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Blood Grouping Tests in Disputed Parentage
Qualifications of Experts

II. An Error Involving the Rare Rh-Hr Blood Types Rh_zRh_1 and Rh_zrh

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The application of blood grouping tests in medicolegal cases of disputed parentage has become more or less routine, so that courts no longer hesitate to order such tests wherever indicated, and courts accept the results of the tests when they exclude parentage. At the same time, there has been an unfortunate tendency to assume that these tests can be done at any blood bank or laboratory where clinical blood grouping tests are carried out, so that courts have not infrequently referred such cases to laboratories or clinical pathologists with only limited experience in the field. This false belief has been fostered by the ready availability commercially of the antisera needed for conducting such examinations. This has led individuals with little or no experience in the field to accept the assignment from a court to conduct these examinations, and some of the errors which have resulted were described in a previous report (1).

Experience has shown that while blood grouping tests are indeed simple to carry out, the reactions are of a delicate nature, and subject to error (2). Such errors can be avoided only by using reagents of high potency and specificity, including ample controls with each test, and by doing each test more than once. The present author does each test at least four times, namely, in duplicate on two separate days using different sets of reagents and when the results exclude parentage or are in any way ambiguous, the tests are repeated again with different reagents. In addition, all readings are taken blind (3) to rule out the possibility of any bias, and no report is submitted unless the findings are unequivocal and all possibility of error has been excluded. It is apparent that, if the reagents used have to be purchased, the cost of carrying out the examination in the manner described would be prohibitive. Thus, a satisfactory examination at a reasonable fee can be carried out, in general, only by specialists in the field, who prepare their own testing reagents.

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Technical skill in the performance of the blood grouping tests is not sufficient to guarantee accurate results. Experience has shown that of greater importance than technical skill is understanding and insight into the nature of blood group reactions and their proper interpretation. This is illustrated by the confusion that prevails even at the present time regarding Rh-Hr serology and terminology. The average worker assumes that the exact terminology used is unimportant. This naive position fails to take into account that terminology *interprets* observations, and that a nomenclature that misrepresents facts can lead to errors. Moreover, terminology teaches by implication, so that an incorrect terminology teaches wrong things. For example, the C-D-E notations teach incorrectly that each antigenic substance has but a single corresponding antibody and that conversely to each antibody there corresponds but a single antigenic substance. Therefore, workers using these symbols make no distinction between agglutinogens and blood factors (serological specificities) and use the terms interchangeably. On the other hand, Wiener's Rh-Hr terminology (4, 5) distinguishes carefully between agglutinogens and their serological specificities (blood factors) as well as between phenotypes and genotypes, and thus avoids the fallacies and errors inherent in the Fisher-Race C-D-E notations. It is evident, therefore, that the careful and precise use of blood group terminology is an indication of one's knowledge and understanding of the subject, and as Wiener (1) has pointed out, this may be used as one of the criteria by which to judge the qualifications of an individual who holds himself out to be an expert in medicolegal blood grouping tests.

The purpose of this paper is to describe an unusual medicolegal case of disputed paternity, which serves to exemplify the points that have just been outlined.

Case report

The present author was requested to carry out blood grouping tests in a case of disputed paternity, in which a clinical pathologist had previously examined the blood specimens and submitted a report that the respondent was not the father of the child in question. The mother of the child, who was the petitioner in the case, insisted that the man she had accused was the father, and it was at her request that the second examination was to be carried out. All parties in this case are Caucasian.

In table 1 is shown the report submitted by the clinical pathologist. As can be seen, he based his exclusion of paternity on his finding that the putative father was **hr′** negative while the child was found to be **rh′** negative. Closer examination of

Tab. 1. Report submitted by a clinical pathologist in a case of disputed paternity

Name	Group	Rh_0	Rh prime	Rh double prime	Hr prime	M	N
Putative father	B	Pos	Pos	Pos	Neg	Pos	Pos
Mother	A	Pos	Pos	Neg	Pos	Pos	Pos
Child	AB	Pos	Neg	Pos	Pos	Neg	Pos

the report demonstrates, however, that the clinical pathologist lacked thorough understanding of the subject, and was not properly qualified for these tests. First of all, while the A-B-O groups were reported by him properly in terms of phenotype symbols, the M-N and Rh-Hr findings were reported only in *protocol* form and no attempt was made to express the findings in phenotype symbols. This is inconsistent, and betrays lack of thorough understanding. (The present author includes in his own reports a protocol giving the actual reactions, but also gives a *separate* table in which the results are presented entirely in phenotype symbols.) Moreover, while the clinical pathologist paid " lip service " to the correct Rh-Hr terminology, he used the terminology just as a C-D-E protagonist would, that is, each blood factor is treated as though it represented a separate agglutinogen. Finally, he took no notice of the remarkable fact that the putative father belongs to the rare Rh-Hr type Rh_zRh_1, which was a clue to the possibility that there might be some error in his findings, or, at any rate, called for closer scrutiny of the results.

In table 2 are presented the present author's own findings which, as can be seen, do not exclude paternity, but instead offer circumstantial evidence that the

Tab. 2. Findings of the present author in the case of table 1

Blood of	A-B-O group	M-N type	Rh-Hr type
Putative father	B	MN	Rh_zRh_1
Mother	A_1	MN	Rh_1rh
Child	A_1B	N	Rh_zRh_o

putative father is probably the actual father of the child. The important difference between the findings given in tables 1 and 2 relate to the reactions of the child's red cells with anti-**rh′** serum. Based on the tests for blood factors **Rh_o**, **rh′**, **rh″**, **hr′** and **hr″**, the child's blood was classified as phenotype Rh_zRh_o. These findings suggested that the child might be of phenotype Rh_zrh rather than of phenotype Rh_1Rh_2, while the opposite is usually the case with type Rh_zRh_o individuals (6, 7). Therefore, further tests were carried out with anti **rh_i** and with anti-**hr** sera, and at the same time the tests with anti-**rh′** sera were repeated in order to be certain that we had not made the error rather than the previous expert. These additional findings are shown in table 3.

As can be seen, our expectations were fulfilled, since the child proved to be **rh_i** negative and **hr** positive (cfr. table 3). Thus, the child's phenotype was Rh_zrh, so that his genotype had to be R^zr, R^zR^o or R^or^y. The putative father, on the other hand, had to be one of the following three genotypes: R^zR^1, R^zr' or R^1r^y. The mother, type Rh_1rh, had to be one of the following three genotypes: R^1r, R^1R^o or R^or'. In any event, it was clear that the child must have acquired either an r or an R^o gene from the mother, and either an R^z or an r^y gene from the father. Considering the very

Tab. 3. Reactions of the red cells of the individuals of table 2, with selected Rh-Hr antisera

Blood cells of	Reactions* with anti-**rh′** sera		Reactions with sera		
			Anti-**rh$_i$**	Anti-**hr**	
	# 1	# 2		# 1	# 2
Putative father	+++	+++	++	—	—
Mother	+++	+++	++	+++	+++
Child	++	++	—	+++	+++
Controls: Type rh	—	—	—	+++	+++
Type Rh$_1$rh	+++	+++	++	+++	+++
Type Rh$_2$rh	—	—	—	+++	+++
Type Rh$_1$Rh$_2$	+++	+++	++	—	—
Type Rh$_z$rh	+++	+++	—	+++	+++

* The strength of the reactions is indicated by the number of plus signs, +++ being the strongest reaction possible, namely, one large clump of agglutinated cells.

low incidence of the genes R^z and r^y among Caucasians, the presence of the same rare gene in putative father and child could be considered circumstantial evidence of paternity.

Discussion

Anti-**rh′** serum agglutinates red cells of all individuals carrying any of the genes R^1, r', R^z, or r^y. On the other hand, anti-**rh$_i$** serum agglutinates cells of carriers of genes R^1 and/or r' only. Since genes R^z and r^y are quite rare, in routine tests on a series of blood specimens anti-**rh′** and anti-**rh$_i$** sera would give parallel reactions, and the difference between them is not noticeable unless red cells of the rare type Rh$_z$rh are available for testing the antisera. It is entirely possible that some of the so-called anti-**rh′** (anti-C) sera commercially available are really anti-**rh$_i$** reagents. To one subscribing to the C-D-E notations with their simple but false implication of a one-to-one correspondence between antigen and antibody, the idea of more than one kind of " anti-C " reagent would be unthinkable. Thus, the naive worker would be unaware of and would not notice the fine differences in specificity between anti-**rh′** and anti-**rh$_i$**, both of which might be classified as anti-C by him.

As shown in table 3, if an anti-**rh$_i$** serum were mistaken for anti-**rh′**, as could easily happen, then the child in this case would be classified as type Rh$_2$rh instead of Rh$_z$rh, and paternity would then be excluded erroneously. Perhaps that is what actually happened in the present case. Also, if weak anti-**rh′** reagents are used, it is possible to get false negative reactions with type Rh$_z$rh blood even though the serum is reactive with type Rh$_1$Rh$_2$ blood. Such false negative reactions can be avoided by using reagents of satisfactory specificity and titer, and by using an appropriate sensitive technique of typing.

Whatever was the cause of the error, the observations confirm the point made at the beginning of this article, namely, that understanding and insight are more important than mere technical skill in order to guarantee accurate results. Thus, correct interpretation, as well as careful technique using potent and specific reagents, are essential to guarantee exact results. The correct use of nomenclature is an essential part of the interpretation, so that this may be used as one of the criteria by which to judge the qualifications of an individual who claims to be an expert in the field [cfr. Wiener (1)].

Recently, still another case has come to the author's attention that illustrates this point. A lawyer has written to me as follow: " Recently I received the enclosed report which I feel should be introduced into evidence. The Judge has already told me that he will not allow me to introduce the report on the grounds that it is not a definite exclusion. I do not know whether I should make an issue out of it and bring it to a higher court. " The report read as follows:

	Group	Rh factor
" Mr. C.	O M	D c e
C. H.	O M	D c e
Child	O M	c e

" Conclusion: The mother and Mr. C. have identical types. The child is missing the Rh factor " D " which is present in both adults. Although Mr. C. cannot be unequivocally excluded as the putative father, the chance of his being so is less than 97% ".

This report shows woeful ignorance on the part of the " expert ", which could have been surmised again from his incorrect use of blood terminology. Because mother and putative father are both type Rh_0 and the child type rh, he concluded that the accused man probably is the father! Since the child is type rh, the child's genotype must be *rr*, and the mother is necessarily heterozygous, genotype R^0r. The putative father, if he is the actual father must also be genotype R^0r. If the putative father is white, then he is probably heterozygous, so the statement of the " expert " is certainly false. But the case probably involves negroes, among whom type Rh_0 is common. However, even among negroes such as exist in New York City, at least half of the type Rh_0 individuals are heterozygous. Thus, the assertion of the " expert " that the chance that the putative father is not the true father is 97 percent is entirely incorrect, and based on his ignorance of the subject, as further evidenced by his incorrect use of the Rh-Hr nomenclature.

In a report by the Committee on Medicolegal Problems of the American Medical Association (8), the problem of Rh-Hr nomenclature was summarized as follows: " One of the most pressing problems at the present time is that of blood group nomenclature. This pertains particularly to the important Rh-Hr types, for which two principal methods of designation are in use, namely, the original Rh-Hr nomencla-

ture and the C-D-E symbols. The submission to jurist by different experts of medicolegal reports with conflicting symbolism cannot help by confuse them and shake their confidence in the blood tests. Moreover, reports in two different symbolisms require translation from one into the other when comparisons are to be made; there is always danger in the process. Therefore, the adoption of one uniform nomenclature for medicolegal reports is desirable... The C-D-E notations for the Rh-Hr blood types make no allowance for the difference between a blood factor and an agglutinogen, and incorporate the tacit incorrect assumption that every agglutunogen has but a single corresponding antibody. This has led to a number of paradoxes and errors... On the other hand, the original Rh-Hr nomenclature presents the data objectively... This committee, therefore, recommends that the C-D-E notations for the Rh-Hr types be discarded, and that, in approved medicolegal reports, ..., the original Rh-Hr nomenclature be retained as the sole nomenclature for this blood group system ".

More than seven years have passed since this report and recommendation were published. The continued use in clinical and legal medicine, and in scientific journale of the fallacious C-D-E symbols can only mean, that, as in the case of chiropractic, individuals with a vested interest in these symbols, or who have been so brain-washed that they cannot distinguish mythology from facts (9, 10), are interfering with the adoption of a single uniform nomenclature which is so badly needed to further this subject. The situation has been aggravated by the recent introduction of a third system of nomenclature (11), which involves the use of numbers. In this nomenclature, the twenty odd blood factors are numerically coded, and the symbols for the agglutinogen Rh_1, for example, then becomes 1, 2, -3, -4, 5, -6, 7, -8, -9, -10, -11, 12, 13, 14, 15, 16, 17, 18, 19, -20, 21. It is obvious that what is being reported here is not a symbol for a phenotype or agglutinogen, but a protocol of reactions, so that this method of designation hardly qualifies to be called a nomenclature. Worse, the symbols are given in numbers which convey no meaning except to those who have the original article in hand so that they can consult its tables and " crack the secret code ". Yet Rosenfield et al. (11), as well as others, have published additional articles using this numerical code. It seems surprising that any editor would permit the publication of a scientific article in code numbers that are meaningless, except to those who have available the original article explaining the code, or even when this is available, it is not proper to require the reader to go to so much trouble in order to understand an article. The publication of articles in a private code should not be permitted in scientific journals; the reader is entitled to require the author of an article to take him into his confidence, and not address his article merely to those " in the know ". It is of interest to point out that while the use of numbers for the four A-B-O groups allowed of only 24 possibilities, of which two were actually proposed and used, for 21 Rh-Hr factors the number of possibilities is factorial 21, an astronomical number that dwarfs the national debt into significance.

Tab. 4. Comparison of the designations for phenotype Rh_1rh in three currently used nomenclatures

Wiener's Rh-Hr nomenclature	Fisher and Race's C-D-E notations*	Rosenfield's numbered notations †
Rh_1rh	1. + + + — or + — + +, etc.	Rh: 1, 2, —3, 4, 5,
	2. C+D+E—c+d?e+	6, 7, —8, —9,
	3. C+D+E—c+e+	—10, —11, 12, 13,
	4. CDe/cde or CDe. cde, etc.	14, 15, 16, 17,
	5. $\frac{CDe}{cde}$	18, 19, —20, 21
	6. CDe/cde CDe/cDe Cde/cDe	Suggested shorthand: R_1r
	7. CcDee	
	8. CDce	
	9. CcD or DCc	
	10. DCe/dce or DCd.dce, etc.	
	11. DCcee	
	12. D—Ccee, CcD—ee, etc.	
	13. CDe/ce, DCe/ce, etc.	
	14. CDe/c—e	
	15. CDeF/cdef, DCeF/dcef, etc.	
	16. CcDeef, etc.	
	17. CcDeefG, etc.	
	18. „ Shorthand " (sic!) R_1r	
	19. Ce/D/ce (Lauer)	

* Partial listing only.
† As of 1962; present approved listing not known.

Summary

An unusual case is described in which a man was accused of the paternity of a child, and an expert reported him to be type Rh_zRh_1, the mother as type Rh_1rh and the child as type Rh_2rh, thus excluding paternity. In repeat blood tests, the author confirmed the typing results on the putative father and the mother, but showed the child to be type Rh_zrh instead of type Rh_2rh, so that paternity was not excluded. Instead, the presence in both putative father and child of the rare gene R^z (or r^y) could be considered circumstantial evidence that the accused man actually was the father. It is pointed out that the error could easily have been due to the mistaken use of anti-**rh**$_i$ serum in place of anti-**rh**′ serum. Such an error would be more apt to occur with a worker using the fallacious C-D-E notations, because the tacit assumption implicit in those notations that each agglutinogen has but a single corresponding antibody (and blood factor) would render unthinkable the concept of more than one kind of anti-**rh**′ (anti-C) reagent.

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RIASSUNTO

Viene descritto l'insolito caso di un uomo accusato di essere il padre di un bambino, e di un esperto secondo il quale egli sarebbe stato Rh_zRh_1, la madre Rh_1rh ed il bambino Rh_2rh, escludendone in tal modo la paternità. Ripetendo le determinazioni, l'A. confermò i risultati riguardanti l'eventuale padre e la madre, ma dimostrò che il bambino era Rh_zrh, e non Rh_2rh, di modo che la paternità non veniva ad essere esclusa. Al contrario, la presenza, sia nell'eventuale padre che nel bambino, del raro gene R^z (o r^y) poteva essere considerata una prova della paternità dell'uomo. Si afferma che l'errore potrebbe facilmente essere dovuto ad un errato uso di siero anti-**rh$_1$** invece di anti-**rh′**. Un errore del genere potrebbe più facilmente verificarsi con un ricercatore che usasse le erronee notazioni C-D-E, in quanto il tacito presupposto, in esse implicito, che ciascun agglutinogene non ha che un solo anticorpo (e fattore sanguigno) corrispondente, renderebbe inconcepibile il concetto di più di un tipo di reagente anti-**rh′** (anti-C).

RÉSUMÉ

L'A. décrit le cas singulier d'un homme accusé d'être le père d'un enfant, et d'un expert d'après lequel il aurait été Rh_zRh_1 ,la mère Rh_1rh et l'enfant Rh_2rh, ce qui en excluait la paternité. Ayant répété les déterminations, l'A. confirma les résultats pour le père éventuel et la mère, mais démontra que l'enfant était du type Rh_zrh et non pas Rh_2rh, de façon que la paternité n'en était pas exclue. Par contre, la présence soit chez le père éventuel, soit chez l'enfant, du rare gène R^z (ou r^y) pouvait être considérée une preuve de la paternité. L'A. affirme que cette erreur pourrait être due à l'usage du sérum anti-$\mathbf{rh}_1$ au lieu du sérum anti-$\mathbf{rh}'$. Cette erreur aurait pu facilement se vérifier par l'usage des notations C-D-E, étant donné qu'elles entraînent l'implication que chaque agglutinogène n'a qu'un seul anticorps (et facteur sanguin) correspondant, ce qui rend inconcevable l'idée de plus qu'un type de réagent anti-$\mathbf{rh}'$ (anti-C).

ZUSAMMENFASSUNG

Beschreibung eines ungewöhnlichen Falles von einem Mann, der angeklagt ist, Vater eines Kindes zu sein und einem Sachverständigen, demgemäss der Vater Rh_zRh_1, die Mutter Rh_1rh und das Kind Rh_2rh gewesen sein sollte, wodurch die Vaterschaft auszuschliessen wäre. Verf. wiederholte die Blutgruppenbestimmungen und bestätigte die Ergebnisse für den angeblichen Vater und die Mutter, bewies jedoch, dass das Kind Rh_zrh und nicht Rh_2hr, wodurch die Vaterschaft nicht ausgeschlossen wäre. Im Gegenteil konnte das Vorhandensein des seltenen Gens R^z (oder r^y) sowohl bei dem angeblichen Vater als bei dem Kind als ein Beweis für die Vaterschaft des Mannes angesehen werden.

Verf. meint, der Irrtum liesse sich leicht durch den irrtümlichen Gebrauch des Anti-$\mathbf{rh}_1$-Serums austelle des Anti-$\mathbf{rh}'$-Serums erklären. Solch ein Irrtum könne leicht einem Untersucher unterlaufen, der die irrtümlichen Bezeichnungen C-D-E gebrauche, denn demzufolgen würde er von der Voraussetzung ausgehen, dass jedes Agglutinogen nur einen einzigen Antikörper (und Blutfaktor) besitze, wodurch das Konzept von mehr als einem Anti-$\mathbf{rh}'$- (Anti-C)-Reagenztyp unfassbar gemacht würde.