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# THE MEDICOLEGAL APPLICATION OF BLOOD GROUPING TESTS

I. Davidsohn†

The knowledge of blood groups has found application in medical jurisprudence because there are demonstrable differences in the blood of different individuals which are permanent, unalterable, and inherited according to strict biological rules.

A court that is called upon to decide a bastardy case—one in which an alleged father makes a denial of paternity—may derive considerable assistance from the report of an expert who has conducted blood grouping tests on the mother, the child, and the alleged father. Likewise, in certain types of criminal cases the grouping of a specimen of blood at the scene of a crime or on the person of a suspect or victim may be very helpful in determining criminal responsibility for the offense in question.

## Purpose and Scope of this Paper

In this paper, written primarily for the benefit of police officers, and members of the legal profession, an attempt will be made to explain in as simple language as possible the nature and underlying biological principles of blood grouping tests; and, also, to outline the various possible medicolegal applications.

## Nature and Biological Principles of Blood Grouping Tests

Unlike fingerprints, which are dis-

tinctive in every person, the distinguishing properties of blood are common to groups of persons. Within each group the blood is identical, but it varies among the different groups. When blood of identical groups is mixed no change takes place. The color remains a homogeneous red, and when viewed under a microscope the red blood cells or corpuscles are seen to retain their individuality; they remain round and separate from each other. Conversely, however, when blood of different groups is mixed, the red cells lose their identity and form irregularly shaped clusters, many times the size of the individual red cells. This phenomenon, which may be likened to that which occurs in the curdling of milk, is known as blood incompatibility. If it happens in the body of a person during the course of a blood transfusion, the smallest blood vessels, some of them not much wider than the diameter of a red cell, become plugged and thus interfere with the normal circulation. This may lead to more or less severe manifestations of disease and even to death.

In the test tube a mixture of bloods of different groups loses the red, homogeneous, opaque appearance and becomes a suspension of red clumps in a transparent colorless or pale yellow medium. The clumping or agglutination is due to a union of two factors,

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one in the red cells, the other in the liquid portion of the blood, which is called the serum. This factor in the red cells is known as agglutinin; the factor in the serum is known as agglutinin. The differences between blood groups are based upon the presence of different factors or agglutinogens in the red cells of different persons and upon a corresponding difference in the properties of the serums of different individuals. The reaction between the agglutinin or the agglutinable factor in the red cells and the agglutinin or the clumping substance in the serum is strictly specific, which means that they correspond to each other just like a key and a keyhole. After the clumps in a mixture of bloods of two individuals belonging to different groups have settled, the upper layer of clear serum lacks the ability to clump more blood of the same kind as before, because the agglutinins have been removed by the red cells in the process of clumping.

There are two blood group factors, designated by letters *A* and *B*. A person whose blood possesses factor *A* is considered a member of group *A*; and likewise for *B*. A third group, *AB*, is composed of persons whose blood possesses both factors *A* and *B*. A fourth blood group is characterized by the absence of either factor *A* or *B*, and is known as group *O*. The red cells of persons of blood group *O*, which constitutes about 45 per cent of the population of the United States, cannot be clumped by any other serum, because they lack the factor needed for the reaction. The blood of persons of group *A*, amounting to about 40 per cent of

the population, is clumped by about 55 per cent of all serums. To the blood group *B* belongs about 10 per cent of the population, and its red cells are clumped by about 85 per cent of all serums. The red cells of the final and smallest group, which includes only about 5 per cent of the people, known as group *AB*, are clumped by about 95 per cent of serums. The distribution of the four blood groups varies greatly among different races.

The designation by letters is called the Landsteiner classification, after the discoverer of blood groups. Two other classifications, one of Moss, the other of Jansky, employing numbers I, II, III and IV are still widely used, but the Landsteiner classification is to be preferred and is the one used most generally in the scientific literature.

It is a general rule that blood serum has the ability to clump red cells of groups other than its own. The reason for that is obvious, because it would not be compatible with the life of an individual if his serum would clump his own red blood cells. The serum of group *A* clumps cells with the factor *B*, because it has the agglutinin against *B* (i.e., the agglutinin anti-*B*); the serum of group *B* clumps red cells with the factor *A*, because it has the agglutinin anti-*A*; the serum of group *AB* does not clump any other human red cells because it cannot have either agglutinin anti-*A*, or anti-*B*, since it has both factors *A* and *B* in its blood cells; and the serum of group *O* clumps the cells of all other groups, because not having either *A* or *B* factors in its red cells, it must have in its serum both agglutinins anti-*A* and anti-*B*. From

the foregoing, it is apparent that the presence of factors *A* or *B* in the red blood cells excludes the presence of the corresponding agglutinin anti-*A* or anti-*B* in the serum. On the other hand, the absence of any of the two factors from the red cells is always associated with the presence of the corresponding agglutinin in the serum. If both factors *A* and *B* are absent, as is the case in Group *O*, the serum contains both anti-*A* and anti-*B* agglutinins.

Blood groups are constant; they never change; they are found in young embryos, and can be detected long after death, even after decomposition has set in. It has been established beyond any question that neither disease nor environment can change the blood group. The older reports indicating such a possibility were due to technical errors which can be easily avoided by expert handling.

Recent studies have shown that it is not altogether correct to speak of blood groups, because the same properties that are in the red blood cells are also present in most of the body tissues of all individuals, and in the secretions like saliva, gastric juice, seminal fluid, etc., of some individuals. The detection of blood group properties in saliva and in seminal fluid has been applied for medicolegal purposes, as will be discussed later on in this paper.

The method of detecting group factors in tissues or secretions differs from that in blood. In the latter the group factors are determined by the ability of blood serums of known groups to clump the red cells, while in tissues and secretions it is based on their ability to absorb and remove agglutinins from

the serum. The following is an example of how it is done:

Serum of group *A* has the ability to clump red cells of group *B*. If a tissue suspension of a person of group *B* is added to such a serum, the latter loses its ability to clump red cells of group *B*. The presence of the group factor *A* in tissues can be detected in a similar way by using serum of group *B*.

There is no relation between blood groups and other body characteristics like color of skin or hair, shape of skull and so forth.

Properties in blood comparable to blood groups were found in different animal species but not so regularly as in man except in higher apes, whose blood groups are very similar to those of man.

#### The Value and Application of Blood Grouping Tests for Exclusion of Paternity

Blood groups are inherited according to laws of inheritance known as the laws of Mendel.

Properties transmitted to posterity by inheritance are divided into two classes: (a) Dominant properties which are obvious, readily detectable, and never hidden, and (b) recessive properties, which may be hidden in the individual who received them by inheritance. These recessive properties may become apparent in a descendant to whom they were transmitted, although they were hidden in the ancestor.

The blood group properties *A* and *B* are dominant, which means that when present they must be recognizable or apparent and cannot be hidden. The

property *O*, characterized by the absence of *A* and *B* is recessive, which means that it may be hidden. If a parent has property *A* or *B*, the child may or may not inherit it. On the other hand, the properties *A* or *B* cannot appear in a child unless they are present in one or both parents. This is the first law of inheritance of blood groups. The property *O*, however, can appear in a child though it is not detectable in the parents; it is then assumed to have been hidden in the blood of the parents.

A child gets one of its properties from each parent and both make up its blood group. A parent who has both *A* and *B*, and who therefore belongs to group *AB*, must give either *A* or *B* to his child. Consequently, he cannot be a parent of a child of blood group *O*. This is the second law of inheritance. On the other hand, a parent of group *O* cannot have a child of blood group *AB*, because the child could not get both properties from one parent only. This is the third law of inheritance.

The following table lists the blood group combinations of parents and the blood groups which their legitimate children can and cannot have. If any of the blood groups listed in the "impossible" column is found in a child, and the maternity of the mother is unquestioned, then the accused man can be *excluded* as the possible father.

TABLE I

*The Inheritance of Blood Group Characteristics*

Blood Types of the Parents	Children Possible (Blood Groups)	Children Impossible (Blood Groups)
O x O	O	A, B, AB
O x A	O, A	B, AB

O x B	O, B	A, AB
O x AB	A, B	O, AB
A x A	A, O	B, AB
A x B	O, A, B, AB	None
A x AB	A, B, AB	O
B x B	B, O	A, AB
B x AB	B, A, AB	O
AB x AB	AB, A, B	O

The probability of an exclusion of an innocently accused man varies according to his blood group. It is greater if his blood group is rare, and vice versa.

The possibilities of exclusion were greatly enhanced when two additional blood properties were discovered by Landsteiner and Levine (5). The new properties known as factors *M* and *N* are independent in their occurrence from factors *A* and *B*; in other words, their distribution is the same in the four blood groups *A*, *B*, *AB*, and *O*. They are similar to the *A* and *B* factors with respect to constancy and independence from disease and from environmental influences, but they differ from the *A* and *B* properties in several ways.

Human serums lack agglutinins for the factors *M* and *N*; they lack the ability to clump the red cells which are the carriers of the *M* and *N* factors. This is different from the situation with regard to the factors *A* and *B*, and is undoubtedly the reason why the *M* and *N* factors were discovered so much later. They were discovered in a rather roundabout way—by the injection of human blood into rabbits.

Whenever blood of one animal species is injected into an animal of another species, the latter develops the ability to destroy the injected cells, a phenomenon which may be interpreted as a means of defense. The newly ac-

quired ability to destroy manifests itself in various ways, one of which is clumping. Accordingly, whenever human blood is injected into the blood of a rabbit (or other such animal), the blood serum of the rabbit will acquire the ability to clump human blood. However, in the course of the experiments during which the *M* and *N* factors were discovered, there was the unexpected result that when the clumping ability of the serum was removed by adding to it red cells of certain persons, the serum retained the property of clumping the cells of other persons of the same blood group—a fact which demonstrated that there are differences even within the same blood group. These differences were found to be due to the presence of two properties, *M* and *N*, which are called blood *types* in order to distinguish them from the blood *groups* (*A*, *B*, *AB*, and *O*). Considerable experience and care are necessary for the preparation and use of such serum in determining the presence of these *M* and *N* factors. (6).

Another distinguishing feature of the *M* and *N* blood properties is that either or both of them are present in *every human blood*, thus giving three blood types *M*, *N*, and *MN*, whereas about 45 per cent of the population lacks the *A* and *B* properties. The third difference between the blood factors *M* and *N* on one hand and the factors *A* and *B* on the other, is that the former are present only in the red blood cells, and not in other tissues as are the *A* and *B* factors.

The blood type *M* is found in about 30 per cent of the population of the United States, the type *N* in about 20

per cent, and the type *MN* in about 50 per cent. There are geographic variations among different racial groups.

The new blood types are inherited according to three laws, the first of which is identical with the first law of inheritance of the *A* and *B* properties: the factors *M* and *N* cannot appear in a child unless they are present in one or in both parents. The second law states that a parent of the type *M* cannot have a child of type *N*; the third law that a parent of type *N* cannot have a child of type *M*. These two laws are easily understood when it is recalled that a child must get a blood type property from each of its parents, and therefore a parent who has only one of the two factors must transmit it to his offspring.

The following table lists the parental *M* and *N* combinations and the types of children which can and cannot be an issue thereof.

TABLE II

*The Agglutinogens M and N in Parents and Children*

Blood Types of the Parents	Children Possible (Blood Types)	Children Impossible (Blood Types)
M x M	M	N, MN
M x N	MN	M, MN
M x MN	M, MN	N
N x N	N	M, MN
N x MN	N, MN	M
MN x MN	MN, M, N	None

The application of the laws of inheritance of the blood types *M*, *N*, and *MN* has increased the chances of exclusion by about 100 per cent, from 16 per cent to over 30 per cent. It must be remembered, of course, that the theoretical percentage of possible exclusions is based on the assumption that only

falsely accused men are examined. In practice this figure is not achieved because some of the accused men are the actual fathers.

The following table lists the chances

of exclusion of innocently accused men if blood group factors *A* and *B* are determined alone, and if they are determined in combination with blood type factors *M* and *N*.

TABLE III

*Chances of Proving Non-Paternity with the Blood Groups\**  
(where alleged father has been falsely accused)

O	A	B	AB
23.5%	7.7%	14.6%	39.9%

Chances of Proving Non-Paternity with the Agglutinogens *A*, *B*, *M* and *N*

Group Type	O			A			B			AB		
	M	N	MN	M	N	MN	M	N	MN	M	N	MN
Chances												
%	50.0	54.6	23.5	39.6	45.1	7.7	44.1	49.3	14.6	60.7	64.3	39.9

While the chances of exclusion of an innocently accused individual belonging to blood group *O* are 23.5 per cent, the chances are increased to 50.0 per cent or even to 54.6 per cent if the *M* and *N* types are determined. In the case of group *A* the chances of exclusion rise from 7.7 to 39.6 or even to 45.1 per cent by the application of the *M* and *N* factors, while in group *AB* the rise amounts to 60.7 or 64.3 per cent, as compared with 39.9 per cent when the *A* and *B* factors alone are determined. However, the chances of an accused person belonging to type *MN* are not enhanced by the determination of the *M* and *N* factors, as compared with the determination of the *A* and *B* factors alone, because such a person can have children of type *M*, *N*, and *MN*.

*Exclusion of Paternity Without Examination of the Mother*

Paternity may be excluded even

without the examination of the blood of the mother, when the blood group of the alleged father is *AB* and that of the child *O*, or vice versa, or when the blood type of the alleged father is *M* or *N* and the blood type of the child is the opposite.

*When Exclusion is Impossible*

In some instances the examination of the blood of the mother is unnecessary or futile. For instance, if the alleged father and the child belong to the same blood group and type combination, or if they belong to the same blood group and the alleged father belongs to blood type *MN*, in such a case exclusion of paternity is not possible, no matter what the blood group or blood type of the mother might be.

*Exclusion of Paternity Without Examination of Either Father or Mother*

Occasionally an exclusion is possible even without examination of the blood

\* Modified from Wiener, A. S., *Blood Groups and Blood Transfusion* (2d ed., 1939), pp. 255, 260.

group of either parent. When the bloods of the paternal grandparents are available, and when they belong to type *M*, or when both belong to type *N*, the grandchild cannot belong to the opposite type.

*Blood Grouping Tests in Disputed Maternity*

Although blood group tests are used most commonly in cases of disputed paternity, they may help occasionally in cases of disputed maternity. When the alleged mother belongs to group *AB* and the child is found to be of group *O*, or vice versa, or when the alleged mother belongs to type *M* and the child to type *N*, or when the alleged mother belongs to type *N* and the child to type *M*, maternity is disproved.

*Blood Grouping Tests in Cases of Mix-Up of Infants*

In June of 1930, Mrs. W. gave birth to a boy in a Chicago Hospital. Half an hour later, Mrs. B. bore a son in the same hospital. When the mothers and children returned in due time to their homes, Mrs. W. noticed that her child had on its neck a mark *B* and Mrs. B. noticed about the same time that her boy was marked *W*. To solve the mystery blood grouping tests were applied. They showed:

<i>Group</i>		<i>Group</i>	
Mr. B. ....	<i>AB</i>	Mr. W. ....	<i>O</i>
Mrs. B. ....	<i>O</i>	Mrs. W. ....	<i>O</i>
Baby brought from hospital	<i>O</i>	Baby brought from hospital	<i>A</i>

Now a group *A* child could not possibly be the offspring of Mr. W., but it could be a child of Mr. B. Likewise, a

group *O* baby could not be the offspring of Mr. B., but it could very well be the child of Mr. W. The interchange of the infants in the hospital became obvious. In cases of interchange of infants—as in maternity hospital cases such as this—a solution is possible in almost 70 per cent of cases.

There is reason to believe that discoveries of additional blood factors may further increase the chances of excluding paternity. A few new blood properties have actually been reported, but the knowledge of them is still incomplete. However, it is probable that one recent development will soon be sufficiently confirmed to become generally accepted.

It has been known for some time that the factor *A* in blood groups *A* and *AB* occurs in two forms. One of them, called subgroup *A*<sub>1</sub> is much more readily clumped than the other, which is known as subgroup *A*<sub>2</sub>. The former is about four times as common as the latter. Evidence has been advanced recently that the inheritance of the subgroups *A*<sub>1</sub>, *A*<sub>2</sub>, *A*<sub>1</sub>*B*, and *A*<sub>2</sub>*B* is governed by laws which when applied to litigations on paternity would increase the chances of exclusion by about 3 per cent. However, further studies will be needed before their reliability will become unquestioned and before they are suitable for medicolegal purposes.

As a result of combinations of the four blood groups *A*, *B*, *AB* and *O*, and the subgroups *A*<sub>1</sub>, *A*<sub>2</sub>, *A*<sub>1</sub>*B* and *A*<sub>2</sub>*B*, with the blood types *M*, *N* and *MN*, eighteen blood group and type combinations are possible, as listed in Table IV.

TABLE IV

*Blood Group and Type Combinations*

<i>Group</i>	<i>Type</i>	<i>Group</i>	<i>Type</i>
1. A <sub>1</sub> B	.....M	10. A <sub>2</sub>	.....N
2. A <sub>2</sub> B	.....M	11. A <sub>1</sub>	.....MN
3. A <sub>1</sub> B	.....N	12. A <sub>2</sub>	.....MN
4. A <sub>2</sub> B	.....N	13. B	.....M
5. A <sub>1</sub> B	.....MN	14. B	.....N
6. A <sub>2</sub> B	.....MN	15. B	.....MN
7. A <sub>1</sub>	.....M	16. O	.....M
8. A <sub>2</sub>	.....M	17. O	.....N
9. A <sub>1</sub>	.....N	18. O	.....MN

Although the agglutinins in the serum are not always fully developed until the child is several months to one year old, the blood groups and blood types can be determined at birth from the reactions of the cells. However, due to the fact that the subgroup A<sub>2</sub> may not be fully developed at birth, it is advisable in affiliation cases not to give an opinion until the child is at least one month of age.

It is important to emphasize that the determination of blood groups and blood types may *exclude* paternity, but does not determine it. Failure to exclude paternity does not imply that the accused person is the father of a given child. Any man of the same blood group may be the father. For that reason it is advocated that laws relative to the use of blood grouping tests in courts provide for their use only in cases where exclusion is shown and that the introduction of results of tests be prohibited when exclusion is not possible. The failure to provide for elimination of evidence of negative results may prejudice a judge or jury against innocently accused men whose exclusion is not possible.

### The Value and Application of Blood Grouping Tests in Criminal Cases

Since the blood group factors A, B, M, and N are highly resistant to drying heating, and to the action of other physical and chemical influences, it is possible to detect their presence even in blood stains. Consequently the tests are of considerable potential value in criminal investigations.

The determination of blood groups in stains may be helpful under various circumstances. Stains may be found on the clothing of suspects. Their claim that it is their own blood may be disproved by finding that their own blood belongs to a different group than the blood stain. Stains on the clothing of a victim may be similarly examined for the purpose of determining whether or not they are of a blood group similar to that of the blood of the victim or of the suspect. Because of the potential value of blood grouping tests in cases of this nature it is advisable that blood grouping tests be made of the blood from a victim so that even after disposal of the body itself the blood specimen would be available for comparison purposes in the event such tests are later deemed necessary.

The presence of factors A and B in seminal fluid makes it possible to utilize the tests in rape cases. For instance, the blood group of seminal stains on the body or clothing of a victim may be determined and compared with that of a suspect. As in the case of paternity determination, however, the results can only prove exclusion, but the possibility of ascertaining such a fact in any given case is well worth the effort.

In somewhat the same manner, and also with similar practical limitations, group tests can be made of saliva. And there are records of successful determinations of blood groups in traces of saliva on gummed edges of envelopes and on cigarette stubs.

*Precautions Which Should Be Taken  
by Law-Enforcement Officials*

All blood stains on bodies, or on the clothing of victims or suspects, or on other objects, should be submitted for blood grouping tests as soon as possible, even if the investigators are not certain at the time of the investigation that the results of blood grouping tests will be needed later. The fresher the stain, the better are the chances for determination of blood groups. Then, too, even on a very small fresh stain it may be possible to effect a determination of the blood group, whereas in older stains much more material may be required than is actually available. In any event, there is no reason why an early determination should not be made, for the labor involved in the examination of a fresh stain is small in comparison to the great and sometimes insurmountable difficulties which are encountered in attempting to determine the blood groups of old stains.

Where an examination is to be made of stains on clothing, the investigator should submit as much of the clothing as possible. Moreover, unstained cloth from the immediate vicinity should also be available for examination, since in-

terfering substances such as sweat, for instance, may conceivably affect the accuracy of the test results.

Obviously it is advisable to determine the blood groups and types of the individual or body on which the questioned stain is found. Such determinations should be made as a matter of routine in all cases of death by violence.

Only properly qualified experts should be entrusted with the task of conducting blood grouping tests. A thorough knowledge of the subject, of the many involved intricacies and the sources of errors, as well as considerable experience and mastery of the technic are essential prerequisites for a successful application of the determination of blood groups and for a reliable interpretation of the results.

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