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Methods Available for Solving Medicolegal Problems of Disputed Parentage

Until recently, serologists confronted with a forensic problem of disputed parentage limited their tests to a few blood group systems only, especially the A-B-O system, M-N types, and Rh-Hr system. The tests were used mainly for providing evidence of exclusion of parentage, that is, if one or another of the blood groups of the respondent in a paternity action did not match with that of the child, the accused man could be excluded as father and exonerated of the charge of paternity by the court. If, however, the blood groups of the accused man matched, within the laws of heredity, those of the mother and child, the serological findings were interpreted as inconclusive. Because of the limited number of blood types such findings were not generally considered admissible by the court as evidence. There were occasional cases in which the accused man and the child shared a rare blood factor which the mother lacked; in such cases, the findings were usually considered circumstantial evidence though not absolute proof that the respondent was the father.

During the past decade there has been both remarkable progress in this field and a change in attitude of the courts. Not only has knowledge of the human blood groups markedly increased, but a number of other kinds of tests have been introduced, including so-called serum groups, leukocyte types, and isozymes. One recent report [*1*] lists as many as 50 systems that could be tested for when confronted with a problem of disputed paternity or maternity. In some European countries the serology expert is expected to carry out many of the newly developed tests, and when the tests fail to exclude paternity, the expert must report to the court the results of his calculations of the likelihood of the respondent being the father. To facilitate such calculations of likelihood of paternity, large volumes of logarithmic tables have been published [*2*].

Five previous reports [*3–7*] prepared by medicolegal committees required to investigate the forensic aspects of blood grouping tests for disputed paternity have been published in the *Journal of American Medical Association.* The last such report appeared almost 20 years ago, and a new committee (since disbanded) was formed to bring this subject up to date. The purpose of this report is to explain the principles of the tests and discuss the practical value and limitations, especially of the newly introduced tests, for the benefit of physicians, lawyers, and courts having need for them.

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Chances of Excluding Paternity

Assuming that the test which one plans to use gives clear, reproducible results and that the hereditary mechanism of the types defined is clear-cut, the usefulness of the test will depend mainly on the distribution of the types in the population from which the litigants derive. Thus, if the trait in question has a very high or very low frequency, the test will have hardly any value because of the limited degree of polymorphism; the individuals tested will usually either be all positive or all negative. Therefore, the formulas for the chances of excluding paternity are usually given in terms of the gene frequencies. For convenient reference, the principles underlying various formulas will be explained briefly here [*8,9*].

Gene frequencies can be calculated by one of two methods: square root formulas or direct count. (Calculations of square roots are no longer formidable since this is now readily carried out in a fraction of a second by using inexpensive pocket calculators.)

Consider first the case of a blood group system for which there is only a single reagent, anti-**D**, determining a character D transmitted by a corresponding dominant gene *D*. Then, there are two phenotypes and three corresponding genotypes, as shown below.

Phenotypes	Genotypes
D+	*DD* and *Dd*
D−	*dd*

If we designate the frequency of gene *D* by the symbol *p,* and the frequency of gene *d* by symbol *r,* it is obvious that, assuming random intermarriage, the frequency of the type D− in terms of gene frequencies must be r^2. Thus,

$$r = \sqrt{\bar{\mathrm{D}}-}$$

and

$$p = 1 - r = 1 - \sqrt{\bar{\mathrm{D}}-}$$

where the overbar indicates "frequency of."

For the heredity of the four A-B-O groups, one must postulate three allelic genes *O, A,* and *B,* to which may be assigned the gene frequencies *r, p,* and *q,* respectively. In this case, there are four phenotypes and six corresponding genotypes, the frequencies of which are given by the expanded trinomial $(p + q + r)^2$. The gene frequencies again are derived from the same square root formulas, since, for example,

$$p = 1 - \sqrt{\bar{A}-} = 1 - \sqrt{\bar{0} + \bar{B}}$$

Similarly,

$$q = 1 - \sqrt{\bar{B}-} = 1 - \sqrt{\bar{0} + \bar{A}}$$

and

$$r = \sqrt{\bar{0}}$$

The second method of calculating gene frequencies, which uses direct count, is applicable to systems having two contrasting, codominant characters, so that there are three phenotypes and three corresponding genotypes. Direct count is possible here because there is only a single genotype corresponding to each phenotype. For example, three Gc phenotypes and genotypes are given by the table below.

Phenotypes	Genotypes
Gc(1-1)	Gc^1Gc^1
Gc(1-2)	Gc^1Gc^2
Gc(2-2)	Gc^2Gc^2

If p is used to represent the frequency of gene Gc^1 and q the frequency of gene Gc^2, then the frequencies of the three phenotypes are given by the expanded binomial $(p + q)^2$. Then, it is readily seen by direct count that

$$p = \text{Gc(1-1)} + \tfrac{1}{2}\text{Gc(1-2)}$$

and

$$q = \text{Gc(2-2)} + \tfrac{1}{2}\text{Gc(1-2)}$$

The formulas for the chances of excluding paternity are readily derived in terms of the gene frequencies. In the case of a blood group system having only two types, D+ and D−, paternity will only be excluded when both the mother and the accused man are D− and the child is D+. Obviously, the frequency with which both mother and the falsely accused respondent will be D− is $r^2 \times r^2 = r^4$, while the chance that the child will be D+ depends on the frequency p of gene D derived from the actual father. Therefore, the chance of exclusion of a falsely accused man is pr^4.

Since $p = 1 - r$, the chance of exclusion is $r^4 - r^5$, and the maximum chances of exclusion are readily derived by applying the differential calculus. It is easy to show that the maximum chances of exclusion are 0.08192 or about 8.2%, when r equals 0.8 and p equals 0.2.

In practice, these maximum chances are often not achieved. For example, in the case of Kell types, the chances of exclusion for whites are only 3.5%. For blacks, who have a very low percentage of Kell-positive individuals, the exclusion rate is only 0.5%; for Chinese, who have hardly any Kell-positive individuals, the exclusion rate is virtually zero.

In the case of four A-B-O groups, the formula for the chances of excluding paternity can be derived similarly. For simplicity and to conserve space, the reader is referred to the literature for details [*10*]. The maximum chances of excluding paternity are about 20%, and tests for the subgroup of A increase the chances by only about 2 or 3%. Fortunately, the distribution of the A-B-O blood groups in most populations is favorable, so that the chances of exclusion are not much below the theoretical maximum.

A frequent situation is one involving a system with two contrasting, codominant alleles, having three phenotypes and three corresponding genotypes, as for the group specific component system Gc already cited here, or the haptoglobins. If, as usual, p and q represent the frequencies of two respective alleles, the chance P of excluding paternity is given by the formula below.

$$P = pq(1 - pq)$$

In this case the maximum chance of excluding paternity is 18.75%, namely, when $p = q = 0.5$.

It is necessary now to calculate the chances of excluding paternity when tests are done for more than one system. It is easy to see that the chances are not obtained by simply adding together the chances for each individual system, because there are cases of overlap in which the same man is excluded by more than one of the tests. The correct procedure is to determine the chances of nonexclusion for each system, and the product of these individual chances gives the chance that none of the tests will exclude an innocent man. Therefore, by subtracting this product from unity (or from 100%) one obtains the chance that at least one test will exclude a falsely accused man, as in the formula

$$P_E = 1 - (1 - P_1)(1 - P_2) \cdots (1 - P_n)$$

where P_E is the probability of exclusion, n is the number of independent systems tested for, and $P_1, P_2 \cdots P_n$ are the individual chances of exclusion for each system. It is not difficult to see that as more and more independent tests are included, the chances of excluding paternity become closer and closer to 100% (the ideal situation where every falsely accused man would be excluded, so that those not excluded must necessarily be the actual fathers) without achieving that goal. Unfortunately, attempts to raise the exclusion rate become less and less rewarding. For example, if the exclusion rate is already at the 90% level, and a new test is added which gives a 10% exclusion chance, the total chances of exclusion will be raised only to 91%, because $\frac{1}{10}$ of the men excluded by the new test will already have been excluded by one or more of the other tests. Thus, as more and more tests are added, and as the cost in money and effort inexorably increases, this additional effort becomes progressively less and less rewarding. Thus it becomes necessary to decide when further effort is worthwhile.

A more serious problem is that each test has a certain possibility of error, even in the hands of experts. To be sure, the chance of error with any individual test is usually quite small, but as more and more tests are added the chance increases that at least one of the tests may be in error. Table 1 lists an evaluation of the seriousness of that problem. In

TABLE 1—*Theoretical chances of exclusion of paternity and increasing rate of mistakes in testing corresponding to the number of systems used in forensic tests.*

No. of Systems	Chances of exclusion, %	Chances of error, %
1	15.0	0.5
2	27.75	1.0
3	38.59	1.5
4	47.80	1.99
5	55.63	2.48
6	62.29	2.97
7	67.95	3.46
8	72.76	3.94
9	76.85	4.42
10	80.32	4.90
12	85.78	5.85
14	89.73	6.79
16	92.58	7.72
18	94.64	8.64
20	96.13	9.55
25	98.29	11.80
30	99.25	13.99

this table it is assumed that a series of tests have been carried out for a series of independent systems, each with an exclusion rate of 15%, and a chance of error of 1 to 200. As shown in Table 1, by the time ten independent systems have been used the exclusion rate will have reached 80.3%, but the chance that one or more of the tests might be in error may be as great as 4.9%. By the time the number of tests has reached 20, the exclusion rate has reached 96.13%, but almost one in ten of the reports may be in error. Attempts to push the exclusion rate still higher brings one finally to a point where the exclusion rate goes up even more slowly than the chances of error. Table 1 represents an idealized situation which underestimates the chance of error by placing all tests on the same level of difficulty and reliability. When the A-B-O blood groups which are now taken for granted were the only tests used, there was a significant number of errors with resulting miscarriages of justice [*11*]. Obviously, the chances of error are greater with newly introduced tests which have not yet been perfected and standardized.

Individual Systems: Usefulness and Pitfalls

Blood Groups

By now more than a hundred different reagents have been described defining individual specificities of human red cells. The agglutinogens detected by those reagents fall into various blood group systems. Not all of these tests are of equal usefulness for problems of disputed parentage: first, some of the tests are poorly defined and not readily reproducible and second, most of the reagents are difficult to obtain and many of them are available only in the laboratories of their discoverers. In this section, we propose to indicate which tests are most valuable and reliable for application to forensic problems of disputed parentage and to point out special peculiarities and pitfalls of the tests.

A-B-O groups—These blood groups, known since the turn of the century, were the first to be discovered and applied and may be considered the most reliable. Their distribution in the general population is favorable, so they provide a substantial chance of excluding paternity—close to 20%. Most experts also test for subgroups of A, even though these are often incompletely developed in newborn babies. When dealing with older children, and when the findings are supported by tests with anti-**H** lectin, exclusion of paternity based on the subgroups of A are entirely reliable. The tests have also been used in problems of disputed maternity, as in kidnapping cases, and maternity is excluded if any of the following combinations is found.

Woman	Child
Group AB	Group 0
Group 0	Group AB
Subgroup A_1B	Subgroup A_2
Subgroup A_2	Subgroup A_1B

The application of the A-B-O blood groups depends on the assumption that these are always clearly defined and remain unchanged throughout life. Actually, this is not strictly correct. Reports have been published describing the change in the reactivity of red cells with blood typing reagents in the course of leukemia, such that the red cells of a person known to be group A may simulate group O in their reactions. However, this is hardly likely to be a pitfall in a medicolegal case, since a patient with leukemia has never, so far, been involved in a paternity case. Another pitfall of similar kind is so-called acquired B,

in which the red cells of a person of subgroup A_1 reacts as A_1B due to the acquisition by the red cells of a weak B reactivity from colon bacteria in patients with carcinoma of the bowel or colitis. Such cases, again, are readily recognized by tests on the individual's serum for the presence of isoagglutinins (anti-**B** is present) as well as by the atypical nature of the reactions of the red cells with anti-**B** reagents. Similar reactions are given by the blood of people having the extremely rare genetic type cis-AB in which parents reacting as group AB can have children of group O, or vice versa.

A blood group of considerable theoretical significance but of little practical importance because of its extreme rarity is the so-called Bombay type. In such individuals homozygosity for an extremely rare suppressor gene causes failure of development of the A, B, and H antigens, both on the red cell surface and in the secretions. If such blood is mistyped as group O, false exclusion of parentage could result, but the true situation is easily recognized if complete testing of red cells and serum is carried out.

In addition to the subgroups A_1 and A_2, a series of graded variations of the agglutinogen A have been described. Of these the most interesting and important is the agglutinogen A_3, characterized by a mixed field agglutination picture of the red cells (clumps on the background of unagglutinated cells) in tests with anti-**A** reagents. This anomaly of blood typing is easily recognized by complete typing of red cells and serum, as is the error of mistyping group A_2B blood as group B. Because of the extreme rarity of the subgroup A_3, the simultaneous presence of this type in a child and his putative father could be considered strong circumstantial evidence, though not absolute proof, of paternity. As for the rare blood type characterized by failure of the red cells to agglutinate with ordinary anti-**A** and anti-**B** reagents despite virtual regular agglutination by group O serum, the various designations of A_4, A_x, A_0, and A_z should all be discarded and replaced by the designation, group C, because of the reactivity of the red cells with the anti-**C** agglutinin present in the serum of group O individuals only [*12*]. As in the case of agglutinogen A_3, the anomaly is easily recognized, and the presence of the rare blood type in putative parent and child would be strong circumstantial evidence of parentage, even though it appears that there is more than one hereditary mechanism for this rare blood type.

Variants of the agglutinogen B have also been described, including an agglutinogen B_3 having properties analogous to those of agglutinogen A_3.

At one time, when serology experts introduced evidence regarding the A-B-O blood groups into the courtroom excluding paternity, the alert defense attorney would at times invoke the possibility of mutation, even though this has an estimated frequency of only about one in 40 000. It is evident that the defense attorney now has much more ammunition he can use, but the competent serology expert should have no difficulty in rebutting such arguments.

A-B-H Secretor Types—Despite the clear-cut nature of the heredity of this characteristic as a simple Mendelian dominant and the high reproducibility of the test, these tests are only rarely used in paternity disputes. The reason is that the collection of the saliva samples is troublesome and time consuming, especially from infants and younger children. Moreover, the tests themselves are time consuming and must be carried out by the titration method to yield reliable results. Therefore, considering the low rate of exclusion[3] by such tests, they are not sufficiently rewarding in proportion to the cost in time and effort.

It has come to our attention that some workers who claim to be experts in the field are totally unaware that immediately after collection, the saliva specimens should be placed in a boiling water bath for 20 minutes to destroy bacteria and blood group enzymes; such boiled saliva may be stored in a refrigerator and tested later at one's

[3] In whites and blacks the frequency of nonsecretors is about 25%, giving the chances of exclusion as $pr^4 = (0.5)\ (0.5)^4 = 0.03125$, or only 3.1%. In Mongoloid populations, which have no nonsecretors, the test is of course entirely useless.

leisure. In a recent homicide case, where group O semen was found at the scene of the crime, the "expert" retained by the defense attorney asserted that the group O defendant could not be the real criminal because their test on unboiled saliva shipped to them through the mail showed the defendant to be a nonsecretor. Actually, tests on properly prepared saliva proved that the accused man was a group O secretor.

M-N-S-s Types—All experts test routinely for the three M-N types defined by anti-**M** and anti-**N** reagents, while tests for the S-s-U antigens are not routinely done in this country because of the unavailability of potent reagents of specificities anti-**s** and anti-**U**.

Tests for the three M-N types are very much worthwhile since the distribution of these types in most populations is favorable, yielding an exclusion rate close to the maximum possible of 18.75% for systems determined by a pair of codominant allelic genes. The reagents ordinarily used are prepared from rabbit immune serums and give clear-cut and readily reproducible reactions with only rare exceptions. Nevertheless, errors continue to occur in tests done by experts designated by our American courts, most often due to the use of imperfect anti-**N** reagents. All human red cells appear to have an N-like specificity, even those of type M, so that when the anti-**N** reagents are prepared from the raw immune rabbit serums there is a tendency to overabsorb them, yielding reagents giving false negative reactions. When attempts are made to avoid this pitfall, the antiserum may be underabsorbed with resulting false positive reactions.

Since all human red cells have N-like agglutinogens, it is evident that the original theory of Landsteiner and Levine [*13*] of two contrasting alleles, *M* and *N*, cannot be correct. Instead, based primarily on tests for homologues of M and N in nonhuman primates, and based on certain biochemical findings, it has now been proposed that the genes *M* and *N* are inherited independently of one another, but that N is related to **M**, as **H** is related to A and B agglutinogens. Table 2 compares the original [*13*] and new [*14*] theories of heredity of the three M-N types.

TABLE 2—*Comparison of two theories of the inheritance of the three M-N types.*

Phenotypes	Genotypes	
	Original Theory	New Theory
M	*MM*	*MM NN*
MN	*MN*	*Mm NN*
N	*NN*	*mm NN*

Although the difference between the two theories is of considerable theoretical significance, it is easy to see that both theories yield the same results as far as problems of parentage are concerned. Therefore, it still remains the practice to use the less cumbersome symbols of the original theory, bearing in mind that this is done only to facilitate communication. To the expert, knowledge of the newer theory is essential for insight into the cause for limitations and difficulties provided by anti-**N** reagents; the situation can be ameliorated by the use of anti-**N** lectins prepared from seeds of *Vicia graminea* or leaves of the Korean *V. unijuga*. The latter reagents are particularly useful for identifying the rare type MN_2 which is most prevalent in Chinese and which has not infrequently been misdiagnosed as type M because of its weak reactions with anti-**N** reagents, with resulting false exclusion of parentage. The existence and explanation of certain other rare apparent exceptions to the rule that type M parents cannot have type N children will be discussed in connection with factors **S** and **s**.

Where feasible, tests for the S-s types should be carried out because the three S-s types, SS, Ss, and ss, behave as if they are transmitted by codominant allelic genes *S* and *s* and are thus comparable in usefulness to the three M-N types. The S-s and M-N types are closely associated and family studies indicate that they are transmitted together by four allelic gene couplets, *MS, Ms, NS, Ns,* respectively, or what amounts to the same thing, by four allelic genes. It was originally believed that just as type M parents do not have type N children or vice versa, similarly type SS parents cannot have type ss children, or vice versa. However, this had to be modified after the agglutinogen U was discovered; this agglutinogen is present in all whites but absent in a small percentage of blacks. It was soon realized that U was part of the M-N-S-s system when first the association between U and N was noticed, and especially when it was later found that all blacks who were U negative also lacked both S and s, a type not previously known to exist.

Apparently, U is associated with both S and s, though there are certain very rare individuals who lack both S and s and who are, nevertheless, U positive. At any rate, it is clear that while the rule that type SS parents cannot have type ss children remains valid for whites, it does not always hold for blacks, for example, when a parent is genotype *Su* and the child genotype *su*, or vice versa. Similarly, it is now known that there are very rare exceptions to the rule that type M parents cannot have type N children, namely, when the parent is genotype MM^g and the child genotype NM^g, or vice versa, where the extremely rare gene M^g determines an agglutinogen lacking both the specificities **M** and **N** and having in their place the specificity $\mathbf{M^g}$. Since such cases are so extremely rare it remains the practice to exclude paternity when the accused man is found to be type M and the child type N or vice versa, but if for any reason the possibility must be considered that the case may be one of the rare exceptions, tests with anti-$\mathbf{M^g}$ serum can readily resolve the problem. Anti-$\mathbf{M^g}$ is not easily available and therefore is not used routinely; it can be found as a natural isoagglutinin in the serum of a small percentage of nonimmunized individuals.

By now a great multiplicity of antigens and specificities related to the M-N-S-s system have been discovered. Most of these have not proved to be relevant or useful for medicolegal application, mainly because the reagents are not readily available and insufficient studies have been carried out. Obviously, the knowledgeable attorney can pose any number of embarrassing questions, so that the expert must be thoroughly versed in all aspects of this seemingly simple, but actually highly complex, subject.

Rh-Hr System—This is probably the most important and interesting blood group system from the point of view of forensic serology because of its complexity and the high frequency of exclusions of falsely accused men. By now, more than 30 different specificities have been defined, but for routine testing in forensic cases usually only five of these reagents are used, anti-Rh_o, anti-**rh**′, anti-**rh**″, anti-**hr**′ and anti-**hr**″, though in special cases other reagents are needed, especially anti-**hr**. The seemingly complex serology and genetics are readily mastered when one keeps in mind the central position in the system of the factor Rh_o and the natural arrangement of the phenotypes and genotypes when preparing tables. For example, the eight principal allelic genes are arranged naturally in the order, *r, r′, r″, r^y, R^o, R^1, R^2,* and R^z. Tables of genotypes and phenotypes are readily constructed and need not be memorized since each element of the table falls naturally into its place. To be sure, as for the A-B-O blood groups, other methods of designating the Rh-Hr types have been proposed but this problem has been disposed of in previous reports published in the *Journal of American Medical Association* [*5,6*] and need not concern us further here.

Because of the large number of phenotypes defined by the tests, the chances of excluding paternity using the Rh-Hr tests alone is as high as 25%. The unequal distribution of types causes complications and may be a pitfall when carrying out the tests. Thus, two reagents of two different specificities may seem to be the same if they give parallel

reactions on the more common types but differ in their reactions on the rarer types. For example, the alleles r^y and R^z are very rare, except among some Mongoloid populations, and this has caused confusion and error in actual cases of disputed paternity. Examples of pairs of reagents which have been confused with each other are shown in Table 3. The most common is confusion of anti-**rh**$_i$ with the more standard useful reagent, anti-**rh**′ since many of the commercially available antiserums labeled as anti-**rh**′ are actually of specificity anti-**rh**$_i$. In one of the most common type of cases the putative father is typed as Rh_zRh_1, the mother is typed Rh_2rh, and the child also is typed Rh_2rh. The expert then asserts that paternity is excluded because the accused man is hr′-negative, and, therefore, presumably homozygous for factor **rh**′ and unable to have a child who is rh′ negative. However, proper tests will show that the child is actually rh′ positive but of type Rh_zrh, so that the child's red cells react with true anti-**rh**′ reagents but not with anti-**rh**$_i$.

TABLE 3—*Pairs of Rh-Hr antiserums which have been confused with each other.*

		Reactions of Antiserums									
		1st Pair		2nd Pair		3rd Pair		4th Pair		5th Pair	
Allelic Gene[a]	Corresponding Agglutinogen	Anti-**rh**′	Anti-**rh**$_i$	Anti-**rh**″	Anti-**rh**$_{ii}$	Anti-**hr**′	Anti-**rh**″+ Anti-**hr**′	Anti-**hr**″	Anti-**rh**′ + Anti-**hr**″	Anti-**hr**	Anti-Hr$_0$[b]
r	rh	−	−	−	−	+	+	+	+	+	+
r′	rh′	+	+	−	−	−	−	+	+	−	+
r″	rh″	−	−	+	+	+	+	−	−	−	+
r^y	rh^y	+	−	+	−	−	+	−	+	−	+
R^0	Rh_0	−	−	−	−	+	+	+	+	+	−
R^1	Rh_1	+	+	−	−	−	−	+	+	−	−
R^2	Rh_2	−	−	+	+	+	+	−	−	−	−
R^z	Rh_z	+	−	+	−	−	+	−	+	−	−

[a] R^y and R^z are very rare in whites and blacks and less rare in Monogoloids, r' and r'' are also rare in all populations; R^0 is uncommon in whites but common in blacks.
[b] Hypothetical.

In the case cited, not only do the correct results fail to exclude paternity, but they provide strong circumstantial evidence that the accused man actually is the father since he shares with the child a very rare gene, R^z (or r^y), which the child's mother lacks.

A somewhat similar example was the false claim of the discovery of so-called anti-d serums, the existence of which had been predicted by Fisher and Race (cited in Ref *15*). Actually, the hypothetical anti-d (anti-**Hr**$_0$) has never been shown to exist, and in most cases the antibody mistaken for anti-d (**Hr**$_0$) was probably anti-**hr** which among whites, but not blacks, gives reactions similar to those expected of the hypothetical anti-**Hr**$_0$ (Table 3).

Among the other reagents that have been proved to be most useful are those of specificities anti-**hr** and anti-**rh**^{w}. By testing with anti-**hr** reagent, the common phenotype Rh_zRh_0 and the rare phenotype rh_yrh can both be subdivided into two types, as follows:

Major Phenotype	Subtype	hr Reaction	Corresponding Genotypes
Rh_zRh_o	Rh_zrh	+	R^zr, R^zR^o, and R^or^y
	Rh_1Rh_2	−	R^1R^2, R^1r'', and R^2r'
rh_yrh	rh_yrh (proper)	+	r^yr
	rh′rh″	−	$r'r''$

The important fact to bear in mind is that of the two subtypes of phenotype Rh_zRh_o, type Rh_1Rh_2 is quite common while type Rh_zrh is rare. In contrast, the relative frequencies of the rare subtypes rh′rh″ and rh_yrh are about equal. Therefore, in a case where, for example, the putative father is type Rh_1Rh_1, the mother is type Rh_zRh_o, and the child is type Rh_1rh, paternity would be excluded except in the unusual event that the mother is subtype Rh_zrh instead of the far more common subtype Rh_1Rh_2. This problem can easily be resolved by testing the mother's blood with anti-**hr** serum, since, if in conformity with expectation, the mother proves to be hr-negative, paternity can be excluded with certainty. If desired, such cases can be further strengthened by tests with anti-$\mathbf{rh_i}$ reagents, since the mother's blood would be expected to be rh_i positive as well as rh′ positive.

Ordinarily, paternity is considered to be excluded if the accused man is rh′ negative and the child is hr′ negative or vice versa, or if the accused man is rh″ negative and the child hr″ negative or vice versa, because of the reciprocal relationship between the blood factor pairs rh′-hr′ and rh″-hr″. However, a number of very rare Rh-Hr allelic genes have been discovered which are characterized by having as gene products agglutinogens lacking one or both of the factor pairs rh′-hr′ and rh″-rh″. Therefore, if for example the accused man is type Rh_1Rh_1 and the child in question is Rh_2Rh_2, paternity is not necessarily excluded because the man could be genotype $R^1\overline{\overline{R}}^o$ and the child genotype $R^2\overline{\overline{R}}^o$. While as a rule this possibility may be disregarded as being too far-fetched because of extreme rarity of genes such as $\overline{\overline{R}}^o$ and $\overline{\overline{r}}$, there are occasions where this possibility must be taken into account. For example, in an immigration case one of us (A. S. W.) was able to show the presence of the $\overline{\overline{R}}^o$ gene when a study on the family showed its transmission through three generations, and in this way a possible miscarriage of justice was avoided [*16*].

If suitable reagents are available, tests for $\mathbf{rh^{w1}}$ may occasionally yield very useful information. For example, where the mother lacks the agglutinogen Rh_1, while the child and putative father both have agglutinogen Rh_1, the presence of the associated specificity $\mathbf{rh^{w1}}$ in the man or child but not in both would serve to exclude paternity, while the presence of the specificity in both would be strong circumstantial evidence, though not absolute proof, of paternity because of the low frequency of the specificity $\mathbf{rh^{w1}}$ in the general population.

Of the multiplicity of additional Rh-Hr specificities which have been described in the literature, probably the most important and interesting are the cognates of $\mathbf{Rh_o}$, the numerous specificities $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, $\mathbf{Rh^C}$, $\mathbf{Rh^D}$, and so forth closely associated with the agglutinogen Rh_o. While these factors are occasionally of clinical importance, the rarity of the atypical blood types involving those specificities renders them of little forensic importance.

Kell Blood Types—Recent observations have shown this system to include as many as 13 serological specificities, so that it has a complexity approaching those of the M-N-S-s and Rh-Hr systems. However, for various reasons, only one of these specificities is actually being tested for in forensic cases of disputed paternity. This specificity **K** has the

frequency of about 9% among whites, so that the chance of excluding paternity by the Kell test is only about 3.8%. Since the agglutinogen K is much less frequent in blacks and virtually absent in Chinese and other Mongoloids, the test has practically no value at all in those races. As for the contrasting specificity **k** it has a frequency of close to 99.8%, and when tests are done with anti-**k** as well as anti-**K** so as to define three phenotypes, KK, Kk, and kk, the chance of exclusion is raised by only less than 0.5%, from 3.8 to 4.2%. Thus, as expected, there is little or nothing to be gained by testing with anti-**k** because it detects a specificity lacking in only 1 of 500 individuals.

Very rare are individuals of so-called type K_o, with red cells lacking both specificities **K** and **k**, and there is also an antibody called anti-**Ku** because of its almost universal activity for human red cells except those of type K_o. Blood of type K_o also lacks the two contrasting pairs of blood factors, Kp^a-Kp^b and Js^a-Js^b; in fact, this observation is part of the evidence that those four factors are part of the Kell system. More recently a series of blood factors have been described with the common property that their corresponding antiserums react on all other red cells except those of the individuals themselves and red cells of type K_o. Thus, these newer specificities are all cognate blood factors of **Ku**, shared by both agglutinogens K and k, and analogous to the cognate factors of $\mathbf{Rh_o}$ of the Rh-Hr system. None of these newly discovered Kell specificities have found application in problems of the disputed parentage, because of either unfavorable distribution or lack of reagents giving clear-cut, easily reproducible reactions. In summary, therefore, despite its complexity, the Kell blood system offers forensic application for only single specificity **K** with a chance for exclusion of paternity of only about 3.8% in whites, and practically none for other races.

P Blood Groups—The agglutinogen P appears to be identical with the so-called agglutinogen Q described by Japanese workers (cited in Ref *8*); in this article only the symbol P will be used. Individuals of the very rare type Tj^a-negative produce an antibody anti-$\mathbf{Tj^a}$ reactive for red cells of all other individuals. Since it has been found that absorption of anti-$\mathbf{Tj^a}$ serum with P-negative red cells leaves behind a fraction reactive for P-positive but not P-negative red cells, it is evident that Tj^a is part of the P blood groups system and the blood types have therefore been renamed as shown below.

Original Designation	Present Designation	Comment
P-positive	P_1	
P-negative	P_2	serum may contain anti-$\mathbf{P_1}$
Tj^a-negative (very rare)	p	serum may contain anti-$\mathbf{P_1}$ + **P**

Thus, the serum originally designated anti-**P** is now called anti-$\mathbf{P_1}$ while anti-$\mathbf{Tj^a}$ is called anti-**P**. There are three P types analogous to the A_1-A_2 subgroups except that type p is extremely rare. Thus, tests with the very rare anti-**P** (anti-$\mathbf{Tj^a}$) serum contribute virtually nothing to the exclusion rate because they detect a very high frequency factor. For practical purposes, therefore, tests are limited to the reagent anti-$\mathbf{P_1}$, and paternity is excluded when the mother and the putative father are both type P_2 while the child is type P_1.

Extremely rare individuals have also been found whose blood resembles those of the rare type p, judging from the antibodies that their serums contained, except that the red

cells are agglutinated by most serums containing anti-**P** antibodies. These reactions have been ascribed to a so-called anti-$\mathbf{P^k}$ antibody in the serums and a corresponding agglutinogen P^k on the red cells. However, one of us (A. S. W.) maintains that it is unnecessary to invoke a special P^k antigen and antibody and prefers to interpret the reactions observed as due to the presence on the red cells of a very rare agglutinogen having only the specificity $\mathbf{P_1}$ but lacking specificity **P** ($\mathbf{Tj^a}$).

Since about 21% of whites are P-negative, the chance of excluding paternity by the P test is about 2.5%. Among Japanese and Chinese in whom the frequency of P-negative is higher, the chances may reach as high as 8%; whereas for blacks, with the P-negative type of much lower frequency, the test has virtually no value.

Although some European workers carry out P tests routinely in cases of disputed parentage, the present authors do not consider the test reliable enough for routine use. The reagents generally react only at low temperature, and are, as a rule, of only low titer; thus, it is difficult to free the reagents entirely of nonspecific cold autoagglutinins. Since, in addition, there are individuals whose red cells give reactions of intermediate intensity with anti-$\mathbf{P_1}$ reagents, it is not always possible to draw a sharp line between positive and negative reactions.

Duffy Types—Two antibodies, anti-$\mathbf{Fy^a}$ and anti-$\mathbf{Fy^b}$, define two contrasting specificities which determine three blood types designated Fy(a+b−), Fy(a+b+), and Fy(a−b+). If one assumes that the situation is comparable to that for the M-N types, the Duffy types would offer about the same chance of excluding paternity because of their favorable distribution. However, occasionally individuals of type Fy(a−b−) have been encountered, more often in blacks, and the existence of the silent allele *fy* reduces somewhat the chances of exclusion.

Many European experts test for $\mathbf{Fy^a}$ and $\mathbf{Fy^b}$ routinely. However, reagents of satisfactory potency and specificity are not readily available; the tests must be done by antiglobulin method and therefore cannot be used for testing individuals having red cells coated with autoantibodies, such as patients with autoantibody disease.

Kidd Types—Analogous to the situation for the Duffy system, there are two anti-serums, anti-$\mathbf{Jk^a}$ and anti-$\mathbf{Jk^b}$, which determine three blood types, namely, Jk(a+b−), Jk(a+b+), and Jk(a−b+). The findings in family studies fit the expectations for the inheritance by a pair of contrasting alleles, Jk^a and Jk^b. Moreover, the distribution of the types is favorable so that the chances of exclusion of paternity are close to the theoretical maximum of about 18%. However, the interpretation of the findings must be qualified because there are rare individuals of type Jk(a−b−), pointing to the rare occurrence of an amorph gene *jk*.

While many European investigators do test for the Kidd types routinely, the reliability of the tests is limited by the peculiar nature of the reactions which depend on the presence of complement. Potent and specific reagents are difficult to obtain, and the test must be done by the antiglobulin method in the presence of complement. Obviously, such tests should not be undertaken by serologists with limited experience with the peculiarities of the reactions.

Xg^a Types—These blood types are unique in that they are hereditarily transmitted by a dominant sex-linked gene located on the X chromosome. Therefore, Xg^a typing can be used for excluding paternity for baby girls but not for baby boys. As far as male children are concerned, the test can be used to exclude maternity if the supposed mother proves to be Xg^a-negative while the baby boy is Xg^a-positive. In the case of female children, the Xg^a test can be used to exclude paternity under two conditions: if the supposed father is Xg^a positive while the female child is Xg^a negative, or when the female child is Xg^a positive while her mother and putative father are both Xg^a negative [*15*].

If, as usual, p is used to represent the frequency of the dominant allele Xg^a and r the

frequency of the amorph gene *xg*, the chance *P* of excluding paternity by the Xg^a test is given by the following formula:

$$P = pr^2 + pr^3$$

Therefore, applying the differential calculus, the maximum chance of excluding paternity can be shown to be 25%, when $r = \frac{1}{2}\sqrt{2}$ or 0.7071, and $p = 0.2929$.

Since this test for paternity is applicable only to female children, the maximum chances of exclusion for *all* children are half as high, or about 12.5% [*17*].

In summary, tests for Xg^a agglutinogen would be very much worthwhile for problems of disputed paternity involving female children but are useless for such problems involving male children. The main difficulty is that potent and specific anti-$\mathbf{Xg^a}$ reagents are not readily available, except in the laboratories of the discoverers of this blood type.

Other Blood Groups—Numerous other blood types have been discovered, so that more than a hundred blood group specificities have so far been defined. In most cases, the reagents are available only in the laboratories of their discoverers; in other cases the specificities defined are either of extremely high or very low frequencies and therefore offer little chance for exclusion of paternity. A striking example is the sharply defined Vel negative blood type which is shared by all but 1 among about 6 000 individuals.

An interesting blood type is the so-called Diego blood type. In this case, two contrasting blood factors have been found, Di^a and Di^b. The agglutinogen Di^a is practically limited to individuals of Mongoloid origin, among whom it may attain frequencies exceeding 10%. Tests for Di^a are therefore useful only for cases involving Mongoloids, for whom the chance of excluding paternity would be about 5%, or for resolving problems of suspected Mongoloid-white racial mixtures. Tests for Di^b have practically no value because of the extremely high frequency of the antigen Di^b. At any rate, the very limited availability of suitable reagents precludes the routine use of tests for the Diego types.

As for the application of blood grouping tests for determining the probable racial origin of individuals, as in paternity disputes and inheritance cases, the usefulness of blood grouping tests is limited. In exceptional cases, the demonstration of the presence of the specificity **Henshaw (He)** on the red cells or the absence of **U** would point to a Negroid origin, since those types are virtually limited to blacks. In contrast, the combination type rh with P-negative would strongly point to a white derivation because type rh is extremely rare in Mongoloids, while the P-negative is quite rare in blacks. Finally, as pointed out above, the demonstration of the presence of $\mathbf{Di^a}$ would point strongly to a Mongoloid derivation of the individual.

Serum Groups

The possibilities of excluding paternity have been increased considerably by discovery of the so-called serum groups [*18,19*], defined by individual difference in serum proteins. These serum differences are usually demonstrated by precipitation tests, analogous to the agglutination tests used for demonstrating red cell groups. The tests in question are carried out by a variety of techniques including immunodiffusion and immunoelectrophoresis. Haptoglobin types, hemoglobins, transferrin types, and albumin variants are defined by electrophoresis without involving immunological techniques. The Gm and Inv serum groups are unique in that they are detected by hemagglutination inhibition tests.

Haptoglobin Types

These were among the first serum types discovered after the introduction of starch gel electrophoresis as a diagnostic tool in clinical medicine. The types are determined by

subjecting serum to electrophoresis, preferably on a starch gel, then applying a stain containing benzidine or other comparable chemical, and developing a color by adding hydrogen peroxide. Three main types are usually distinguishable, and according to the genetic theory these are determined by two codominant allelic genes, Hp^1 and Hp^2. Thus, corresponding to three possible genotypes, there are three demonstrable phenotypes, designated Hp(1-1), Hp(1-2), and Hp(2-2). The situation is genetically comparable to that for the three M-N blood types and the favorable distribution of the phenotypes makes the tests very much worthwhile for application to problems of disputed paternity.

The existence of the haptoglobin types was discovered as long ago as 1955, and in the intervening 20 years considerable information and experience have been accumulated regarding the types. However, the number of families tested is small compared to those tested for the blood groups. Another difficulty is the incomplete development of the haptoglobin type in the newborn; in fact, only about 10% of newborn can be typed and the percentage gradually rises until the age of four to six months, when the haptoglobin types are fully developed. The situation is further complicated by the existence of variants; for example, the Hp^1 allele has been subdivided into two kinds, Hp^1F and Hp^1S, so designated to indicate that the haptoglobin determined by the former has a faster mobility than the haptoglobin determined by the latter. Still other variants have been described of the Hp^2 allele. A more serious complication is the existence of rare individuals with hereditary ahaptoglobinemia, apparently homozygous for an amorph gene Hp^o, analogous to the genes $\overline{\overline{R}}^o$ and $\overline{\overline{r}}$ of the Rh-Hr system and K^o of the Kell system. Thus, when a putative father is type Hp(1-1) and the child type Hp(2-2) or vice versa, parentage is not necessarily excluded, assuming that the man and child are both carriers of the rare gene Hp^o. Finally, in certain diseases the individuals may have an acquired form of ahaptoglobinemia, and obviously the tests cannot be applied under conditions such as when the child is still too young for reliable testing.

It is apparent that tests for haptoglobins are less reliable than tests for the standard blood groups for problems of disputed parentage because of the complications outlined above. However, the tests are being applied by European experts, because they are relatively simple to carry out, and the findings can prove helpful, provided that one recognizes their limitations.

Serum Immunoglobulin Types

These serum types are unique because of the vast amount of information that has accumulated since the first such specificity was described in 1956. Also, the serological method of testing is unusual; the hemagglutination inhibition test is somewhat comparable to that used for determining the A-B-H and Lewis saliva types. In the case of immunoglobulin types, however, the indicators used are group O Rh-positive human cells coated with immunoglobulins from an anti-Rh_0 serum. The serum used for coating the red cells must be derived from a sensitized Rh-negative person of known Gm type, whose antibodies are of the nonagglutinating IgG variety. It is necessary also to have an antiserum containing antibodies for one of the Gm factors present in the anti-Rh_0 serum used for coating. Such antiserums can be prepared by immunizing monkeys or rabbits, but the preferred reagents are from normal human beings. The inhibition test is carried out by mixing the serum to be tested with the antiserum, e.g., anti-Gm(1) and then adding the indicator cells coated with anti-Rh_0 from a donor of the homologous Gm type, in this case Gm(1). If no agglutination occurs, the unknown serum contains the Gm antigen in question, Gm(1), while the occurrence of agglutination indicates the absence of Gm(1) or the Gm factor tested.

The multiple immunoglobulin specificities that have been identified to date fall into two systems, of which the most important are the so-called Gm and Inv systems, com-

parable in their genetic behavior to the blood group systems. The Gm system is of greatest theoretical and practical importance because of the large amount of knowledge and understanding that has accumulated regarding these types. As many as 23 Gm specificities have been found, and, remarkably, the chemical basis for these specificities has been established by inference from amino-acid sequencing of Bence-Jones proteins from patients who have myeloma, the Gm specificities of which were determined. This was possible because of the monoclonal origin of most myeloma proteins, in contrast to the heterogeneity of the immunoglobulins in the serums of normal individuals. In this way, it has been possible to pinpoint the location on immunoglobulin molecule chains of the determinants responsible for all the numerous Gm specificities, as shown in Table 4.

TABLE 4—*Specificities of the immunoglobulin systems.*

WHO Number	Original Designation	Location of Antigen
Gm		
1	a	γG1 (Fc)
2	x	γG1 (Fc)
3	$b^w = b^2$	γG1 (Fd)
4	f	γG1 (Fd)
5	$b = b^1$	γG3 (Fc)
6	$c = c^5$ Gm-like	γG3 (Fc)
7	r	γG1 (Fc)
8	e	γG1 (Fc)
9	p	γG1 (Fc)
10	α	γG3 (Fc)
11	$B = b^0$	γG3 (Fc)
12	γ	γG3 (Fc)
13	b^3	γG3 (Fc)
14	b^4	γG3 (Fc)
15	s	γG3 (Fc)
16	t	γG3 (Fc)
17	z	γG1 (Fd)
18	Ronen 2	γG1 (Fc)
19	Ronen 3	γG3 (Fc)
20	20	γG1 (Fc)
21	g	γG3 (Fc)
22	y	γG1 (Fc)
23	n	γG2 (Fc)
...	b^5	γG3 (Fc)
...	c^3	γG3 (Fc)
Inv		
1	1	Kappa chain
2	a	Kappa chain
3	b	Kappa chain
Am	...	γA_2
Isf	...	γG1 (Fc)

Most of the Gm specificities are located on the immunoglobulin of the γG1 class, others are on molecules of the γG3 class, and only one, Gm(23), on molecules of the γG2 class. Moreover, in all but three cases the determinants have been located in the Fc portion of the molecule, while the remaining three determinants have been shown to be in the Fd portion. As can be seen from Table 4, the nomenclature of the Gm specificities is a vexing problem, and it has not been possible to work out a neat system of symbols comparable to those for the Rh-Hr types. The recommendation of the World Health Organization (WHO) to number the Gm specificities in the order of their discovery had to be adopted as the last resort, and this constitutes a confession of one's lack of insight into the problem. Despite this difficulty, certain facts and regularities have emerged (Table 5).

TABLE 5—*Gm alleles in various races.*

Race	Alleles	Gm Specificities and Their Locations on the Immunoglobulin Molecules		
		γG1	γG3	γG2
Caucasian	$Gm^{3,4,5,11,13,14,22,23}$	3, 4	5, 11, 13, 14, 22	23
	$Gm^{3,4,5,11,13,14,22}$	3, 4	5, 11, 13, 14, 22	...
	$Gm^{1,17,21}$	1, 17	21	...
	$Gm^{1,2,17,21}$	1, 2, 17	21	...
Negro	$Gm^{1,5,11,13,14,17}$	1, 17	5, 11, 13, 14	...
	$Gm^{1,5,6,11,17}$	1, 17	5, 6, 11	...
	$Gm^{1,5,6,11,14,17}$	1, 17	5, 6, 11, 14	...
	$Gm^{1,11,13,17}$	1, 17	11, 13	...
Mongolid	$Gm^{1,11,13,15,16,17}$	1, 17	11, 13, 15, 16	...
	$Gm^{1,3,4,5,11,13,14,22,23}$	1, 3, 4, 22	5, 11, 13, 14	23
	$Gm^{1,2,17,21}$	1, 2, 17, 21	...	...
	$Gm^{1,17,21}$	1, 17, 21	...	...

Two principal genetic theories have been proposed to account for the heredity of the Gm types. According to the first theory, there are series of allelic genes, with each allele having a product characterized by multiple Gm specificities, analogous to the multiple allele theory for the Rh-Hr blood types in which each allelic gene determines a corresponding agglutinogen with multiple serological specificities. The alternative theory postulates the various alleles consist of so-called cistrons, each having three subloci corresponding to the three gamma G classes, γG1, γG2, and γG3, on which the various Gm determinants are located. Fortunately, for practical purposes, the consequences of both theories are the same, though it would be of theoretical value if convincing evidence could be adduced to resolve this question.

In general, each human race has at least four allelic Gm genes (Table 5), and aside from the alleles $Gm^{1,2,17,21}$ and $Gm^{1,17,21}$ which are shared by whites and Mongoloids, the sets of alleles in all three races are different. This makes the Gm tests valuable for determining an individual's racial origin as well as for solving problems of disputed parentage. The tendency of certain Gm specificities to be closely associated, such as Gm(3) with Gm(4) and Gm(1) with Gm(17), suggests that there may have been duplication when supposedly different Gm specificities were numbered, or points to the existence of associations among the specificities comparable to those of the cognate factors of Rh_0 of the Rh-Hr system. In practice, testing for all of the set of such associated specificities would have little or no practical value for resolving problems of disputed parentage because they make little or no contribution to the polymorphism detected. For this reason, as well as for the lack of availability of appropriate testing reagents, in practice Gm testing has usually been limited to only two or three of the numerous specificities, namely Gm(1), Gm(2), and Gm(5). This, of course, considerably reduces the usefulness of the tests, which obviously have a great potential.

Family studies support the multiple allele theory for the Gm types as given in Table 5. However, one rare family has been found in which the father was Gm(1,21), the mother Gm(3,5,13,14), three children type Gm(3,5,13,14) like the mother, one child Gm(1,21) like the father, and one child Gm(−), that is, devoid of demonstrable Gm specificities. This indicates that the two parents were carriers of a rare allele, Gm−, analogous to the genes $\bar{\bar{R}}^0$ and $\bar{\bar{r}}$ of the Rh-Hr system and K^0 of the Kell system. The existence of such rare amorph Gm alleles must be borne in mind by the expert when interpreting the results of his tests.

The so-called Inv types differ from Gm in that their determinants are located on the light chains of the K type (kappa) instead of on heavy chains. Moreover, the situation is much simpler in that only three Inv specificities have so far been described, the inheritance of which appears to be by triple alleles Inv^1, $Inv^{1,2}$, and Inv^3, independent of the Gm types. Apparently, if there is also an Inv^2 allele, this allele must be very rare. Because of the limited availability of suitable reagents, experts who use Inv types generally limit their tests to Inv(1) only. This considerably restricts the usefulness of these tests.

Other serum types determined by hemagglutination inhibition are the so-called Isf and Am types. The Isf determinant, like almost all Gm determinants, appears to be located on the Fc fragment of molecules of the γG subclass. Nevertheless, Isf seems to be inherited independently of the Gm and the Inv types. In contrast, Am, the determinant of which is located on IgA immunoglobulins of the γA2 subclass, remarkably appears to be closely linked genetically with the Gm serum types. Because of the limited experience with these types and unavailability of suitable reagents, the Isf and Am serum types have little or no forensic value.

It is important to point out that while the IgG concentration in the serum of newborn babies is about the same as for the mother, this does not represent the baby's own IgG immunoglobulins but maternal IgG passively acquired by the fetus in utero by transplacental transfer. Thus, while in exceptional cases a newborn baby's genotypic Gm type has been successfully determined, for example, mother Gm− and baby Gm+, generally tests on the baby's serum will not give the baby's own Gm type but the mother's. Since immunoglobulin molecules have a half-life of about 30 days, Gm typing for problems of disputed paternity should be delayed until the baby is about six months old, at which time the maternal immunoglobulins will have disappeared from its circulation and the baby's own Gm type will have been fully established.

Gc Serum Types

These types were orginally detected by immunoelectrophoresis in 1959. The serum to be tested is first separated into its components by electrophoresis in agar gel, and the so-called Gc (group-specific components) properties identified by using a rabbit immune antihuman precipitating serum. Two different such components have been identified, the faster moving designated Gc(1) and the other Gc(2). Genetic studies have shown that the three phenotypes, Gc(1-1), Gc(2-1), and Gc(2-2), are hereditarily transmitted by contrasting codominant allelic genes, Gc^1 and Gc^2. In this regard, the situation is analogous to that for the principal three haptoglobin types and M-N blood types, and because of the favorable distribution of the types the Gc tests are useful markers for use in problems of disputed parentage.

As to be expected, further study has shown the situation to be more complicated than appeared at first because of the demonstration of a number of Gc variants which could be differentiated from the original two kinds, Gc(1) and Gc(2), by differences in electrophoretic migration. A simplification of the technique of testing is to use simple electrophoresis of the serum tested, since the Gc components can readily be recognized as bands in the postalbumin region without resort to the immunodiffusion against rabbit immune antihuman precipitating serums.

The Gc tests are used by most European serologists and are recommended if the expert is aware of the complications possible when variants are present.

Ag Types

The specificities of this system of serum types are detected by passive hemagglutination inhibition tests. The antigens in question are low density lipoproteins (LDL), which are passively coated onto red cells that are used as indicators as the red cells coated with

anti-$\mathbf{Rh_0}$ antibody are used as indicators in the Gm tests. Most of the antiserums used have been derived from patients sensitized by multiple blood transfusions. When originally discovered the Ag antigens were detected by precipitation and immunodiffusion in agar; however, the inhibition method is presently preferred because of its greater sensitivity. As many as ten different specificities characteristic of Ag serum antigens have been reported to date. In the most recent report, it was found necessary to postulate a large series of multiple alleles, giving rise to a minimum of 90 different phenotypes, of which all but four were actually observed. Unfortunately, while this promises to be a very useful system for use in problems of disputed parentage, the required reagents are unavailable except in the laboratories of their discoverers.

Lp Types

The serum factors of this system determine polymorphism of human lipoproteins which is independent of Ag. The antigen is detected by immunodiffusion, for which the original antiserum was produced by immunizing rabbits with human β-lipoproteins and absorbing the antiserum with serum from certain human subjects. Later it was found that antiserum of similar specificity could be produced without difficulty in horses. However, the antigen detected by the horse antiserum proved to be not identical with that detected by the rabbit serum. The original antigen was named Lp^a and the additional antigen detected by the horse serum Lp^x. The association between the two antigens is evident from their distribution in the population: Lp(a+x+), 20%; Lp(a+x−), 14%; and Lp(a−x−), 66%. Evidently, heredity is by triple alleles, analogous to the alleles for the subgroups of A of the A-B-O blood group system.

The distribution of types is favorable for medicolegal applications, but actual application must await greater experience and availability of the required reagents.

Xm Serum Groups

By immunizing rabbits with human serum, an antiserum was obtained which reacted by immunodiffusion against an antigen which, like Lp, is located in the serum lipoproteins. The antigen, designated Xm(a), proved to be inherited as a sex-linked Mendelian dominant. The gene frequencies, which range between 20 and 30%, indicate that tests for this antigen will be rewarding for problems of disputed paternity involving female children, as already discussed in the case of the Xg blood type.

Albumins

Genetic variations in human albumin demonstrable as a second albumin band of slower electrophoretic mobility was reported as long ago as 1955. This original bisalbuminemia and other similar variants are quite rare, so that they have very limited value for demonstrating genetic polymorphisms which could be of use in problems of disputed parentage. However, because of this very rarity, the concomitant presence of the anomaly in a child and putative father could be used as circumstantial evidence of paternity.

Other Serum Protein Markers

Polymorphisms have been described for other serum protein constituents, such as transferrins and ceruloplasmins. However, among normal individuals all but one of the variants of these serum proteins is rare, so that they have little value for resolving problems of disputed paternity. A more recently reported serum polymorphism which promises to be useful for problems of disputed parentage are the C3 (third complement) variants.

Red Cell Isozymes

A substantial contribution to the chances of excluding paternity by blood tests resulted from the discovery of enzymes in red cells which have more than one molecular form in the population. Polymorphisms of the enzymes in question are demonstrated by electrophoresis of hemolysates of the red cells, showing differences in the bands developed by staining after addition of the appropriate substrates for the enzyme action. The polymorphic enzymes, named isozymes, are genetically determined, and this makes possible their application for forensic problems of disputed parentage. The most useful of the isozymes are the acid phosphatases because of their high degree of polymorphism; however, by running electrophoresis simultaneously for multiple isozymes, it becomes practicable also to include other less useful isozymes, such as adenosine deaminase (ADA), adenylate kinase (AK), and 6-phosphogluconate dehydrogenase (PGD). In the case of glucose-6-phosphate dehydrogenase (G-6-PD), the tests are only worthwhile among blacks in whom there is a significant degree of polymorphism. Similarly, it is worthwhile to look for hemoglobin variants in black subjects because as many as 10% could be carriers of sickle-cell trait; such electrophoresis of hemoglobin is readily done in parallel with electrophoresis of the variants of red cell isozymes.

Acid Phosphatase Isozymes.

As indicated above, these are the most important of the red cell isozymes because of their high degree of polymorphism. There are three main variants transmitted by corresponding allelic genes designated as P^A, P^B, and P^C, respectively, though two additional rarer variants have also been found, designated P^R and P^D. The chances of excluding paternity are substantial (close to 35%). However, caution is necessary because of the relative novelty of the test and limited experience with reading the stained gels and the interpretation of the findings. Moreover, the discovery of a few families indicating the existence of a very rare amorph gene P^O must be taken into account when interpreting the observations. Obviously, the test should only be undertaken in forensic cases by workers thoroughly qualified by experience with the test.

Phosphoglucomutase Isozymes

Tests for phosphoglucomutase isozymes are worthwhile where feasible because of the significant polymorphism in all populations. Moreover, there appear to be at least two systems of these isozymes inherited independently of one another, so that the gene symbols are assigned different numerical subscripts to indicate the difference in gene locus. To these tests the same general remarks are applicable as given above for the acid phosphatase isozymes.

Glucose-6-Phosphate Dehydrogenase

Among the isozymes, G-6-PD is of special interest because of its sex-linked heredity, like Xg and Xm. For the very same reason, however, the test is applicable only to problems of disputed parentage involving daughters. Because of virtual absence of G-6-PD polymorphism in whites and Japanese, the test is useful only in cases involving blacks, but the chance of exclusion is only 1%. However, if tests are done for the variants B, A, and A−, which are inherited by triple allelic genes, the chance of exclusion may be as high as 17% (for blacks only).

Other Isozymes

This is a rapidly developing field, and the list of isozymes systems is constantly increasing in length. It is doubtful that any one laboratory is prepared and qualified to

carry out all the known tests. Moreover, as has been pointed out, when the chances of excluding paternity are already high, there is little to be gained by adding further tests since this makes the total expense prohibitively high and progressively less rewarding.

Leukocyte Antigens

Individual differences in white cell antigens were studied intensively because of their presumed role in the success or failure of organ and tissue transplants [*20*]. A variety of techniques have been used, including agglutination of leukocytes, complement fixation by platelets, and cytotoxicity of lymphocytes. The cytotoxicity test has proved to be the simplest to do and read, and it also gives the most reproducible results. This test depends on the ability of the antibodies to kill viable lymphocytes having the corresponding antigen in the presence of complement, as demonstrated by the ability of the dead cells to take up a dye which will not stain living cells. A great multiplicity of antigenic specificities have been discovered, almost all of which remarkably determine antigens of one system designated HL-A. As the result of the combined efforts and ingenuity of many investigators, the antigenic specificities which have been defined have been shown to make up two mutually exclusive segregant series; the first series, named LA, comprises more than 15 mutually exclusive antigens, presumably transmitted by corresponding allelic genes; and the second, labeled the Four series, also includes at least 12 specificities. According to the genetic theory most widely accepted, heredity of the so-called HL-A types is by multiple alleles with each gene having two subloci, one for a factor of the HL series, and the second for a factor of the Four series. The two subgenes are almost completely linked, so that if, for example, a parent has the genotype HL-A1, W5/HL-Al0, HL-A7, then half of his children will receive from him the chromosome determining factors HL-A1 and W5, while the other half will receive the chromosome determining factors HL-A10 and HL-A7.

It is evident from the great multiplicity of specificities that the number of possible genotypes and phenotypes is legion. According to one estimate, there are more than a hundred haplotypes (the linked factors of the subloci of the same segment of a chromosome transmitted as a unit), giving rise to more than 8000 genotypes and 4000 phenotypes. Some workers claim, therefore, that the chance of excluding paternity by the HL-A tests equals or exceeds the chance obtained with all the blood and serum groups combined. According to one investigator, the chance of exclusion by the blood and serum groups is about 92%, and so that this would be raised by HL-A tests to more than 99%.

It would seem from these considerations that the virtual solution of problems of disputed parentage is now at hand. Unfortunately, this solution is beset with numerous pitfalls, and few laboratories, if any, are equipped to carry out all the necessary tests. The performance of all the tests mentioned in this report would be a laborious task indeed, and too costly in time and material for routine use. Furthermore, the HL-A tests are reputed to have the reproducibility of only about 90%, so that the possibility of errors is a real one indeed.

Likelihood of Paternity

If it were practical to do all the known blood tests, a man falsely accused of paternity would have more than a 99% chance of being exonerated. In some European courts, when the defendant is not excluded by the tests, the judge requires the expert to calculate the likelihood that the defendant actually is the father. Because likelihood of paternity is closely related to the chance of excluding paternity, there has been a tendency to confuse the two. A simple example should suffice to show the

difference. Suppose that the various blood types of the mother and her child have been determined, and that a 12-year-old boy is selected at random and fully typed and by chance proves to be compatible with the types of mother and child. Obviously, the likelihood that the boy in question was the father of the child would remain zero despite this happenstance.

The value of the likelihood W that the defendant in a paternity case who is not excluded by any of the blood tests actually is the father can be calculated from the a priori chances of paternity P_A and the chances of exclusion by the tests P_E by the following formula:

$$W = \frac{P_A}{P_A - (1 - P_A)(1 - P_E)}$$

In the case of the 12-year-old boy, P_A was zero, so that W also was necessarily zero, irrespective of the results of the blood tests. In a case where two men are named by the mother as the possible father, either of whom could with equal probability be the father, $P_A = 0.5$, and the formula above is simplified to the one below:

$$W = \frac{1}{2 - P_E}$$

The following are the results of the blood tests on the mother and child in an actual case studied by a European expert, in which two men were involved as possible fathers.

	A-B-O	M-N-S	Rh-Hr	P	K	Fy^a	Hp	Gc	Gm
Mother	B	M.s	Rh_1Rh_1	+	−	−	1−1	1−1	a−x−b+
Child	B	MN.s	Rh_1Rh_1	+	−	−	2−1	1−1	a−x−b+

It is evident that the father of the child had to belong to type N or type MN and to be rh′ positive, haptoglobin type Hp(2-2) or Hp(2-1), and Gc type Gc(1-1) or Gc(2-1). The chance that a falsely accused man would satisfy all these requirements equals (0.80)(0.70)(0.80)(0.75) = 0.33, so that the chance of exclusion is only about 0.67. Therefore, if one of the defendants were tested and not excluded, the probability of his being the father would be raised from the a priori likelihood of 0.5 to only 0.75 because $1/(2 - 0.67) = 0.75$. In this case, therefore, it was not remarkable that when both men were tested neither was excluded, thus leaving the problem where it was originally. This raised the question whether it was possible to calculate from the blood types of the two defendants (see table below) their relative chance of being the father of the child. This can actually be done very simply as follows.

	A-B-O	M-N-S	Rh-Hr	P	K	Fy^a	Hp	Gc	Gm
First Man	0	N.s	Rh_1Rh_1	+	−	−	2−2	1−1	a−x−b+
Second Man	0	MN.s	Rh_1rh	+	−	+	2−1	2−1	a+x−b+

By comparing the blood types of mother and baby, it can be seen that the baby must have received the following genes from the father: *N*, R^1 (or r'), *k*, *fy*, Hp^2, Gc^1, and *gm*. It is noteworthy that the first man is homozygous for all these genes, while the second man is heterozygous for all these genes, except for gene *k* (and he may even be homozygous for Fy^a which would exclude him). Therefore, the odds are at least about 2^6 to 1, or about 64 to 1, that the first man is the father rather than the second.

General Conclusions

There has been a virtual explosion in the development of methods for resolving problems of disputed parentage by blood tests since the last report on the subject was published. This has been the result of three breakthroughs which have added to red cell blood grouping tests, serum grouping, red cell isozyme grouping, and leukocyte grouping. Even though calculations demonstrate that it is progressively more difficult to increase the chances of excluding paternity the higher the level of exclusion already attained, the vast multiplicity of systems and specificities that have been discovered make it theoretically possible, at the present time, to approximate closely the ideal of 100% exclusion rate for men falsely accused of paternity. However, few laboratories if any are equipped to carry out all the necessary tests, and it is doubtful that any single individual is fully qualified to carry out and interpret all the tests. In addition, the high cost of a "complete" test makes it prohibitive and impractical. A more serious difficulty is the real possibility of mistakes, which increases in likelihood as the variety and number of test procedures increase, thus raising the danger of miscarriage of justice.

Progress in the field continues as more and more tests are discovered and introduced for use. One possible solution to the problem is the establishment of special institutes devoted to blood grouping, serum grouping, isozyme typing, and leukocyte typing. Such central reference institutes could overcome the problem of training qualified experts, producing and standardizing antiserums and other reagents, and the tests could find applications also for clinical medicine, for example, HL-A typing of patients requiring tissue or organ transplants and their donors. The methods could also find application for the more refined typing of dried stains of blood and secretions like semen in criminal cases.

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