Heredity of the M-N-S Blood Types

Theoretico-Statistical Considerations

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By injecting human blood of group O into rabbits Landsteiner and Levine (1928a) obtained antisera which after suitable absorption yielded reagents for three previously undescribed agglutinogens of human blood. Two of these agglutinogens, designated by the letters M and N, were found to be related to one another, and determined three types of human blood, M, N, and MN. By family studies Landsteiner and Levine (1928b) showed that the three M-N types are inherited by a pair of allelic genes, giving rise to three genotypes corresponding to the three phenotypes as follows: type M, genotype *MM;* type N, genotype *NN;* and type MN, genotype *MN*. This theory has been substantiated by studies on a large series of families, and the M-N types have found their most important application in legal medicine for solving problems of disputed parentage (Wiener, 1943).

For a long time, the M-N types were considered to constitute a simple system of only three types, as originally described by Landsteiner and Levine. The development of new techniques for detecting immune isoantibodies (Wiener, 1945; Coombs et al., 1945; Morton and Pickles, 1947) led to the demonstration that the M-N system is actually far more complicated. Recently, Walsh and Montgomery (1947) detected in the serum of a mother of an erythroblastotic baby not only an Rh antibody but also a second antibody which gave reactions different from those of any of the known blood group antibodies. A sample of the serum was tested by Sanger and Race (1947) who showed that the antibody in question gave reactions related to the M-N system. The new agglutinogen was designated by the symbol S and the corresponding antibody as anti-S. It was shown that by using anti-S serum each of the three M-N types could be subdivided, resulting in a more complex system of 6 M-N-S types. To account for the hereditary transmission of these 6 M-N-S types, Sanger et al. (1948) postulated a series of tightly linked gene couplets or allelic pseudogenes, *MS*, *Ms*, *NS*, and *Ns*, respectively. More recently, Levine et al. (1951) studied the serum of an Rh-positive mother of an erythroblastotic baby and detected an antibody in it giving reactions reciprocally related to the agglutinogen S. The new agglutinogen and corresponding antibody have been designated s and anti-s, respectively. The discovery of serum anti-s has consider-

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ably increased the interest and importance of the M-N-S system, because when all four antisera are available, it becomes possible to differentiate as many as 9 phenotypes corresponding to the 10 theoretically possible genotypes.

The M-N-S types are of considerable theoretical importance, because they present the same problems regarding serology, genetics, and nomenclature as the Rh-Hr types (cf. Wiener, 1951). The discovery of agglutinogens S and s has considerably enhanced the usefulness of the M-N system for the solution of problems of individual identification, disputed parentage, interchange of infants, and zygosity of twins. Thus far, only limited quantities of the antisera have been available, so that the number of studies carried out have been few. However, the essential facts are known and it is possible to make certain theoretico-statistical calculations. The purpose of this paper is to present such a theoretico-statistical analysis of the M-N-S types. While, as will be pointed out, it is possible to devise a simplified nomenclature for the M-N-S system analogous to Wiener's nomenclature for the Rh-Hr types, for clarity in this paper the original nomenclature of Sanger and Race will be used with only minor changes.

HEREDITY OF THE M-N-S TYPES

In table 1 are given the serological reactions of the M-N-S types, showing the 9 possible phenotypes and 10 corresponding genotypes. If we let a represent the frequency of gene MS, b represent the frequency of gene NS, c represent the frequency of gene Ms, d represent the frequency of gene Ns, then the frequencies of the 9 phenotypes in terms of the gene frequencies a, b, c, and d are as given in the column headed "formula" in table 1. With the aid of these formulae we have calculated the approximate distribution of the 9 M-N-S types in England, based on the frequencies of the 4 genes as given by Fisher (1951). These gene frequencies were computed by Fisher from the observations of Sanger and Race on a large series of individuals tested with anti-M, anti-N, and anti-S sera, at a time when anti-s had not yet been found.

The formulae can be used to verify the genetic theory by applying them to data obtained by testing a large series of individuals with all four reagents. Thus, the frequencies of the four genes can be estimated from the frequencies of the phenotypes as follows:

$$a = \sqrt{\overline{\mathrm{M.S}}} \tag{1}$$

$$b = \sqrt{\overline{\mathrm{N.S}}} \tag{2}$$

$$c = \sqrt{\overline{\mathrm{M.s}}} \tag{3}$$

$$d = \sqrt{\overline{\mathrm{N.s}}} \tag{4}$$

Having determined the gene frequencies with the aid of formulae (1) to (4), the frequencies of the other five phenotypes can then be calculated in order to determine how closely the theoretically expected values agree with those actu-

ally observed. Moreover, if the genetic theory is correct, the sum of the calculated gene frequencies, $a + b + c + d$, should equal 100 percent.

If we let $D = 1 - (a + b + c + d)$, then it is necessary to calculate the value of σ_D in order to test whether the genetic theory is valid. The value of σ_D is determined as follows:

Let the frequency of type M.S = A, of type N.S = B, M.s = C, and N.s = D.

then,

$$a = \sqrt{\bar{A}}$$
$$b = \sqrt{\bar{B}}$$
$$c = \sqrt{\bar{C}}$$
$$d = \sqrt{\bar{D}}$$

By using the same reasoning as was used when analyzing the genetics of the three M-N types (Wiener, 1931), it can be shown that:

$$\sigma_a = \tfrac{1}{2}\sqrt{\frac{1 - A}{N}}$$

where N represents the number of persons tested

Similarly,

$$\sigma_b = \tfrac{1}{2}\sqrt{\frac{1 - B}{N}}$$

and there are similar formulae for σ_c and σ_d.

Moreover,

$$r_{ab} = -\sqrt{\frac{AB}{(1 - A)(1 - B)}}$$

$$r_{ac} = -\sqrt{\frac{AC}{(1 - A)(1 - C)}}$$

with similar formulae for r_{ad}, r_{bc}, r_{bd}, and r_{cd}.

But
$$\sigma_D^2 = \sigma_a^2 + \sigma_b^2 + \sigma_c^2 + \sigma_d^2 + 2r_{ab}\sigma_a\sigma_b + 2r_{ac}\sigma_a\sigma_c + 2r_{ad}\sigma_a\sigma_d + 2r_{bc}\sigma_b\sigma_c + 2r_{bd}\sigma_b\sigma_d + 2r_{cd}\sigma_c\sigma_d$$

So that

$$\sigma_D^2 = \frac{1 - A}{4N} + \frac{1 - B}{4N} + \frac{1 - C}{4N} + \frac{1 - D}{4N} - \frac{\sqrt{AB}}{2N} - \frac{\sqrt{AC}}{2N} - \frac{\sqrt{AD}}{2N} - \frac{\sqrt{BC}}{2N} - \frac{\sqrt{BD}}{2N} - \frac{\sqrt{CD}}{2N}$$

Substituting $A = a^2$, $B = b^2$, $C = c^2$, and $D = d^2$

$$\sigma_D{}^2 = \frac{4 - (a + b + c + d)^2}{4N}$$

and

$$\sigma_D = \frac{\sqrt{3}}{2\sqrt{N}} \tag{5}$$

Because anti-s sera has only recently been found, there are as yet no data to be tested with the aid of these formulae.

The theory can also be tested by studies on families. There are 45 different kinds of matings which can occur, and the M-N-S types which can occur among the children of each mating are readily determined. Nevertheless, it may be of some value to have a table summarizing the possibilities, and for that reason table 2 was prepared. This table is easily constructed because the genotype of an individual is evident from his phenotype for 8 of the 9 types, while for individuals of type MN.Ss there are only two genotypes possible. Theoretically no more than four types can occur among the children of a single family. However, in the matings with one or both parents belonging to type MN.Ss, namely, Nos. 11, 26, 28, 29, and 35, as many as 6 or 8 possible M-N-S types are given for the offspring. The reason for the seeming paradox is that these matings actually represent more than one kind of family; in some the parents of type MN.Ss belong to genotype *MSNs* while in others the genotype of the parents of type MN.Ss is *MsNS*. According to a personal communication from Race, he and his co-workers have recently tested a series of families with all four sera, anti-M, -N, -S, and anti-s, and the results obtained conform with the theoretical expectations.

INDIVIDUAL IDENTIFICATION

A simple type of problem which can be solved with the aid of blood grouping tests is the following. Suppose a fresh blood stain is found from which blood suspensions can be prepared and completely typed with all available antisera, and it is desired to know whether this stain contains the blood of a particular individual. Obviously, only a negative answer can be given, that is, if the blood type of the stain and of the individual are different, then the blood stain is not his; on the other hand, if the groups are the same the blood stain is not necessarily his because of the possibility of coincidence. In cases where the blood spot does not contain the blood of the suspected individual, the chances of exonerating him by blood group tests is given by the general formula:

$$I = 1 - \sum_{i=1}^{i=k} A_i{}^2 \tag{6}$$

where $A_1, A_2, A_3 \cdots A_k$ represent the respective frequencies of the k types into which blood can be classified with the reagents available.

For example, in the case of the M-N types,

$$I_{M,N} = 1 - (M^2 + MN^2 + N^2)$$

If m represents the frequency of gene M, and n represents the frequency of gene N, then,

$$I_{M,N} = 2mn(2 - 3mn) \tag{7}$$

Similarly, in the case of the three S-s types, we have

$$I_{S,s} = 2pq(2 - 3pq) \tag{8}$$

where p represents the frequency of gene S, and q represents the frequency of gene s.

If I_1 represents the chance of solving a problem of disputed identity by one system of blood types, and I_2 the chance by using a second independent system of blood types, then the combined chances of proving non-identity by using both systems is given by the formula:

$$I = 1 - (1 - I_1)(1 - I_2) \tag{9}$$

However, this formula cannot be applied to the M-N-S types, because S-s is not independent of M-N. One can calculate $I_{M,N,S,s}$ directly by applying the general formula (6). To determine the value of the chances in terms of gene frequencies one must then substitute the formulae given in table 1 for the frequencies of each phenotype. This, upon simplification, yields the following formula for the chances of establishing non-identity by the M-N-S types:

$$I_{M,N,S,s} = 1 - (a^2 + b^2 + c^2 + d^2)^2 + (a^4 + b^4 + c^4 + d^4) - 8abcd \tag{10}$$

Applying these formulae to the Caucasoid population given in table 1, one finds that the chances of proving non-identity by the three M-N types are 62.3 percent, by the three S-s types they are 59.0 percent, while for the nine M-N-S types combined the chances are 83.6 percent. It may be of interest to note that had we assumed that S-s was independent of M-N and used formula (9), we would have found the chances for the M-N-S types combined to be 84.5 percent, which is not much different from the correct value.

DISPUTED PARENTAGE

As in individual identification, so also in disputed parentage blood tests may be used for purposes of exclusion only. Problems of disputed parentage may present themselves in a variety of forms. At times, the question may be posed whether a certain woman is the mother of a certain child; such a situation has occurred, for example, when a wet-nurse kidnapped a baby and claimed it to

be her own child. In a second more common type of case it is desired to determine whether or not a given man is the father of a certain child. Usually in such cases the mother of the child is known, but at times it is not possible to test the mother's blood. In a third type of problem, the question may be raised whether a man and woman are the parents of a child; this could occur, for example, when a couple claims that a certain child whose parents are unknown is theirs, a situation which has arisen not infrequently as an aftermath of the last war. Finally, the blood tests are useful for detecting interchange of babies in hospitals; in such cases there are usually two babies and two pairs of parents

TABLE 1. THE M-N-S TYPES

3 M-N TYPES			9 M-N-S TYPES			CORRESPONDING GENOTYPE	FREQUENCIES	
Designation	Reaction with sera		Designation	Reaction with sera			Formula*	Among Caucasoids† (percent)
	Anti-M	Anti-N		Anti-S	Anti-s			
M	+	−	M.S	+	−	*MSMS*	a^2	6.1
			M.Ss	+	+	*MSMs*	$2ac$	14.0
			M.s	−	+	*MsMs*	c^2	8.0
MN	+	+	MN.S	+	−	*MSNS*	$2ab$	4.0
			MN.Ss	+	+	*MSNs* and *MsNS*	$2ad + 2bc$	23.8
			MN.s	−	+	*MsNs*	$2cd$	22.1
N	−	+	N.S	+	−	*NSNS*	b^2	0.6
			N.Ss	+	+	*NSNs*	$2bd$	6.2
			N.s	−	+	*NsNs*	d^2	15.2

* The symbols a, b, c, d, represent the frequencies of genes *MS*, *NS*, *Ms*, and *Ns*, respectively.

† Based on the gene frequencies reported by Race and Sanger (1951) and Fisher (1951) for London.

to be tested. The chances of arriving at a decision with the aid of the M-N-S types for each of these problems will be considered separately.

Chances of Excluding Maternity. From the mathematical standpoint this is the simplest situation to analyze. The question which is presented here is whether a certain woman is the mother of a certain child. A comparable situation exists when one wishes to determine whether a given man is the father of a certain child in cases where the mother's blood is not available for examination.

Firstly, when the three M-N types alone are considered it is evident that a decision is possible only when the supposed mother belongs to type M and the child to type N, or when the supposed mother belongs to type N and the child to type M. The chances of encountering one or the other of these cases are given by the simple formula:

$$P_{M,N} = 2M \times N \tag{11}$$

TABLE 2. HEREDITY OF THE M-N-S TYPES

MATING	M-N-S TYPES POSSIBLE IN CHILDREN	M-N-S TYPES IMPOSSIBLE IN CHILDREN
1. M.S x M.S	M.S	M.Ss, M.s, MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s
2. M.S x M.Ss	M.S, M.Ss	M.s, MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s
3. M.S x M.s	M.Ss	M.S, M.s, MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s
4. M.Ss x M.Ss	M.S, M.Ss, M.s	MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s
5. M.Ss x M.s	M.Ss, M.s	M.S, MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s
6. M.s x M.s	M.s	M.S, M.Ss, MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s
7. M.S x MN.S	M.S, MN.S	M.Ss, M.s, MN.Ss, MN.s, N.S, N.Ss, N.s
8. M.S x MN.Ss	M.S, M.Ss, MN.S, MN.Ss	M.s, MN.s, N.S, N.Ss, N.s
9. M.S x MN.s	M.Ss, MN.Ss	M.S, M.s, MN.S, MN.s, N.S, N.Ss, N.s
10. M.Ss x MN.S	M.S, M.Ss, MN.S, MN.Ss	M.s, MN.s, N.S, N.Ss, N.s
11. M.Ss x MN.Ss	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s	N.S, N.Ss, N.s
12. M.Ss x MN.s	M.Ss, M.s, MN.Ss, MN.s	M.S, MN.S, N.S, N.Ss, N.s
13. M.s x MN.S	M.Ss, MN.Ss	M.S, M.s, MN.S, MN.s, N.S, N.Ss, N.s
14. M.s x MN.Ss	M.Ss, M.s, MN.Ss, MN.s	M.S, MN.S, N.S, N.Ss, N.s
15. M.s x MN.s	M.s, MN.s	M.S, M.Ss, MN.S, MN.Ss, N.S, N.Ss, N.s
16. M.S x N.S	MN.S	M.S, M.Ss, M.s, MN.Ss, MN.s, N.S, N.Ss, N.s
17. M.S x N.Ss	MN.S, MN.Ss	M.S, M.Ss, M.s, MN.s, N.S, N.Ss, N.s
18. M.S x N.s	MN.Ss	M.S, M.Ss, M.s, MN.s, MN.s, N.S, N.Ss, N.s
19. M.Ss x N.S	MN.S, MN.Ss	M.S, M.Ss, M.s, MN.S, N.S, N.Ss, N.s
20. M.Ss x N.Ss	MN.S, MN.Ss, MN.s	M.S, M.Ss, M.s, N.S, N.Ss, N.s
21. M.Ss x N.s	MN.Ss, MN.s	M.s, M.Ss, M.s, MN.S, N.S, N.Ss, N.s
22. M.s x N.S	MN.Ss	M.S, M.Ss, M.s, MN.S, MN.s, N.S, N.Ss, N.s
23. M.s x N.Ss	MN.Ss, MN.s	M.S, M.Ss, M.s, MN.S, N.S, N.Ss, N.s
24. M.s x N.s	MN.s	M.S, M.Ss, M.s, MN.S, MN.Ss, N.S, N.Ss, N.s
25. MN.S x MN.S	M.S, MN.S, N.S	M.Ss, M.s, MN.Ss, MN.s, N.Ss, N.s
26. MN.S x MN.Ss	M.S, M.Ss, MN.S, MN.Ss, N.S, N.Ss	M.s, MN.s, N.s
27. MN.S x MN.s	M.Ss, MN.Ss, N.Ss	M.S, M.s, MN.S, MN.s, N.S, N.s
28. MN.Ss x MN.Ss	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s	None
29. MN.Ss x MN.s	M.Ss, M.s, MN.Ss, MN.s, N.Ss, N.s	M.S, MN.S, N.S

TABLE 2—*Continued*

MATING	M-N-S TYPES POSSIBLE IN CHILDREN	M-N-S TYPES IMPOSSIBLE IN CHILDREN
30. MN.s x MN.s	M.s, MN.s, N.s	M.S, M.Ss, MN.S, MN.Ss, N.S, N.Ss
31. MN.S x N.S	MN.S, N.S	M.S, M.Ss, M.s, MN.Ss, MN.s, N.Ss, N.s
32. MN.S x N.Ss	MN.S, MN.Ss, N.S, N.Ss	M.S, M.Ss, M.s, MN.s, N.s
33. MN.S x N.s	MN.Ss, N.Ss	M.S, M.Ss, M.s, MN.S, MN.s, N.S, N.s
34. MN.Ss x N.S	MN.S, MN.Ss, N.S, N.Ss	M.S, M.Ss, M.s, MN.s, N.s
35. MN.Ss x N.Ss	MN.S, MN.Ss, MN.s, N.S, N.Ss, N.s	M.S, M.Ss, M.s
36. MN.Ss x N.s	MN.Ss, MN.s, N.Ss, N.s	M.S, M.Ss, M.s, MN.S, N.S
37. MN.s x N.S	MN.Ss, N.Ss	M.S, M.Ss, M.s, MN.S, MN.s, N.S, N.s
38. MN.s x N.Ss	MN.Ss, MN.s, N.Ss, N.s	M.S, M.Ss, M.s, MN.S, N.S
39. MN.s x N.s	MN.s, N.s	M.S, M.Ss, M.s, MN.S, MN.Ss, N.S, N.Ss
40. N.S x N.S	N.S	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s, N.Ss, N.s
41. N.S x N.Ss	N.S, N.Ss	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s, N.s
42. N.S x N.s	N.Ss	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s, N.S, N.s
43. N.Ss x N.Ss	N.S, N.Ss, N.s	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s
44. N.Ss x N.s	N.Ss, N.s	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s, N.S
45. N.s x N.s	N.s	M.S, M.Ss, M.s, MN.S, MN.Ss, MN.s, N.S, N.Ss

Or, expressed in terms of gene frequencies,

$$P_{M,N} = 2m^2n^2 \tag{12}$$

Similarly for the three S-s types

$$P_{S,s} = 2S \times s \tag{13}$$

Or, expressed in the terms of gene frequencies,

$$P_{S,s} = 2p^2q^2 \tag{14}$$

The chances of excluding maternity by the nine M-N-S types together is not merely the simple sum of $P_{M,N}$ and $P_{S,s}$, because this would entail counting twice instances of double exclusion by both M-N and S-s. The correct value is obtained by subtracting from the sum the frequency of such double exclusions as follows:

$$P_{M,N,S,s} = 2M \times N + 2S \times s - 2MS \times Ns - 2Ms \times NS \tag{15}$$

Or, expressed in the terms of gene frequencies,

$$P_{M,N,S,s} = 2[(a + c)^2(b + d)^2 + (a + b)^2(c + d)^2 - a^2d^2 - b^2c^2] \tag{16}$$

For the Caucasoid population under consideration, the chances of excluding maternity by the three M-N types are 12.4 percent, the chances of excluding maternity by the three S-s types are 9.7 percent, while the combined chances of excluding maternity by the nine M-N-S types are 19.2 percent. Again, interestingly enough had we assumed that S-s was independent of M-N, the result obtained, namely, 20.9 percent, would not have been much different from the correct value.

Chances of Excluding Paternity. The most common problem with which the medicolegal expert is confronted is that of disputed paternity. In almost all such cases the mother of the child is known, and her blood is available as well as that of the putative father and child. Naturally, the chances of excluding paternity are considerably greater when the mother's blood is available than when it is not. The chances of excluding paternity of a falsely accused man by the M-N types have been previously derived by Wiener, et al. (1930). If m represents the frequency of gene M, and n the frequency of gene N, then:

$$P_M = n(1 - mn) \tag{17}$$

where P_M signifies the chance of excluding paternity when the accused man is known to belong to type M.

Similarly,
$$P_N = m(1 - mn) \tag{18}$$

where P_N represents the chance of excluding paternity when the accused man belongs to type N,

and
$$P_{MN} = 0 \tag{19}$$

From these three values one can derive the average chances of excluding paternity, that is, the chances when the M-N type of the putative father is not known. This is given by the following formula:

$$P_{M,N} = mn(1 - mn) \tag{20}$$

For the Caucasoid population under consideration these formulae yield the following results. A falsely accused man of type M has a 35.3 percent chance of being exonerated, a man of type N has a 39.8 per cent chance, while a man of type MN has no chance. The average chances of being excluded by the M-N types for a falsely accused man are 18.7 percent.

In the analogous problem involving the three S-s types, the same formulae can be used by substituting S for gene M and s for gene N. If the falsely accused man belongs to type S, he has a 52.4 percent chance of being exonerated by the S-s types; a type s man has a 25.6 percent chance of being exonerated, while a type Ss man can never be excluded by these tests. For the three S-s types the average chances of excluding paternity are 17.2 percent.

Again, to determine the chances of exclusion using the nine M-N-S types,

one does not merely add the separate chances for M-N and S-s, because the instances of double exclusion must be subtracted from the sum. When deriving the needed formula, and in making the arithmetic calculations a table of frequencies of mother-child combinations is helpful. Thus, such a table of mother-child combinations was used when deriving the formulae for the three M-N

TABLE 3. FREQUENCIES OF MOTHER-CHILD COMBINATIONS

M-N-S TYPE OF MOTHER	M-N-S TYPE OF CHILD								
	M.S	M.Ss	M.s	MN.S	MN.Ss	MN.s	N.S	N.Ss	N.s
M.S.	a^3	a^2c	0	a^2b	a^2d	0	0	0	0
M.Ss	a^2c	$ac(a+c)$	ac^2	abc	$ac(b+d)$	acd	0	0	0
M.s	0	ac^2	c^3	0	bc^2	c^2d	0	0	0
MN.S	a^2b	abc	0	$ab(a+b)$	$ab(c+d)$	0	ab^2	abd	0
MN.Ss	a^2d	$ac(b+d)$	bc^2	$ab(c+d)$	$ad(a+d)+bc(b+c)$	$cd(a+b)$	b^2c	$bd(a+c)$	ad^2
MN.s	0	acd	c^2d	0	$cd(a+b)$	$cd(c+d)$	0	bcd	cd^2
N.S	0	0	0	ab^2	b^2c	0	b^3	b^2d	0
N.Ss	0	0	0	abd	$bd(a+c)$	bcd	b^2d	$bd(b+d)$	bd^2
N.s	0	0	0	0	ad^2	cd^2	0	bd^2	d^3

TABLE 4. FREQUENCIES OF MOTHER-CHILD COMBINATIONS*

M-N-S TYPE OF MOTHER	M-N-S TYPE OF CHILDREN								
	M.S	M.Ss	M.s	MN.S	MN.Ss	MN.s	N.S	N.Ss	N.s
M.S	.0151	.0173	0	.0049	.0237	0	0	0	0
M.Ss	.0172	.0370	.0198	.0057	.0329	.0272	0	0	0
M.s	0	.0198	.0227	0	.0065	.0312	0	0	0
MN.S	.0049	.0057	0	.0066	.0134	0	.0016	.0078	0
MN.Ss	.0237	.0329	.0065	.0135	.0694	.0361	.0019	.0167	.0374
MN.s	0	.0272	.0312	0	.0361	.0740	0	.0089	.0428
N.S	0	0	0	.0016	.0018	0	.0005	.0025	0
N.Ss	0	0	0	.0078	.0167	.0089	.0025	.0148	.0123
N.s	0	0	0	0	.0374	.0428	0	.0123	.0589

* Calculated for population given in table 1.

types. In table 3 are given the formulae for the frequencies of the various mother-child combinations for the more complicated situation involving the 9 M-N-S types, while in table 4 the actual numerical frequencies for the Caucasoid population under consideration have been calculated. When we consider each of the 9 M-N-S types individually the chances of excluding paternity for men belonging to these types are as follows:

$$P_{M.S} = n(1 - mn) + q(1 - pq) - \tfrac{1}{2}d(a^2 + b^2 + c^2 + d^2 + 1) + bcd \qquad (21)$$

where m and n represent the frequency of genes M and N, respectively; p and q the frequencies of genes S and s; a, b, c, and d, the frequencies of genes MS, NS, Ms, and Ns, respectively; while $P_{M.s}$ is the chance of excluding paternity when the falsely accused man is known to belong to type M.S.

Similarly, $$P_{M.Ss} = n(1 - mn) \tag{22}$$

$$P_{M.s} = n(1 - mn) + p(1 - pq) - \tfrac{1}{2}b(a^2 + b^2 + c^2 + d^2 + 1) + abd \tag{23}$$

$$P_{MN.S} = q(1 - pq) \tag{24}$$

$$P_{MN.Ss} = 0 \tag{25}$$

$$P_{MN.s} = p(1 - pq) \tag{26}$$

$$P_{N.S} = m(1 - mn) + q(1 - pq) - \tfrac{1}{2}c(a^2 + b^2 + c^2 + d^2 + 1) + acd \tag{27}$$

$$P_{N.Ss} = m(1 - mn) \tag{28}$$

$$P_{N.s} = m(1 - mn) + p(1 - pq) - \tfrac{1}{2}a(a^2 + b^2 + c^2 + d^2 + 1) + abc \tag{29}$$

The average chance of excluding paternity by the 9 M-N-S types, that is, when the man's exact type is not known, is given by the formula:

$$P_{M,N,\,S,s} = mn(1 - mn) + pq(1 - pq) - \tfrac{1}{2}(a^2 + b^2 + c^2 + d^2 + 1)(a^2d + ad^2 + b^2c + bc^2) + abcd \tag{30}$$

Substituting the values for the gene frequencies of the Caucasoid population under consideration, we obtain the following results:

$P_{M.S}$ = 63.3 percent
$P_{M.Ss}$ = 35.3 percent
$P_{M.s}$ = 56.4 percent
$P_{MN.S}$ = 52.4 percent
$P_{MN.Ss}$ = 0
$P_{MN.s}$ = 25.6 percent
$P_{N.S}$ = 76.5 percent
$P_{N.Ss}$ = 39.8 percent
$P_{N.s}$ = 49.9 percent
Average chances, $P_{M,N,\,S,s}$ = 31.5 percent

It is interesting to note again that were the M-N and S-s types independent the calculated chance of excluding paternity would be 32.7 percent, which is not much different from the correct value. Fisher has calculated the combined

chances of excluding paternity by the M-N-S types for the Caucasoid population under consideration to be 33.1440 percent.

Chances of Excluding Parentage. Here both of the supposed parents, the putative mother as well as the putative father, are questionable, as in a kidnapping case. The chances of excluding parentage are higher than in the first two cases analyzed, because there are two putative parents who may be excluded. For the three M-N types, the general formula of the chance of excluding parentage is readily derived, and is given below:

$$P = \frac{T}{4}(4 - 5T + 3T^2) \tag{31}$$

where T represents the frequency of the phenotype MN in the general population. The same formula can be used for calculating the chances of excluding parentage by the S-s types, the symbol T then representing the frequency of type Ss. Applying this formula to the Caucasoid population under consideration, we find that the chances of excluding parentage by the three M-N types are 28.1 percent, while the chances of excluding parentage by the three S-s types are 26.2 percent. To derive a general formula for the chances of excluding parentage by the 9 M-N-S types combined is a laborious task. In the first two problems which were considered, the chances of exclusion by assuming that S-s was independent of M-N proved to be close to the correct value, so that probably the same would be true in the present case. Thus, under the assumption of independence between S-s and M-N, the combined chances of excluding parentage becomes 46.9 percent, while Fisher (1951) gives for the chances of detecting interchange of infants (by which he really means the chances of excluding parentage) by the 9 M-N-S types the value of 45.6499 percent.

Chances of Detecting Interchange of Infants. Blood grouping tests are most useful for solving this type of problem, because with two pairs of parents and two children available for testing the chances of arriving at a decision are high. The chances of detecting interchange of infants with the aid of the three M-N types have already been determined by Wiener (1951) whose formula is as follows:

$$P_{M.N} = \frac{T}{8}(16 - 28T + 20T^2 - T^3) \tag{32}$$

where T represents the frequency of type MN in the population. The same formula can be used for the S-s types, with T representing the frequency of type Ss in the population.

No attempt has been made to derive a general formula for the chances of detecting interchange of infants by the 9 M-N-S types combined, because with

45 matings to be analyzed the derivation of such a formula would be a prodigious task. However, for reasons already indicated, a rough estimate of the chances can be made by assuming independence between S-s and M-N. Applying formula (32) to the Caucasoid population under consideration, one finds that the chances of detecting interchange of infants by the three M-N types is 42.9 percent for the three S-s types the chances are 41.0 percent, while with the nine M-N-S types combined as many as two thirds of such problems could be solved.

ZYGOSITY OF TWINS

Still another type of problem to which blood grouping has been applied is for determination of the zygosity of twins. Here, as in the cases of disputed parentage, only negative conclusions may be drawn, that is, if the blood groups of the twins are different they cannot be identical twins, but if the blood groups are the same they may or may not be uniovular. The chances, when one is dealing with fraternal twins, that the blood groups will disclose this fact have been derived for the A-B-O groups and M-N types by Wiener (1935) and by Wiener and Leff (1940).

In the case of the three M-N types the chances of proving dizygosity of fraternal twins are given by the formula:

$$Z_{M,N} = \frac{T}{8}(8 - 3T) \tag{33}$$

where T represents the frequency of type MN in the population. The same formula can, of course, be used for the three S-s types in which case T represents the frequency of type Ss.

Applying the same method to derive the chances of proving dizygosity of fraternal twins by means of the nine M-N-S types the following rather involved formula is obtained:

$$Z_{M,N,S,s} = 1 - \tfrac{3}{4}(a^2 + b^2 + c^2 + d^2)^2 - 2(a^3 + b^3 + c^3 + d^3) + \tfrac{7}{4}(a^4 + b^4 + c^4 + d^4) - 2(ab + ac + ad + bc + bd + cd)^2 + 4abcd \tag{34}$$

where a, b, c, d, as before, represent the frequencies of the four genes. Applying these formulae to the Caucasoid population under consideration one finds that the chances of proving dizygosity by the three M-N types is 40.5 percent, by the three S-s types 36.7 percent, while for the nine M-N-S types the chances are 56.2 percent. It may be of interest to point out that for the latter case Fisher gives the chance of 55.7231 percent. Incidentally, in this case the assumption of independence between S and s and M-N would yield the incorrect high value of 62.3 percent.

SEROLOGY, GENETICS, AND NOMENCLATURE

As Landsteiner (1945) has pointed out, when the blood of an animal is agglutinated by two sera containing antibodies of different specificities, this may be interpreted in two different ways, namely, either the blood contains two different agglutinogens or the blood contains a single agglutinogen and the factors with which the antibodies react are partial antigens within this single agglutinogen. One way to differentiate the two cases is by heredity studies. For example, in the case of group AB blood, since the factors A and B undergo Mendelian segregation it is evident that such blood contains two separate agglutinogens A and B. On the other hand, in the case of blood of subgroup A_1, the blood factors A_1 and common A never separate and therefore must be partial antigens within a single agglutinogen; similarly blood of subgroup A_2 contains the partial antigens common A and O (or A_2). That is why the simpler designations A_1 and A_2 were substituted for the original designations AA_1 and AA_2 by Landsteiner. Similarly, typical blood of type Rh_1 should never be designated as Rh_orh' or CD because the factors rh′ (C) and Rh_o (D) in such blood are partial antigens and do not represent separate agglutinogens, as proved by their genetic behavior. The designation Rh_orh' (or CD) would be permissible, however, in the rare instances where the individual is known to belong to genotype R^or'.

From their genetic behavior it is apparent that M-N and S-s are also partial antigens within a single agglutinogen molecule. Designations such as M.S are therefore misleading, because they imply the possibility of segregation between M and S. The argument that the genes for M-N and S-s may be closely linked within the same chromosome is invalid, because over a period of thousands of generations crossing-over would yield the same equilibrium distribution in the population as independent assortment. The fact is that in the case of the M-S types, as well as the Rh-Hr types and the subgroups of A, there is no indication that such an equilibrium is being reached or even approached. Thus, it is apparent that the factors M and S are not separable.[2]

The parallelism between the M-N-S types and the Rh-Hr types becomes apparent if we substitute M for rh′, N for hr′, S for rh″, and s for hr″, except that there is no property corresponding to Rh_o in the M-N-S system. Moreover, a simple and rational nomenclature for the M-N-S types and genes could readily be devised as follows: Let L (the letter L is selected in honor of Landsteiner) represent the gene responsible for the agglutinogen containing factors M and S, l the gene corresponding to the complex agglutinogen N.s, l^S be the gene for N.S, and l^M be the gene for agglutinogen M.s, so that the table of equiva-

[2] This viewpoint has received support from work carried out by Pickles (1952) while this paper was in press. Pickles showed that the S factor like the M and N factors is destroyed by proteolytic enzymes, indicating that M, N, and S are partial antigens within a protein molecule. On the other hand, the A-B-O and Rh-Hr blood factors, resist the action of proteolytic enzymes.

lents shown in table 5 can be drawn up. With this as a basis, table 6 was prepared to show the reactions of the nine M-N-S types and the corresponding genotypes expressed in these notations.

Landsteiner demonstrated how a single antigen of relatively simple chemical structure can stimulate the production of multiple antibodies differing in specificity. Moreover, he showed that when a single antibody cross-reacts with two different agglutinogens this does not necessarily indicate the presence in

TABLE 5. SIMPLIFIED NOMENCLATURE FOR M-N-S- GENES AND AGGLUTINOGENS

GENES		CORRESPONDING AGGLUTINOGEN		REACTION WITH SERA			
Simplified notations	Longhand designations	Simplified notations	Longhand designations	Anti-M	Anti-N	Anti-S	Anti-s
L	MS	L	M.S	+	−	+	−
l^S	NS	S	N.S	−	+	+	−
l^M	Ms	M	M.s	+	−	−	+
l	Ns	l	N.s	−	+	−	+

TABLE 6. SIMPLIFIED NOMENCLATURE FOR THE M-N-S TYPES

4 M-S TYPES			9 M-N-S TYPES			CORRESPONDING GENOTYPE
Designation	Reaction with sera		Designation	Reaction with sera		
	Anti-M	Anti-S		Anti-N	Anti-s	
l	−	−	l	+	+	ll
S	−	+	SS	+	−	l^Sl^S
			Sl	+	+	l^Sl
M	+	−	MM	−	+	l^Ml^M
			Ml	+	+	l^Ml
L	+	+	LL	−	−	LL
			LS	+	−	Ll^S
			LM	−	+	Ll^M
			Ll	+	+	Ll and l^Sl^M

the agglutinogens of identical substances, because cross-reactions can also result from similarities in chemical structure. This may be compared to the manner in which a master or skeleton key opens many different locks of related structure, or the way that mixed crystals are formed, for example, by different benzene derivatives in which halogens and methyl groups are mutually substituted. These observations preclude a one-to-one correspondence between antibodies, agglutinogens, and genes, as has been assumed by British investigators for the Rh-Hr blood factors. On the other hand, a one-to-one correspondence is possible between genes and agglutinogens with the understanding

that agglutinogens in general behave as though they have mosiac structure and may react with a number of antibodies of different specificities. These concepts have received brilliant confirmation from the recent remarkable investigations by Briles et al. (1950) on blood types in chickens and by Stormont et al. (1951) on bovine blood groups. In Stormont's investigations on bovine blood groups, for example, of 38 different blood factors studied 21 were members of a system designated the B system, and 7 members of a second system, the C system. The blood factors were inherited in blocks of divergent sizes forming unit agglutinogens with complex mosaic structures. A minimum of 80 allelic genes had to be postulated to account for the complex agglutinogens of the B system and 22 for the C system, so that the situation is quite comparable to though more complicated than that of the Rh-Hr system in man.

COMMENT

Due to the lack of general availability of the necessary antisera, the matters discussed in this paper are for the time being largely of theoretic interest only. Several patients have been encountered thus far who were sensitized to agglutinogen S, and from them a few samples of serum have been obtained which give clear-cut and specific reactions by the tube agglutination method. Despite the low titer of these sera the sharpness of the reactions makes reliable results possible in the hands of trained workers. On the other hand, only a single example of anti-s has been found to date, and this was usable only by the antiglobulin technique. The serum was used by Race et al. (1951) for a family study, and he reports that his findings support the genetic theory discussed here. However, based on experience over a period of years with the antiglobulin test, the present author considers that any blood factor which is demonstrable only by this method is not ready for medicolegal application.

SUMMARY AND CONCLUSION

1. The nine M-N-S types are described, and the genetic theory of Sanger and Race is discussed.

2. Formulae are given for computing gene frequencies and their probable errors, in order to subject the theory to a statistical test.

3. Formulae are derived for the chances of solving a number of problems by the nine M-N-S types, namely, disputed identity, disputed paternity and maternity, interchange of infants, and zygosity of twins.

4. Reasons are presented why factors M-N-S must be considered partial antigens within unit agglutinogens, inherited by multiple allelic genes.

For the time being the considerations discussed here are largely of academic interest due to the lack of availability of antisera, particularly against the agglutinogen s.

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