1 is now enlarged with the description of a third variety of anti-Rh serum reacting with 30 per cent. of random white individuals.[1,9,10] This serum subdivides, but in unequal portions, each of the four Rh varieties, thus making eight different subtypes of Rh.

Wiener[2] has stated that "the anti-Hr sera have a place in the scheme of Rh blood types similar to that of the anti-O sera in the blood group scheme." In support of his view, he states that ". . . anti-O sera, like anti-Hr sera, are usually of low potency." In this he obviously is in error, because anti-Hr sera are usually of weak activity because of statistical considerations and not out of genetic necessity. Levine[3,7] has shown that 92 per cent. of all mothers of erythroblastotic infants are Rh negative in tests with the potent diagnostic anti-Rh_0 sera. Of the remaining 8 per cent. Rh positive mothers, only a fraction (let us assume 3 per cent.[11]) are Hr negative and immunized by the Hr factor in fetal blood. It is, therefore, obvious that the chances for detecting equally potent anti-Hr and anti-Rh sera are 1:30. This value may be considered as tentative and the disproportion may be much greater because the Rh factor is far more antigenic than Hr. Actually, there are more incompatible matings for Hr (80 per cent. Hr+ × 20 per cent. Hr– or 16 per cent.) than for Rh (85 per cent. Rh+ × 15 per cent. Rh– or 13 per cent.).

In support of the views presented, Levine[12] has observed two potent anti-Hr sera, and reference to one of them has been reported elsewhere. Another anti-Hr serum now being investigated has a titer of at least 1:512, a value which approximates those of the most potent anti-Rh sera.[13]

This anti-Hr serum failed to yield quantitative titration differences of bloods homozygous and heterozygous for Hr factor. Qualitative differences, however, were observed, Rh negative and Rh_2 bloods giving stronger reactions. By analogy all bloods homozygous for N give stronger reactions with anti-N sera than the heterozygous type MN.

On purely theoretical grounds of a serologic nature, Wiener's views can not be accepted because anti-Hr and anti-Rh sera share the identical properties of origin (isoimmunization) and greater activity at 37° C. (warm agglutinins). Furthermore, there is no

[5] R. R. Race and G. L. Taylor, *Nature*, 152: 300, 1943.
[6] See Table 1.
[7] P. Levine, *Jour. Pediatrics*, 23: 656, 1943.
[8] K. Landsteiner and P. Levine, *Jour. Exp. Med.*, 48: 731, 1928.
[9] A. S. Wiener and E. B. Sonn, *Jour. Immunol.*, 47: 461, 1943.
[10] A. S. Wiener, SCIENCE, 54: 316, 1943.
[11] This value is probably too high.
[12] P. Levine, *Arch. Path.*, 32: 227, 1941.
[13] For this serum the writer is indebted to Dr. Peter Vogel.

reason to assume the existence of any differences in their pathological effects on the fetus and newborn infant. On the other hand, there are several striking characteristics which differentiate human anti-0 agglutinins from normal anti-A and anti-B isoagglutinins (incidence, potency and action at various temperatures). Certainly, there is no basis for excluding the Hr gene from any theory of the heredity of the various subtypes of the Rh factor.

Most workers believe that the Rh factor may be inherited as a series of multiple alleles, and indeed this is to be expected on the basis of past experience with agglutinable factors in animal blood detected by isoimmunization. It can be assumed that agglutinable properties detected by heteroimmunization like M and N exhibit simple genetic behavior in contrast to the agglutinable factors in chicken, rabbit and human blood detected by isoimmunization. In the latter group of the cases, the blood properties seem to be inherited as a series of multiple alleles.

Presumably, the Hr factor was not included in the Rh genetic scheme because it did not seem to fit into the theory originally suggested by Wiener on the basis of studies which, until very recently, did not include anti-Hr sera. Undoubtedly, the genetics of the Rh-Hr system may be still more complicated than is indicated in the schemes of Wiener, Race and Taylor.

Further support of this view was recently provided by Waller, Levine and Garrow,[14] who described an anti-Rh serum of unusual specificity, produced by an Rh_2 mother of an erythroblastotic infant. This serum contained an anti-Rh′ agglutinin, but in addition, an entirely new agglutinin which acted on some Rh_2 and many Rh negative bloods. Accordingly, this agglutinin was distinctly different in its specificity from anti-Rh″. Furthermore, the blood of this immunized woman reacted with anti-Rh″ sera.

In general, the experimental work in this field is hampered by the dearth of potent reagents of all varieties which must be derived mainly from immunized mothers, at best a most uncertain source. Workers are naturally tempted to carry out heredity and racial studies with whatever rare reagents may be available at the time. This is well illustrated in recent numerous publications on the Rh_2 factor carried out with a particular serum which contained anti-Rh″ but also anti-Rh_0 agglutinins. The dilution method for the separation of the anti-Rh″ agglutinin failed to give clear-cut differentiation.[15] Another example is the use of a weak anti-Hr agglutinin, the activity of which disappeared in the course of the study.[16]

Our knowledge of the Rh factor is slowly being extended so that no final theory can be accepted because the subject is still in a fluid state. The calculation of gene frequencies in support of the current theory of multiple alleles may perhaps be proven to be correct. On the other hand, such calculations may also be misleading as happened in the case of the two heredity theories of the four blood groups. Thus, the gene frequencies, on the basis of the now discarded theory of v. Dungern and Hirszfeld, seemed to fit the observed values obtained from studies of families and races. It is significant that the current theory of Bernstein was accepted as a result of statistical studies involving the rarest of the four blood groups, *i.e.*, AB. The state of affairs in the case of the genetics of the RhHr system is certainly far more involved, both from the point of view of technic and the availability of potent reagents. An additional complicating factor is the existence of several subtypes of remarkably low incidence.

Unfortunately, there are already several terminologies of the Rh subtypes, *i.e.*, those of Wiener[2] and Murray, Race and Taylor.[17] In the more or less distant future, it will undoubtedly become necessary for an international committee of geneticists and serologists to recommend a uniform terminology. This, however, can be done only after the analysis of family and racial studies, carried out with all varieties of maximally active reagents. Such studies must, of necessity, be on a vast scale in order to evaluate the role of the very rare subtypes, determined by the factors Rh′, Rh″ and the new factor of Waller, Levine and Garrow.

Because this subject is clinically important, one can hardly expect the clinician to commit to memory at this stage several genetic schemes of phenotypes and genotypes. Fortunately, this is not necessary, nor indeed, is it desirable. It follows from the statistical studies of mothers of erythroblastotic infants and the direct correlation of the frequency of erythroblastosis fetalis in any race to the incidence of Rh negative individuals,[18, 19] that the anti-Rh_0 serum is the most important single reagent for the diagnosis of erythroblastosis fetalis and prevention of the associated and frequently fatal intra-group transfusion reactions. Ninety-two per cent. of all mothers of erythroblastotic infants are Rh negative in tests with the diagnostic anti-Rh_0 serum. In order to produce evidence of isoimmunization in the remaining 8 per cent. of Rh positive mothers of erythroblastotic infants, at least three different blood factors may be involved, (1) the prop-

[14] R. K. Waller, P. Levine and I. Garrow, *Am. Jour. Clin. Path.*, 14: 756, 1944.

[15] R. K. Waller and P. Levine, SCIENCE, 100: 453, 1944.

[16] A. S. Wiener, I. Davidsohn, and E. L. Potter, *Jour. Exp. Med.*, 81: 63, 1945.

[17] J. Murray, R. R. Race and G. L. Taylor, *Nature*, 155: 112, 1945.

[18] P. Levine, SCIENCE, 96: 452, 1942.

[19] P. Levine and H. Wong, *Am. Jour. Obstet. and Gynec.*, 45: 832, 1943.

erties A or B of fetal blood, non-secretor type, (2) the Hr factor and (3) finer differences of the Rh factor. The report to the clinician in the exceptional Rh positive mothers can be worded as an incompatibility detected by a particular reagent. In any event, it will be necessary for these bloods to be referred to a serologic specialist who may or may not have on hand potent anti-Hr and the other two varieties of anti-Rh sera. So far as the clinician is concerned, one may recommend the simple genetic theory based on the behavior of the diagnostic (anti-Rh_0) serum, which contains but a single antibody. A more detailed analysis which requires the use of other anti-Rh sera or anti-Hr serum can be supplied by the specialist in the field.

SCIENTIFIC EVENTS

THE IPATIEFF HIGH PRESSURE AND CATALYTIC LABORATORY OF NORTHWESTERN UNIVERSITY

THE funds for the founding of the High Pressure and Catalytic Laboratory came from Northwestern University and private sources. A part of the apparatus was contributed by the Universal Oil Products Company. The idea for such a laboratory was sponsored by Professor W. V. Evans, of the department of chemistry, and permission to establish such a laboratory was obtained from the president of the university. The aims of the laboratory have been:

1. To study catalytic reactions under normal and high pressures because of their theoretical as well as their industrial importance.
2. To give students in chemistry and engineering not only theoretical but practical knowledge of the main types of catalytic reactions and the properties of catalysts.

During the first five years of the existence of the laboratory the work has dealt mainly with the application of catalysts in the field of terpenes, as follows:

1. A method for obtaining terpenes from solutions of terpene alcohols by dehydration in the presence of very dilute inorganic salts such as magnesium chloride, ammonia chloride, etc.
2. A new method of determining the presence of three, four and five methylene rings in di-cyclic terpenes.
3. A study of alkylation of terpenes with aromatics in the presence of various catalysts.
4. A new cyclic isomerization of limonene into a new di-cyclic terpene.
5. A study of the transfer of hydrogen in the terpene series in the presence of no hydrogenation catalyst.

From the student's point of view the following programs are in progress:

A. Students perform experiments on hydrogenation, oxidation, isomerization, polymerization, alkylation, etc.
B. They become acquainted with and prepare the main types of catalysts.

The equipment of the laboratory consists of the following:

1. Ipatieff type bombs of various sizes and models which can withstand pressures up to 400 atmospheres at 500° C. temperature.
2. Special type bombs for the study of the solubility of gases and critical temperatures, which allow the removal of small portions of the reactants during the reaction for study.
3. Turbo mixer type bombs which rapidly mix the reactants during a reaction.
4. Special apparatus for the study of continuous reactions under pressures up to 130 atmospheres.
5. Special bomb-proof units where these high pressure reactions can be carried out.

The laboratory is under the control of Dr. V. N. Ipatieff, an authority on high pressure reactions and a pioneer in the field of catalysis.

Dr. Ipatieff is assisted by Professor Pines, who gives lectures on catalysis in the department of chemistry. Professor Pines has been associated with Professor Ipatieff in his major discoveries of the past fifteen years.

The war has interfered with the development of this laboratory both by taking away prospective students and by making it impossible to secure needed apparatus. As soon as it is possible to do so, the laboratory will be enlarged, and its accommodations increased. A large number of students and research associates taking graduate work along the lines of catalysis and high pressure are expected to take part.

NEW LECTURE ROOM VISUAL AIDS AT COLORADO AGRICULTURAL AND MECHANICAL COLLEGE

EIGHTEEN mural paintings depicting the epochs of geologic time through representations of various plants and animals from the pre-Devonian period through succeeding epochs to modern times have been painted on 500 square feet of the walls of the botany building lecture room at the Agricultural and Mechanical College, Fort Collins, Colo.

Since they were painted during the summer of 1944, the murals have continued to attract increased attention and have been accorded growing favorable comment by students, faculty and visiting botanists and geologists.

The 7-foot panels done in oil by Dr. L. W. Durrell,