

ABO-Rh Interaction in an Rh-Incompatibly Mated Population*

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THE INVESTIGATION of the possible deleterious effects of blood group incompatibility between mother and fetus dates back almost to the discovery of the ABO blood groups themselves (Dienst, 1905; von Dungern and Hirsfeld, 1909). Yet certain aspects of the problem—the biological mechanism of selection, the interaction of different selection pressures, and the impact of these pressures on allele frequencies in succeeding generations—still remain a challenge today.

Even more interesting than the effects of incompatibility at single blood group loci are the effects of the *dual incompatibilities*, ABO and Rh. As customarily defined, *Rh-incompatibility* is the situation where the fetus is Rh positive (Rh₀ or D positive) and the mother is Rh-negative (really Rh₀ or D negative); and *ABO-incompatibility*, where the fetus carries an A or B antigen lacking in the mother.

The keen observation by Levine (1943) that the Rh-negative mothers of erythroblastotic offspring are less frequently ABO-incompatibly mated than expected has been confirmed by numerous investigations in which a sample of Rh-sensitized mothers and their families has been compared to random expectancy or control groups, mainly or partly Rh-compatibly mated (Wiener, 1945; Race, Taylor, Cappell and MacFarlane, 1943; Broman, 1944; van Loghem and Spaander, 1948; Speiser and Jancik 1953; as well as Malone, 1949; Lucia and Hunt, 1950 a, b, c; Brendemoen, 1952; Wiener, Nappi, and Gordon, 1953; Heistö, 1955; Nevanlinna, 1953; Nevanlinna and Vainio, 1956; Reepmaker, 1956). While such studies yield evidence consistent with the hypothesis of a protective action of ABO-incompatibility against Rh-sensitization, they do not exclude other explanations. This investigation was therefore undertaken to clarify the interplay of the two incompatibilities by utilizing a *nonsensitized* Rh-incompatibly mated series as a control for the *sensitized* Rh-incompatibly mated series—a different approach from other investigations.

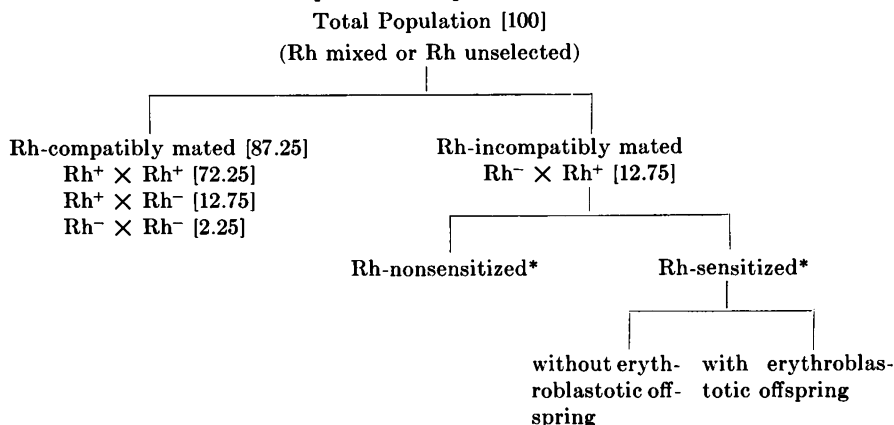
The classification of population samples on the basis of Rh mating status is given in Chart 1.

The ABO-compatible, ABO-incompatible, and potentially ABO-incompatible combinations are listed in Chart 2. The percentages of ABO-incompatible offspring expected from various population segments (mothers, fathers, offspring) are given in Table 1.

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CHART 1. Classification of Population Samples on the Basis of Rh Mating Status



Rh mixed or unselected samples: L. and H. Hirszfeld and coworkers (1925, 1928); Levine (1943); Waterhouse and Hogben (1947); Johnstone (1954a); Boorman and coworkers (1945, 1949); Bryce, Jakobowicz, McArthur and Penrose (1950); Kirk, Kirk and Stenhouse (1953); Kirk, Shield, Stenhouse and Jakobowicz (1955); Reed and Kelly, (1958).
 Rh⁺ × Rh⁺ (primarily because of the low frequency of Rh⁻ in the Japanese population): Matsunaga (1955, 1956, 1958, 1959).

Rh⁻ × Rh⁻ and Rh⁻ × Rh⁺: Bresler (presented at 1959 meetings American Society of Human Genetics)

Rh-sensitized (Rh⁻ × Rh⁺): Malone, 1949; Lucia and Hunt, 1950; Brendemoen, 1952; Wiener, Nappi, and Gordon, 1953; Heistö, 1955; Nevanlinna and Vainio, 1956; Reepmaker, 1956.

Rh-sensitized (with erythroblastotic offspring): Levine, 1943; Wiener, 1945; van Loghem and Spaander, 1948; Speiser and Jancik, 1953.

* Rh-nonsensitized and Rh-sensitized (Rh⁻ × Rh⁺): This study.

MATERIAL

Source and Ascertainment

The group studied here is taken from the records of the Baltimore Rh Typing Laboratory where cards of Rh-negative nonsensitized women and their pregnancy histories (the Rh NS series) are filed separately from those of the Rh-sensitized women (the Rh S series). The Rh-nonsensitized series (Rh NS), derived from the laboratory records for the years 1945 through July, 1954, consists of families of Rh-negative nonsensitized women whose husbands are known to be Rh-positive (with the exception of a few mother-offspring combinations included in which the paternal blood type was unknown). The Rh-sensitized series (Rh S), derived from the records for the years 1945 through July 1955, inclusive, consists of families similarly Rh-incompatibly mated but in which, in addition, the mothers have been shown to possess Rh antibodies.

It is to be noted that sizes of Rh NS and Rh S series are *not* in proportion to one another as in the population, because ascertainment is clearly not equal in the two series. Where Rh immunization or pregnancy difficulties are anticipated,

CHART 2. ABO-Incompatible and ABO-Compatible Combinations

Mother-Offspring Combinations:	ABO-Compatible	ABO-Incompatible
	O-O	O-A
	A-O	O-B
	A-A	A-B
	B-O	A-AB
	B-B	B-A
	AB-A	B-AB
	AB-B	
	AB-AB	
Father-Offspring Combinations:	ABO-Compatible	Potentially ABO-Incompatible
	O-O	A-A
	O-A	A-AB
	O-B	B-B
	A-O	B-AB
	A-B	AB-A
	B-O	AB-B
	B-A	AB-AB
Matings:	ABO-Compatible	Potentially ABO-Incompatible
	O × O	O × A
	A × A	O × B
	B × B	O × AB
	AB × AB	A × B
	A × O	B × A
	B × O	A × AB
	AB × O	B × AB
	AB × A	
	AB × B	

Note: The father-offspring and mating combinations are designated as “potentially” ABO-incompatible since the same combination may be involved in either an ABO-compatible or an ABO-incompatible situation, e.g., A father-A offspring is ABO-compatible if the mother is A or AB but ABO-incompatible if the mother is O or B.

TABLE 1. PERCENTAGE OF ABO-INCOMPATIBLE OFFSPRING EXPECTED* IN THE Rh NS AND Rh S SERIES

Population Segment	Expected Percentage of ABO-Incompatible Offspring	
	Rh NS Series	Rh S Series
Mothers		
O	32.50	32.48
B	24.15	24.08
A	8.17	8.23
AB	0	0
Fathers		
O	0	0
A	33.00	33.05
B	44.25	44.21
AB	70.85	70.86
Offspring		
O	0	0
A	32.06	32.12
B	43.01	42.97
AB	80.59	80.60

* Expected based on the Hardy-Weinberg equilibrium and ABO allele frequencies for U. S. Whites and Negroes (Glass and Li, 1953). Because the racial composition was slightly different in the matings recorded for the Rh NS and Rh S series, the expected values are not identical.

both the attending obstetrician and the family are more cooperative in regard to laboratory and case history follow-up studies. Accordingly, no attempt will be made to combine the Rh NS and Rh S series; instead each series will be considered separately and in comparison with other series. It is reasonable to assume, however, that in neither series was cooperation by the physician or patient biased by ABO blood group constitution, even if it was known.

The terms "multiple ascertainment" and "single ascertainment" refer to parent-offspring combinations. Multiple ascertainment indicates that each offspring of known blood group and sex is entered in a separate parent-offspring combination. Thus if one mother has several children of known blood group and sex, the one mother is included several times, once in each mother-offspring combination. Single ascertainment indicates that one mother can be included in only a single mother-offspring combination, irrespective of the number of her offspring. In single ascertainment the oldest child of known blood group and sex has been used to represent the family in mother-offspring combinations.

Size of Samples

When multiple ascertainment is used, there are 3577 mother-offspring combinations in the Rh NS series and 2998 in the Rh S series, representing 3037 and 1335 individual families, respectively.

The Rh NS series includes 2313 families with 5064 births. The births include:

- 2887 offspring of known ABO blood group
- 4842 offspring of known sex
- 2850 offspring of known ABO blood group and sex

The Rh S series includes 1383 families with 4160 births. These births include:

- 2778 offspring of known ABO blood group
- 3678 offspring of known sex
- 2631 offspring of known ABO blood group and sex

The above categories are not mutually exclusive.

Race

Mixed racial groups are contained in both the Rh NS series and the Rh S series. Only white and Negro classifications were available. In the Rh NS series, 86.77 percent of the mothers are White, whereas in the Rh S series 90.20 percent are white (based on mothers in appendix Table I). This slight difference is reversed when matings only are considered, the Rh NS series then yielding 93.27 percent whites and the Rh S series 92.18 percent whites. This reversal suggests that the variation is not of biological significance but rather a function of socioeconomic factors.

In computing the Hardy-Weinberg expectancies, individuals of unknown race were apportioned according to the proportion of races observed in the same series.

Maternal Ages

The mean ages shown in appendix Table IV reveal no significant differences among O, A, B, and AB mothers, within either series. Moreover, the distribution

of maternal ages (grouped in 5-year categories) analyzed by the mothers' ABO blood groups or by mating category showed no significant differences *within* either series.

A comparison *between* series, however, indicates a significantly greater mean age ($28.625 \pm .149$) for the mothers of the Rh S series than for the mothers of the Rh NS series ($27.237 \pm .106$). Since almost all one-pregnancy Rh-incompatible families and a large proportion of two-pregnancy families of this type are not yet sensitized (Glass, 1949), the reason for a lower mean age in the Rh NS series is apparent.

METHOD

Plan of Tabulation

The basic data have been tabulated in Appendix Tables I-VI. It will be necessary to refer to these to comprehend the statistical analyses, and the analytical tables. For the purpose of ready reference, the latter are indicated by section letters and numerals.

Plan of Analysis

The plan of analysis included:

(1) Comparison of the *observed* ABO distribution of mothers, fathers, and offspring, and their combinations, with the *expected* values for each series. Expected values are based on the assumption of a Hardy-Weinberg equilibrium, taking into account the racial composition of the sample and the ABO allele frequencies for U. S. Whites and Negroes, from Glass and Li (1953).

The expected numbers for each cell were computed separately for Whites and Negroes and then summed to yield cell expectancies. For example, the expected frequency of O \times A matings Rh NS series =

$$\sum \{ [r^2(p^2 + 2 pr) \times \text{total Negro matings}] + [r^2(p^2 + 2 pr) \times \text{total White matings}] \}$$

When the following allele frequencies are substituted for p, q, and r:

	Negroes	Whites
p = I ^A	.180	.248
q = I ^B	.132	.078
r = I ^O	.688	.674

and 156, for total Negro matings, and 2157, for total white matings, the equation equals 408.3 matings or .17652 of all matings, after adjustment of the total is made for rounding the final integer. Likewise, the offspring of O \times A matings are apportioned as:

$$\begin{aligned} \text{A offspring of O } \times \text{ A, Rh NS} &= [pr^2(p + r) \times \text{total Negro offspring} \\ &\quad + pr^2(p + r) \times \text{total white offspring}] \end{aligned}$$

$$\begin{aligned} \text{O offspring O } \times \text{ A, Rh NS} &= [pr^3 \times \text{total Negro offspring} \\ &\quad + pr^3 \times \text{total white offspring}] \end{aligned}$$

Computations were made similarly for the expected numbers of other matings, offspring, and parent-offspring combinations (appendix Tables V, VI and all other Hardy-Weinberg expectancies).

Complete tables with formulae, total frequency distributions, and computation for each cell are available and deposited in Welch Library.

It should be noted that the figures for U. S. whites are actually an average of frequencies for New York City (Tiber) and North Carolina (Snyder) and are in all likelihood representative of the Baltimore area. Moreover, they are based on 30,000 individuals and have almost no sampling error. On the other hand, while the frequencies for Negroes, derived from a Baltimore paternity suit series (cases of mother and offspring typed in connection with paternity suits at law), are representative of the Baltimore area and are similar to figures given for North Carolina and New York Negroes (Glass and Li, 1953), they are based on a relatively small sample (600 individuals) and involve some sampling error. However, since the Negro group represents such a small fraction of the population expectancy, attachment of a sampling error to this group would not alter the expected values to any degree.

(2) Comparison of population segments within each series.

(3) Comparison of the *observed* ABO distribution of the Rh S series with the *observed* ABO distribution of the Rh NS series for each population segment and set of combinations.

Note that comparisons of types (1) and (2) may be classified as comparisons *within each series*. Type (3) constitutes comparisons *between the two series* (Rh NS and Rh S).

TABLE A1. COMPARISON OF OBSERVED AND EXPECTED ABO DISTRIBUTIONS IN SUBGROUPS OF THE Rh NS SERIES AND THE Rh S SERIES*

	d.f.	Rh NS		Rh S	
		χ^2	P	χ^2	P
Mothers (Table II)	3	4.704	> .10	24.305	< .001
Fathers (Table II)	3	8.145	< .05	21.779	< .001
Offspring Total (Table III)	3	3.530	> .30	50.637	< .001
Male	3	2.204	> .50	26.482	< .001
Female	3	1.366	> .70	25.631	< .001
Mother-Offspring Combinations, m.a.	13	32.781	< .01	172.615	< .001
Father-Offspring Combinations, m.a.	13	34.408	< .01	74.657	< .001
Matings	15	32.117	< .01	88.991	< .001
Mating-Offspring Combinations	39	71.956	< .001	288.788	< .0001

* Comparisons of the observed and expected ABO distributions were also made using:
 Mothers derived from M-O combinations for each series
 Fathers " " F-O " " " "
 Mother-Offspring combinations based on s.a. for each series
 Father-Offspring " " " " " " "

These yielded a similar pattern of differences and levels of statistical significance to those tabulated above.

Reliability of Sampling

Internal consistency of the samples has been tested by Fisher's method, according to the procedure described by Dobson and Ikin (1946). All χ^2 values obtained were non-significant. Consequently, it may be assumed that the sampling is satisfactory.

Genetic formulae, statistical methods, and significance levels were computed in accordance with the accepted recommendations for standard procedures (Li, 1955; Cochran, 1952; Snedecor, 1950; Fisher, 1953).

RESULTS

For simplicity the results of the analysis of the data of Appendix Tables I-VI are presented below in tabular and outline form with brief, summarizing comments.

The following abbreviations will be used:

Symbol	Meaning
+	Excess of
-	Deficiency of
<i>i</i>	ABO-incompatible
<i>c</i>	ABO-compatible
NSD	No significant difference or not significantly different (i.e., not statistically significant)
SS	Statistically significant
M-O	Mother-offspring
F-O	Father-offspring (likewise, A-O indicates A mother-O offspring or A father-O offspring)
-x-	Mating Category listed as Female ABO blood group × Male ABO blood group
s.a.	Single ascertainment
m.a.	Multiple ascertainment
CPR	Cross-product ratio

*Analysis of the Complete ABO Distribution of Population
Segments and their Combinations*

A. Comparisons within each Rh series

1. Comparisons of population subgroups with expectancy

To determine whether the ABO distributions of the Rh NS and Rh S series fit the Hardy-Weinberg expectancies, each population segment and set of combinations is compared with the theoretical expected values, for each series separately (Table A1).

2. Comparison between mothers and fathers *within each Rh series*

If, as is commonly assumed (Waterhouse and Hogben, 1947; Johnstone, 1955; Matsunaga and coworkers, 1955, 1956, 1958, 1959; Reed, 1956), there is a similar distribution of adult males and adult females among the four ABO blood groups in the general population, then it would be expected that, in the absence of selective forces, mothers and fathers within the same series (Rh NS or Rh S) would have a similar blood group distribution and that male and female

TABLE A2A. COMPARISON OF THE ABO BLOOD GROUP DISTRIBUTION OF MOTHERS WITH FATHERS WITHIN EACH SERIES

	Rh NS Series		Rh S Series	
	Mothers vs. Fathers %	Fathers %	Mothers vs. Fathers %	Fathers %
From M-O, F-O Combinations (Table V)*				
O	44.97	43.08	41.72	49.05
A	38.59	40.24	45.24	39.64
B	12.51	12.48	8.99	8.64
AB	3.92	4.20	4.04	2.68
Total Distribution:	$\chi^2_3 = 2.334, .70 > P > .50$		$\chi^2_3 = 15.473, .01 > P > .001$	

* Comparative distributions for Mothers and Fathers derived from *Matings*, which can be obtained directly from Table II, reveal a similar pattern to those from combinations tabulated above.

Rh NS Series: $\chi^2_3 = 1.598, .70 > P > .50$

Rh S Series: $\chi^2_3 = 18.914, P < .001$

TABLE A2B. COMPARISON OF PROPORTION OF TOTAL* OFFSPRING PRODUCED BY MOTHERS OF THE DIFFERENT ABO GROUPS WITH THAT OF FATHERS OF CORRESPONDING BLOOD GROUPS

Parental Blood Group	Rh NS Series				Rh S Series			
	From Matings (Table II)		From M-O and F-O Combinations (Table V)		From Matings (Table II)		From M-O and F-O Combinations (Table V)	
	Mothers %	Fathers %	Mothers %	Fathers %	Mothers %	Fathers %	Mothers %	Fathers %
O	44.27	42.12	44.81	42.48	41.25	48.46	42.39	49.07
A	39.06	40.54	38.80	40.89	47.15	39.71	43.63	39.43
B	12.99	12.89	12.36	12.88	9.54	9.57	9.81	9.15
AB	3.67	4.44	4.03	3.75	4.21	2.26	4.17	2.34
	$\chi^2_3 = 7.832, .05 > P > .02$		$\chi^2_3 = 4.070, .30 > P > .20$		$\chi^2_3 = 62.242, P < .001$		$\chi^2_3 = 34.675, P < .001$	

* Offspring are classified on the basis of *parental* blood groups, *not* their own blood groups.

parents of the same ABO blood group would produce a similar proportion of the total offspring (Tables A2a and A2b).

B. Comparisons *between series* (Rh NS and Rh S) of the ABO distributions of corresponding population segments and their combinations.

To determine whether the ABO patterns of the Rh NS and Rh S series are similar, the ABO distributions of corresponding population segments (mothers, fathers, etc.) and of sets of combinations (mother-offspring, matings, etc.) are compared between the two series (Table B).

TABLE B. COMPARISON BETWEEN SERIES: Rh NS VERSUS Rh S SERIES

Population Segment	d.f.	χ^2	P	Deviation ¹ in ABO Distributions of Rh S Series when Compared to Rh NS Series
				<i>Deficiency of:</i>
Mothers (Table II)	3	13.210	< .01	<i>i</i> O and B mothers
Fathers (Table II)	3	26.160	< .001	<i>i</i> B and AB fathers
Offspring (Table III)				
Total	3	38.074	< .001	<i>i</i> B and AB offspring
Male	3	18.686	< .001	<i>i</i> B and AB offspring
Female	3	20.135	< .001	<i>i</i> B and AB offspring
Combinations				
M-O Combinations				
s.a. (Table V)	13	119.063	< .001	all <i>i</i> combinations
m.a. (Table V)	13	165.042	< .001	all <i>i</i> combinations
F-O Combinations				
s.a. (Table V)	13	43.483	< .001	all <i>i</i> combinations except AB-A and AB-AB
m.a. (Table V)	13	71.752	< .001	all <i>i</i> combinations except AB-AB contributing 1.3 to total χ^2 .
Matings (Table VI)	15	92.268	< .001	all <i>i</i> categories
Mating-Offspring (Table VI)	39	207.327	< .001	Deficiency most marked in <i>i</i> combinations O × A-A; O × B-B; and O × AB-B

¹ For simplicity of comparison with analysis in Section A pattern of deviation is given in terms of ABO-incompatible (*i*) components.

² Statistical tests were also performed on mothers, fathers, and offspring (total, and male and female separately) derived from M-O combinations (s.a. and m.a., Table V) and F-O combinations (s.a. and m.a., Table V). All revealed a similar pattern of deviations attaining less than the .001 level of significance except the following groups of *female offspring*:
 from M-O (s.a.) and (m.a.) .01 > p > .001
 from F-O (s.a.) .05 > p > .02

TABLE C1A. COMPARISON OF OBSERVED AND EXPECTED ABO-INCOMPATIBLE COMBINATIONS IN THE Rh NS SERIES

Combinations	Total No.	ABO-Incompatible Combinations				χ^2_1	P
		Observed		Expected			
		No.	Per Cent	No.	Per Cent		
Mother-Offspring (Table V)							
m.a.*	3577	820	22.92	746	20.85	9.374	< .01
s.a.*	3037	697	22.95	633	20.85	8.379	< .01
Father-Offspring (Table V)							
m.a.	2828	1064	37.62	1046	36.99	.492	> .30
s.a.	2284	848	37.13	845	36.99	.020	> .80
Matings (Table VI)	2313	898	38.82	827	35.76	9.317	< .01
Mating-Offspring (Table VI)	2887	636	22.03	598	20.73	2.981	> .05

m.a. = multiple ascertainment per family; s.a. = single ascertainment per family.

TABLE C1B. COMPARISON OF OBSERVED AND EXPECTED ABO-INCOMPATIBLE COMBINATIONS IN THE Rh S SERIES

Combinations	Total No.	ABO-Incompatible Combinations				χ^2_1	
		Observed		Expected			
		No.	Per Cent	No.	Per Cent		
Mother-Offspring (Table V)							
m.a.*	2998	355	11.84	623	20.80	145.919	< .001
s.a.*	1335	133	9.96	278	20.80	94.879	< .001
Father-Offspring (Table V)							
m.a.	2645	839	31.72	978	36.96	31.154	< .001
s.a.	1158	352	30.40	428	36.96	21.450	< .001
Matings (Table VI)	1383	359	25.96	495	35.78	57.890	< .001
Mating-Offspring (Table VI)	2778	316	11.38	576	20.75	148.440	< .001

* m.a. = multiple ascertainment per family; s.a. = single ascertainment per family.

TABLE C2. COMPARISON RATIOS OF Rh NS AND Rh S SERIES AS TO PROPORTIONS OF ABO-INCOMPATIBLE AND ABO-COMPATIBLE COMBINATIONS AND OFFSPRING GROUPINGS

Set of Segments in Comparison (RhNS:RhS)	Cross Product Ratio ¹ (X)	95 Per Cent Confidence Limits	Departure of CPR from unity	
			χ^2_1	P
Mother-Offspring (Table V)	2.214	1.933-2.535	132.212	< .001
Father-Offspring (Table V)	1.298	1.162-1.451	21.118	< .001
Matings (Table VI)	1.810	1.564-2.096	63.111	< .001
Offspring Groupings (Table VI)				
<i>i</i> offspring; <i>c</i> offspring	2.201	1.901-2.549	111.454	< .001
Offspring of <i>i</i> categories; offspring of <i>c</i> categories	1.8490	1.651-2.070	113.652	< .001

¹ Cross Product Ratio =

$$\frac{(i \text{ combinations or } i \text{ grouping RhNS})(c \text{ combinations or } c \text{ grouping RhS})}{(i \text{ combinations or } i \text{ grouping RhS})(c \text{ combinations or } c \text{ grouping RhNS})}$$

C. ABO-incompatible versus ABO-compatible combinations *within Rh series* and *between Rh series*

In the total distributions represented in sections "A" and "B" above, the ABO blood groups and blood group combinations appear to depart from expectation depending upon maternal-fetal ABO-compatibility (Chart 2).

To clarify these findings the various blood group combinations are pooled into two classifications, the ABO-incompatible and ABO-compatible types (Chart 2) and examined as follows:

Table C1. Comparison of the observed number of ABO-incompatible types with the Hardy-Weinberg (H-W) expectancies *within each Rh series*.

Table C2. Comparisons *between Rh series* by estimation of the cross product ratios of the proportion of ABO-incompatibles in the Rh NS series and the pro-

portion in the Rh S series for corresponding combinations (Woolf, 1955). The statistical significance of the cross product ratios depends on departure from unity.

Tables C3. Comparisons with H-W expectancy *within* Rh series and *between* Rh series, as to the distribution of ABO-incompatible and ABO-compatible offspring of each parental type (Table C3a) and each mating category (Table C3b) capable of producing both ABO-incompatible and ABO-compatible offspring.

Only those phenotypic parent groups and mating categories capable of producing both ABO-incompatible and ABO-compatible offspring are included. However, while $O \times AB$ matings, which can produce only ABO-incompatible offspring, were excluded, no attempt was made to distinguish between homozygosity or heterozygosity in A or B fathers in other tabulated categories. Therefore, some of the $O \times A$ matings might well be of genotype $OO \times AA$ and thus incapable of producing ABO-compatible offspring.

The *i:c* ratios are given in terms of numbers of individuals (observed and expected) rather than in the form of ratios based on unity. This affords a direct comparison between observation and expectancy.

D. Reciprocal matings

A comparison of the reciprocal mating categories involving one parent of blood group "O" necessarily constitutes a comparison of ABO-incompatible (or potentially incompatible) mating categories with ABO-compatible mating categories containing parents of the same ABO phenotypes. The strategic mating categories (mother \times father) are $O \times A$ and $A \times O$; $O \times B$ and $B \times O$; $O \times AB$ and $AB \times O$.

It will be assumed [as was done in the comparison of the blood group distribution of mothers versus fathers (A,2)] that there are equal proportions of male and female adults of corresponding blood groups and therefore that an equal number of each category of any pair of reciprocal mating categories is to be expected. The possible role of a differential sex ratio (Sanghvi, 1951; Johnstone, 1954; Cohen and Glass, 1956, 1959b) among the various blood groups and of differential selection in infancy and childhood (Struthers, 1951) cannot be ruled out. However, for the present, it is better to choose the simplifying assumption.

The following comparisons are made:

Table D1. Relative frequency of families of reciprocal mating categories, in terms of the ratio "ABO incompatible category: ABO compatible category."

Tables D2a,b. Comparisons within and between series involving blood group distribution of offspring (O offspring and offspring not O) of the categories in "D1" above.

SUMMARY OF RESULTS

A consistent pattern emerges from the analyses of the complete ABO distribution of mothers, fathers, and offspring (population segments) and their combinations within series (A) and between series (B), the pooled ABO-incompatible and pooled ABO-compatible combinations (C), and the reciprocal matings (D).

TABLE C3A. DISTRIBUTION OF OFFSPRING OF POTENTIALLY ABO-INCOMPATIBLE PARENTAL TYPES (TABLE VI)

Parental Types	Comparisons With Expectancy Within Each Series				Comparisons Between Series				
		Rh NS Series <i>i</i> offspring: <i>c</i> offspring		Rh S Series <i>i</i> offspring: <i>c</i> offspring		Cross Product Ratio ¹	95 Per Cent Confidence Limits	Departure of CPR from Unity	
								χ^2_1	P
Mothers	O	Obs.	436:826 NSD	228:932	P < .001	2.158	1.637-2.844	29.795	< .001
		Exp.	410:852	377:783					
	A	Obs.	100:1051 NSD	46:1169	P < .001	2.418	1.779-3.287	31.761	< .001
		Exp.	93:1058	98:1117					
	B	Obs.	100:258 NSD	42:240	P < .001	2.215	1.483-3.307	15.108	< .001
		Exp.	85:273	67:215					
Fathers	A	Obs.	381:805 NSD	221:894	P < .001	1.915	1.582-2.318	44.368	< .001
		Exp.	390:796	367:748					
	B	Obs.	171:198 NSD	70:187	P < .001	2.307	1.638-3.249	22.893	< .001
		Exp.	163:206	113:144					
	AB	Obs.	84:30 NSD	25:34	P < .001	3.808	1.961->5.0	15.594	< .001
		Exp.	81:33	42:17					

$$^1 \text{ Cross Product Ratio} = \frac{(i \text{ offspring Rh NS})(c \text{ offspring Rh S})}{(i \text{ offspring RhS})(c \text{ offspring Rh NS})}$$

TABLE C3B. DISTRIBUTION OF OFFSPRING OF ABO-INCOMPATIBLE MATING CATEGORIES (TABLE VI)

Mating Category	Comparison with Expectancy Within Each Series				Comparisons Between Series				
		Rh NS Series <i>i</i> offspring: <i>c</i> offspring		Rh S Series <i>i</i> offspring: <i>c</i> offspring		Cross Product Ratio ¹	95 Per Cent Confidence Limits	Departure of CPR from Unity	
								χ^2_1	P
O × A	Obs.	283:231 NSD	182:230		1.548	1.193-	10.792	< .001	
	Exp.	296:218	238:174	P < .001		2.010			
O × B	Obs.	100:77 NSD	37:68		2.387	1.450-	11.694	< .001	
	Exp.	94:83	56:49	P < .001		3.930			
A × B	Obs.	71:76 NSD	33:40	.20 > P > .10	1.132	0.645-	1.873	.20 > P > .10	
	Exp.	78:69	39:34			1.989			
B × A	Obs.	98:88 NSD	39:39	.20 > P > .10	1.114	0.656-	1.589	.30 > P > .20	
	Exp.	107:79	45:33			1.890			
A × AB	Obs.	28:20 NSD	13:21	.20 > P > .10	2.262	0.921-	3.167	.10 > P > .05	
	Exp.	24:24	17:17			> 5.0			
B × AB	Obs.	2:6 NSD	3:6	.50 > P > .30	.667	0.080-	0.141	.80 > P > .70	
	Exp.	4:4	4.5:4.5			> 5.0			

$$^1 \text{ Cross Product Ratio} = \frac{(i \text{ offspring, RhNS})(c \text{ offspring, Rh S})}{(i \text{ offspring, RhS})(c \text{ offspring, Rh NS})}$$

Expected values are rounded to nearest whole number.

TABLE D1. NUMBER OF FAMILIES OF RECIPROCAL ABO-INCOMPATIBLE AND ABO-COMPATIBLE CATEGORIES (TABLE VI)

Matings	Comparisons within Each Series				Comparisons Between Series			
	Rh NS		Rh S		Cross Product Ratio†	95 Per Cent Confidence Limits	χ^2_1	P
	No. of Families	Observed Ratio*	No. of Families	Observed Ratio*				
O × A:A × O	405:405	1.00:1.00 (NSD)	197:274	0.72:1.00 (P < .001)	1.391	1.098-1.736	7.623	.01 > P > .001
O × B:B × O	135:118	1.14:1.00 (P > .30)	54:64	0.84:1.00 (P > .20)	1.356	0.875-2.102	1.853	.20 > P > .10
O × AB:AB × O	48:37	1.30:1.00 (P > .20)	6:22	0.27:1.00 (P < .01)	4.757	1.751->5	9.355	.01 > P > .001

* Expected ratio of ABO-incompatible:ABO-compatible categories would be 1.00:1.00. "P" values in these columns refer to the significance of the difference between observed and expected ratios.

† Cross Product Ratio = $\frac{(i \text{ category Rh NS})(c \text{ category Rh S})}{(i \text{ category Rh S})(c \text{ category Rh NS})}$

TABLE D2A. COMPARISON OF BLOOD GROUP DISTRIBUTION OF OFFSPRING OF RECIPROCAL MATING CATEGORIES WITHIN EACH SERIES (TABLE VI)

Reciprocal Categories in Comparison Ratio	Total No. Offspring per Mating	Cross Product Ratio*	95 Per Cent Confidence Limits	χ_1^2	p
<u>Rh NS Series</u>					
O × A	514				
and		1.036	0.811-1.325	0.081	.80 > p > .70
A × O	515				
O × B	177				
and		1.261	0.805-1.975	1.027	.50 > p > .30
B × O	136				
<u>Rh S Series</u>					
O × A	412				
and		-0.489	0.474-0.795	13.701	< .001
A × O	529				
O × B	105				
and		-0.671	0.301-0.868	6.169	.02 > p > .01
B × O	128				

* Cross Product Ratio =
$$\frac{(\text{not-O offspring } i \text{ mating category})(\text{O offspring } c \text{ mating category})}{(\text{not-O offspring } c \text{ mating category})(\text{O offspring } i \text{ mating category})}$$

TABLE D2B. COMPARISON BETWEEN SERIES OF BLOOD GROUP DISTRIBUTION OF OFFSPRING BY MATING CATEGORY (TABLE VI)

Mating Category in Comparison Ratio (Rh NS and Rh S Series)	Cross Product Ratio*	95 Per Cent Confidence Limits	χ_1^2	p
<u>i categories</u>				
O × A	1.548	1.193-2.010	10.792	.01 > p > .001
O × B	2.387	1.450-3.930	11.694	< .001
<u>c categories</u>				
A × O	0.916	0.719-1.169	0.494	.50 > p > .30
B × O	0.967	0.597-1.568	0.018	.90 > p > .80

* Cross Product Ratio =
$$\frac{(\text{not-O offspring, Rh NS})(\text{O offspring, Rh S})}{(\text{not-O offspring, Rh S})(\text{O offspring, Rh NS})}$$

for each mating category specified.

In both the *Rh-sensitized* (Rh S) series and the *Rh-nonsensitized* (Rh NS) series the ABO distribution of every population segment and every set of combinations (mother-offspring, father-offspring, matings and mating-offspring) is different from expectancy, significantly in the former series ($p < .001$), though not always in the latter series [Table A1].

The *Rh S series* shows a *deficiency* of those elements that are *ABO-incompatible* or most markedly associated with ABO-incompatibility, whereas the Rh NS

series shows a trend toward an *excess of ABO-incompatible* types and combinations [Chart 2, Table 1 (ABO-compatibility status); Appendix Tables II, III, V, VI (*observed distributions*)].

In view of the opposite direction of the deviations of the Rh NS and Rh S series from the Hardy-Weinberg expectancies, the contrast in the ABO distributions of the two series is sharpest when comparisons are made directly between them. Each of the comparisons involving population segments or sets of combinations shows a highly significant difference between the two series [Table B and Basic Tables II, III, V, VI].

That the aberrant ABO distributions are not a function of specific parental or offspring blood groups per se but in fact result from the ABO-compatibility status of combinations is illustrated in several ways.

First, the comparison of mothers with fathers within each series shows differences in their respective ABO-distributions (Table A2a) as well as differences in the proportion of total offspring produced by mothers and fathers of corresponding blood groups within each series (Table A2b). In the Rh S series there is a smaller proportion of O mothers (potentially ABO-incompatible, Table 1) and a larger proportion of A (relatively ABO-compatible) and AB (always ABO-compatible) mothers as compared to the frequencies of fathers of the same blood groups—and opposite ABO-compatibility status— $P < .01$. Likewise, in the Rh S series there is a smaller proportion of total offspring produced by O mothers and a larger proportion produced by A and AB mothers as compared to the proportion of total offspring produced by offspring of fathers of corresponding blood groups ($P < .001$).

The Rh NS series shows a tendency in the opposite direction both in the maternal-paternal ABO-distribution and in the relative proportions of offspring produced by the same maternal and paternal blood groups, although borderline significance is attained only in the offspring derived from the tabulation of matings.

Secondly, direct evidence of the role of ABO-compatibility status in the observed pattern comes from the comparison of the pooled observed ABO-incompatible combinations with the Hardy-Weinberg expectancies (Tables C1a and C1b). The *deficiency of ABO-incompatible* combinations in the *Rh S* series is significant at the .001 level in all comparisons. In the *Rh NS* series the *excess of ABO-incompatible* combinations attains statistical significance except for the father-offspring and mating-offspring combinations.

When comparisons are made directly between the *Rh NS* and *Rh S* series (Table C2), all sets of combinations (mother-offspring, father-offspring, matings, and offspring groupings from matings) emphasize the significantly larger proportion of ABO-incompatibles in the former series ($P < .001$). Cross-product ratios range from almost 1.3 in father-offspring to more than 2.2 for mother-offspring and mating-offspring sets.

Thirdly, the proportions of ABO-incompatible and ABO-compatible offspring produced by mothers of each blood group and fathers of each blood group capable of producing both types of offspring (Table C3a), as well as by each mating

category (Table C3b) capable of producing both ABO-incompatible and ABO-compatible offspring, confirm the overall pattern. In the Rh S series O, A, and B mothers and A, B, and AB fathers (Table C3a) and $O \times A$ and $O \times B$ matings (Table C3b) all show a *significantly smaller* proportion of ABO-incompatible offspring than expected. In the Rh NS series a tendency toward an excess of ABO-incompatible offspring (not statistically significant) is observed when classification is based on individual parental types, although the individual mating categories show some irregularities.

Accordingly, the contrasting pattern in the Rh NS and Rh S series is reflected in comparisons of the relative proportions of ABO-incompatibles in the two series. The cross-product ratios for offspring of corresponding parental types indicate approximately twofold or greater (1.915–3.808) differences between the two series. The trend shown by the offspring of mating categories is similar but not so striking, with significance in comparisons between series only in the $O \times A$ and $O \times B$ categories, which yield cross-product ratios of 1.5 to >2.3 .

Fourthly, the *reciprocal mating* categories, both in their deviations from the expected 1:1 ratio of relative frequency and in the blood group distributions of their offspring, show a significant deficiency of ABO-incompatibles in the Rh S series [deficiency of $O \times A$, and $O \times AB$ matings (Table D1) and deficiency of A offspring in $O \times A$ matings and B offspring in $O \times B$ matings (Table D2a) relative to the findings in their reciprocal mating categories]. In the Rh NS series there is a trend (not statistically significant) in the opposite direction. The contrast in the relative frequency of reciprocal mating categories of the two series (Table D1) is highly significant for the $O \times A:A \times O$ group (cross-product ratio = 1.39) and for the $O \times AB:AB \times O$ group (cross-product ratio = 4.76).

The striking ratio in the $O \times AB:AB \times O$ might well be attributable to the fact that the $O \times AB$ category is the only ABO-incompatible mating category that is unconditionally ABO-incompatible—no ABO-compatible offspring can be produced. In the collection of families of Rh-sensitized women reported by Levine (1958) the ratio of $O \times AB:AB \times O$ matings was 1:59 when transfused mothers were eliminated. While no attempt has been made in the Rh S series of the present study to separate all those matings where Rh sensitization may have been caused or influenced by factors other than Rh-incompatible pregnancies in the particular mating recorded, it is noteworthy that all of the six $O \times AB$ matings listed in the Rh S series involved women who had either been transfused (families 1279, 1303, and 1542), or had been married at least once prior to the current marriage (families 866, 922, and 1490).

In regard to the blood group distributions of offspring, the comparisons between reciprocal categories reach levels of statistical significance only in the Rh S series, although the opposing trends in the two series are apparent from the cross-product ratios of ABO-incompatibles to ABO-compatibles (more than unity in the Rh NS and less than unity in the Rh S—Table D2a). Comparisons between series for offspring distribution in each mating category reveal, in the ABO-incompatible categories $O \times A$ and $O \times B$, a marked contrast between the two series, whereas in the reciprocal ABO-compatible categories (in which both

alternative types of offspring O and not-O are alike ABO-compatible) there are no significant differences between the two series in the blood group distribution of the offspring (Table D2b).

A more detailed analysis of the reciprocal mating categories has been made and is to be presented in the near future. It is noteworthy that when the data are analyzed grouping observations by total number of pregnancies per mating and by pregnancy order, the findings are consistent with the pattern indicated by the pooled groups reported here. This suggests that the ABO blood group differences between the two series cannot be attributed to pregnancy order, or maternal age.

DISCUSSION

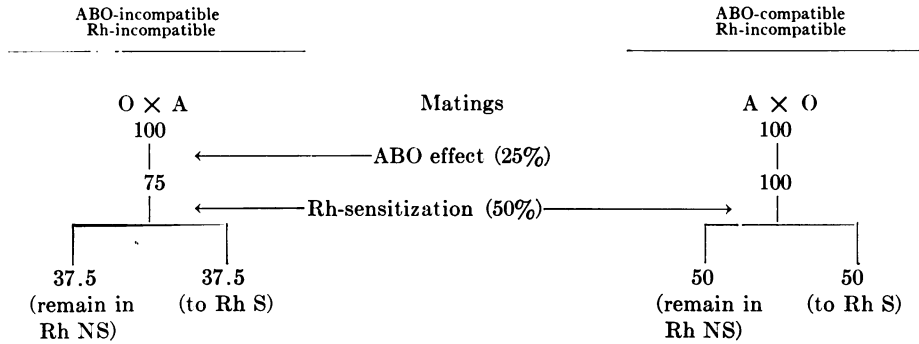
By approaching the data from many points of view the inner consistency of the ABO-Rh relationship is repeatedly demonstrated. Even the nonsignificant trends in the Rh NS series seem fairly well established by means of repeated appearance under different methods of analysis. Clearly, the ABO distributions of the fertile Rh-incompatibly mated population samples, as represented by the Rh NS (not sensitized) and Rh S (sensitized) series not only deviate significantly from one another, but, even more definitively, deviate in opposite directions from theoretical (Hardy-Weinberg) expectancies.

The highly significant deficiency of ABO-incompatibles in the Rh S series of this study is consistent with the findings of other investigators who have examined the families of Rh-sensitized women (Levine, 1945, 1958; Wiener, 1945; Heistö, 1955; Reepmaker, 1956 and others). The opposite tendency, toward an excess of ABO-incompatibles, here seen in the Rh NS series, is, on the other hand, a deviation opposite in direction not only from that in the Rh S series of this study, but also from those in every other Rh-sensitized, Rh-mixed (Waterhouse and Hogben, 1947, Johnstone, 1954a), or Rh-compatibly mated (Matsunaga, 1959) series thus far reported.

Several theories have previously been proposed to explain Levine's original observation of a deficiency of ABO-incompatibly mated Rh-negative mothers of erythroblastotic offspring. All such theories will explain a deficiency of ABO-incompatible types in the Rh-sensitized series. The use of a nonsensitized Rh-incompatibly mated series (Rh NS) now makes possible a decision as to the correct hypothesis.

The deviations from expectation observed in the present data are not explicable on the basis of simple negative selection against A- and B-incompatible types, as Fisher suggested (1944, according to Race, MRC Memorandum No. 27). Such a theory would account for the aberrant ABO distribution in Rh unselected and Rh-compatibly mated samples, but, because it postulates that ABO-incompatibility and Rh-incompatibility act independently of one another, this theory would also require that the Rh NS series show a deficiency of ABO-incompatibles similar to that in the Rh S series. For example, if ABO-incompatibility selected against 25 percent of the ABO-incompatible matings and Rh-incompatibility independently caused 50 percent of Rh-incompatible matings to

become Rh-sensitized, the following might be expected in the reciprocal ABO-incompatible O × A and ABO-compatible A × O matings:



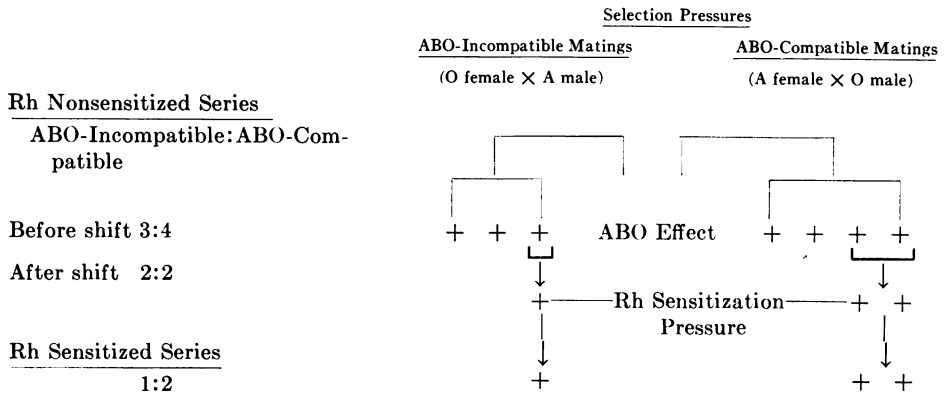
Thus the ratio of ABO-incompatible:ABO-compatible matings would be the same in the Rh NS and the Rh S series, 37.5:50, or .75:1.00. That is to say, both the Rh NS and Rh S series would show a relative deficiency—the same deficiency—of ABO-incompatible types.

Still another proposed explanation for the marked deficiency of ABO-incompatible matings among Rh-sensitized mothers attributes the phenomenon to inherent, most probably genetic, differences in the ability to produce antibodies, i.e., to differences among mothers in “sensitizability.” According to this hypothesis, Rh-sensitized mothers would be not only good Rh antibody-makers but also good anti-A or anti-B makers also. Thus by selection for Rh-sensitized mothers, selection would have been made simultaneously for a strong ABO-incompatibility effect.

The Rh NS series, on the other hand, since it is a group selected for non-sensitization to Rh, would then be expected to be composed of poorly sensitizable or nonsensitizable mothers, showing neither ABO nor Rh response. However, since all mothers are classified as nonsensitized until they produce antibodies, the Rh NS series must be considered in a dual role: it is the “parent” population of the Rh S series as well as a comparable “sister” population. As the parent population, the Rh NS series must contain some families which are potentially destined for the Rh S series, and these families not being true “nonsensitizables,” would be expected to show an ABO-incompatibility effect similar to that of the Rh S series. The resultant Rh NS series should therefore show “no difference” from expectation or possibly a slight trend toward a *deficiency* of ABO-incompatible, as in the Rh S series. On the basis of the sensitizability hypothesis, therefore, one would not at all expect a consistent trend toward an *excess* of ABO-incompatibles in the Rh NS series, such as actually occurs.

The only current theory that can explain both the deficiency of ABO-incompatibles in the sensitized Rh-incompatibly mated population (Rh S series) and at the same time an excess of ABO-incompatibles in the nonsensitized Rh-incompatibly mated population is one that postulates an interaction of ABO-incompatibility and Rh-incompatibility, most probably involving a protective

CHART 3. The preferential shift in Rh-incompatible matings of ABO-compatible matings in comparison with ABO-incompatible matings (after Cohen and Glass, 1959a)



Each “+” represents an equal number of matings.

The primary ABO effect is represented by the reduction in number of +’s (fertile matings) representing the ABO-incompatible matings as compared with the ABO-compatible matings (i.e. 3:4).

The differential in Rh sensitization pressure is represented by the number of +’s shifted relative to the number belonging to the mating category before the shift (i.e., 1/3 for O × A matings; 2/4 for A × O matings).

action of ABO-incompatibility against the Rh effect. A differential Rh-sensitization pressure favoring ABO-incompatible over ABO-compatible matings would produce a preferential shift of ABO-compatible matings from the Rh NS series into the Rh S series. As a consequence, a deficiency of ABO-incompatible matings would occur in the Rh S series, whereas a backlog (or excess) of ABO-incompatible matings would be left in the Rh NS series [an hypothetical example of this shift appears in Chart 3 (Fig. 1, Cohen and Glass, 1959a)].

By manipulating the assumed values for ABO and Rh selection pressures, it may readily be demonstrated that a *difference in negative Rh selection pressure superimposed on the negative AB selection pressure can actually yield either a deficiency, excess, or equality* of the ABO-incompatibly mated families in comparison with their reciprocal ABO-compatibly mated families in the Rh NS series, while the Rh S series still shows a deficiency. Some of the factors that could determine which of the three relations—deficiency, excess, or equality—will occur are the following:

- (1) The degree of negative selection against A and B imposed on ABO-incompatibles in comparison with ABO-compatibles—from the effect of ABO-incompatibility alone.
- (2) The difference between the Rh sensitization pressures for ABO-incompatibles and ABO-compatibles.
- (3) The magnitude or level of Rh sensitization pressures.
- (4) The magnitude of Rh sensitization pressures relative to the primary negative ABO selection pressure in (1) above.

(5) The relative size of (a) the differential between the Rh-sensitization pressures and (b) the AB negative selection pressure.

The findings of this investigation suggest an interaction of these five components of the total selection pressure at such levels as to yield a marked *deficiency* of ABO-incompatibles in the Rh-sensitized series and a slight borderline *excess* of ABO-incompatibles in the Rh-nonsensitized series. This difference in degree of the deviations as well as direction of the deviations in the two series is apparent when the effects of the component selection pressures for each series are considered. In the Rh S series both the simple negative selection against A- and B-incompatibles in early pregnancy and the protective action of ABO-incompatibility against Rh sensitization contribute to the size of the deficiency of ABO-incompatibles. On the other hand, in the Rh NS series, these two components of selection oppose one another. Selection against ABO-incompatibles in early pregnancy tends to diminish the excess of ABO-incompatibles produced by the protective action of ABO-incompatibility against Rh-sensitization.

From the present observations of the ABO-Rh interaction in the Rh NS and Rh S series, the probable effects upon fetal survival and differential blood group distribution of offspring in the total Rh-incompatibly mated segment of the population may be postulated. The concept has been illustrated in a theoretical example [see Chart 4 (Figure 2, Cohen and Glass, 1959a)] in which there is no separation into nonsensitized and sensitized series, but instead the ultimate fate of all Rh-incompatible zygotes is considered.

The illustration is based on the following simplifying assumptions:

(1) ABO-incompatibility tends to manifest its deleterious effects primarily in early pregnancy, sometimes so early that pregnancy is unrecognized (Brambell et al., 1951; Waterhouse and Hogben, 1947; Matsunaga 1955, 1956, 1958, 1959). AB hemolytic disease is relatively so rare (1 in 1100 pregnancies) that it need not be considered in comparison with the early ABO effect (1 in 25 pregnancies).

(2) Rh incompatibility manifests its harmful effects in late pregnancy and early neonatal life, and not at all in the first half of pregnancy (Overstreet, Trent, Hunt, and Lucia, 1947; Glass, 1949).

The effect of simple ABO-incompatibility in early pregnancy is postulated to be such that 20 percent of ABO-incompatible zygotes are eliminated. Superimposed on this effect is a 20 per cent differential in Rh selection pressure favoring the ABO incompatible zygotes; 70 percent of the ABO-compatible Rh-incompatible zygotes survive as compared to 90 percent of ABO-incompatible Rh-incompatibles, which have passed the early 20% negative A-B selection.

As a consequence of these selection pressures the model yields more survivors in ABO-incompatible matings than in ABO-compatible matings: 72 percent survivors in OO \times AA matings in comparison with 70 percent in AA \times OO, 71 percent in OO \times AO as compared with 70 percent in AO \times OO. In addition, there is a higher percentage of A survivors among total conceptions and among all survivors in the progeny of ABO-incompatible matings than in the progeny of ABO-compatible matings.

Percentage of Type A

	Among Total Conceptions	Among Survivors Only
OO × AA	72%	100% A
AA × OO	70%	100% A
OO × AO	36%	50.7% A
AO × OO	35%	50.0% A

} only type possible

Thus with reasonable combinations of ABO-selection pressures and differentials in Rh selection pressures for ABO-incompatible and ABO-compatible offspring it is apparent that the doubly incompatible zygotes, though selected

CHART 4. Opposing selection pressures in Rh-incompatible matings

(after Cohen and Glass, 1959a)

A simplified hypothetical example

Independent ABO Effect: 80% (20% negative selection against ABO-incompatible zygotes)

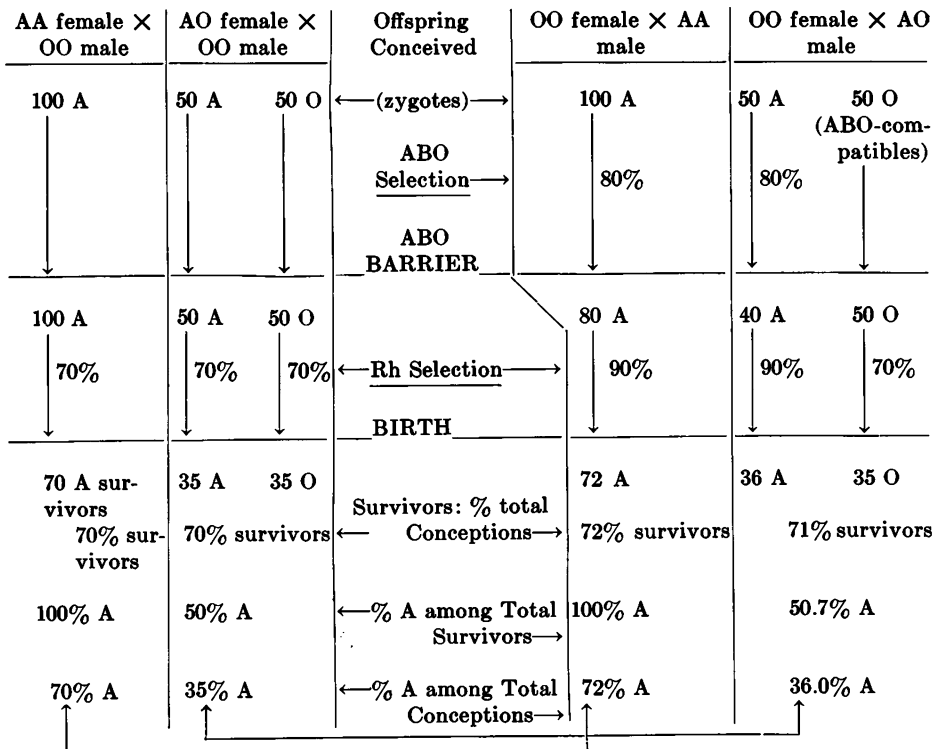
Rh Effect: Differential in Selection Pressure = 20%

ABO-compatible zygotes 70% 30% negative selection

ABO-incompatible zygotes 90% 10% negative selection

ABO-Compatible Matings

ABO-Incompatible Matings



against by two negative selection pressures (ABO and Rh), can actually show a higher rate of survivorship and a higher proportion of non-O survivors than the singly incompatible (ABO-compatible, Rh-incompatible) zygotes.

The statistical findings presented in this investigation and other studies (Grubb and Sjöstedt, 1955) support the view that the double heterozygote (ABO-incompatible, Rh-incompatible) is, in fact, superior in fitness to the single heterozygote (ABO-compatible, Rh-incompatible), as assumed in the model. The evidence is furthermore consistent with the view that the selective disadvantage of ABO-incompatibility in early pregnancy is counteracted by the selective advantage of ABO-incompatibility in protecting against the deleteriousness of Rh-incompatibility in later pregnancy (Cohen and Glass 1959a).

Several hypotheses have been advanced to account for the biological mechanism whereby ABO-incompatibility between mother and fetus can protect an Rh-negative mother against isoimmunization to Rh. For the most part the weight of evidence seems equally favorable to each of two biological mechanisms, competition between antigens (Wiener, 1945) and destruction of ABO-incompatible fetal erythrocytes, when they get into the maternal circulation, by the maternal anti-A or anti-B present there (Race, 1952; Levine, 1958, and others). Neither acquired tolerance (Owen et al., 1954), nor injections of ABO-incompatible Rh-incompatible and ABO-compatible Rh-incompatible blood (Stern, Davidsohn, and Masaitis, 1956), nor the clinical cases of combined AB and Rh hemolytic disease (Gunson, 1957) permit a definite choice between the two explanations. Moreover, since the two theories are not mutually exclusive, it remains possible, as a third alternative, that both are valid. It is feasible that the protective action of ABO-incompatibility may itself be mediated in more than one way and that quantitative as well as qualitative effects need to be considered.

That the ramifications of selective interaction involved in this phenomenon are numerous and are not to be regarded as completely resolved is apparent. In the present investigation of the ABO-Rh interaction, additional findings from the detailed analysis of reciprocal matings, including data on pregnancy wastage and the blood group distribution of offspring classified by pregnancy order and total number of pregnancies, remain to be presented. Empirical estimates and mathematical models of the protective action of ABO-incompatibility against the Rh effect are being formulated. We may hope that other populations will be studied by biometricians, epidemiologists, serologists, and clinicians, as well as by geneticists, for the purpose of clarifying the biological mechanisms involved, refining the estimates for the clinical outcome of pregnancies, and indicating the role of the dual incompatibility phenomena on evolution. The effect of Rh-incompatibility on Rh-rh frequencies in future generations has already been studied by Haldane (1942), Wiener (1942), Glass (1950), and Levine (1958). Nevertheless, it is even now apparent—though still too soon to make precise estimates—that the interaction ABO-incompatibility and Rh-incompatibility must be considered in evaluating the evolutionary trends of allele frequencies at both the ABO and the Rh loci.

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SUMMARY

1. The ABO distributions of Rh-nonsensitized and Rh-sensitized Rh-incompatibly mated population samples not only deviate significantly from one another, but also deviate in opposite directions from Hardy-Weinberg expectancies.

2. Several theories previously proposed to explain the deficiency of ABO-incompatibles in Rh-sensitized samples may be excluded through the utilization of an Rh-nonsensitized as well as an Rh-sensitized series. The only hypothesis which can explain simultaneously both the *deficiency* of ABO-incompatibles in the Rh-sensitized series and the *excess* of ABO-incompatibles in the Rh-nonsensitized series is an interaction between ABO-incompatibility and Rh-incompatibility, such that ABO-incompatibility tends to inhibit Rh-isoimmunization, and possibly also modifies its consequences.

3. The theoretical basis of the superiority of the double heterozygote (ABO-incompatible, Rh-incompatible) over the single heterozygote (ABO-compatible, Rh-incompatible) is considered along with hypothetical models to illustrate (a) the preferential shift of ABO-compatible matings from the Rh-nonsensitized series to the Rh-sensitized series; and (b) differential fetal survival and blood group distribution of offspring as a result of the ABO-Rh interaction.

4. The possible biological mechanisms underlying the ABO-Rh interaction and its possible impact on allele frequencies in future generations are discussed.

REFERENCES

- BOORMAN, K. E. 1949. An analysis of the blood types and clinical condition of 2000 consecutive mothers and their infants. *Ann. Eugen.* 15: 120-134.
- BOORMAN, K. E., DODD, B. E., AND MOLLISON, P. L. 1945. Iso-immunization to the blood group factors A, B, and Rh. *J. Path. Bact.* 57: 157.
- BRAMBELL, F. W., HEMMINGS, W. A., AND HENDERSON, M. 1951. *Antibodies and Embryos*. London: Athlone Press.
- BRENDEMOEN, O. J. 1952. Some factors influencing Rh immunization during pregnancy. *Acta path. microb. scand.* 31: 579-583.
- BRESLER, J. (presentation 1959 meetings American Society of Human Genetics)
- BROMAN, *Acta paediat.* 32, Supp. 2, 1944 (Quoted from Wiener, 1945).
- BRYCE, L. M., JAKOBOWICZ, R., McARTHUR, N., AND PENROSE, L. S. 1950. Blood group frequencies in a mother and infant sample of the Australian population. *Ann. Eugen.* 15: 271-275.
- COCHRAN, W. G. 1952. The χ^2 test of goodness of fit. *Ann. Math. Stat.* 23: 315-345.
- COHEN, B. H., AND GLASS, B. 1956. The ABO blood groups and the sex ratio. *Human Biol.* 28: 20-42.

- COHEN, B. H., AND GLASS, B. 1959a. The relation of the ABO and Rh blood groups to differential reproduction. *J. Med. Educ.* 34: 414-417. Reprinted in *Am. J. Human Genet.* 11: 414.
- COHEN, B. H., AND GLASS, B. 1959b. Further observations on the ABO blood groups and the sex ratio. *Am. J. Human Genet.* 11: 274-278.
- DIENST, A. 1905. Das Eklampsiegift. Vor Säufige Mitteilung. *Zbl. Gyn.* 29: 353-364.
- DOBSON, A. M. AND IKIN, E. W. 1946. The ABO blood groups in the United Kingdom; frequencies based on a very large sample. *J. Path. Bact.* 58: 221-227.
- DUNGERN, E., VON AND HIRSZFELD, L. 1911. Ueber gruppenspezifische Strukturen des Blutes. *Zschr. Immun. Forsch.* 8: 526-562.
- FISHER, R. A. AND YATES, F. 1953. *Statistical Tables for Biological Agricultural and Medical Research*, 4th ed., London: Oliver and Boyd.
- GLASS, B. 1949. The relation of Rh incompatibility to abortion. *Am. J. Obst.* 57: 323-332.
- GLASS, B. 1950. The action of selection on the principal Rh alleles. *Am. J. Human Genet.* 2: 269-278.
- GLASS, B. AND LI, C. C. 1953. The dynamics of racial intermixture—an analysis on the American Negro. *Am. J. Human Genet.* 5: 1-20.
- GRUBB, R. AND SJÖSTEDT, S. 1955. Blood groups in abortion and sterility. *Ann. Human Genet.* 19: 183-195.
- GUNSON, H. H. 1957. Combined Rh and AB hemolytic disease of the newborn. *Am. J. Clin. Path.* 27: 35-41.
- HALDANE, J. B. S. 1942. Selection against heterozygosis in man. *Ann. Eugen.* 11: 333-340.
- HEISTÖ, H. 1955. The relation of ABO-blood groups to Rh immunization in pregnancy. With special reference to A₁ and A₂ subgroups. *Acta path. et microb. scand.* 36: 257-262.
- HIRSZFELD, H. AND HIRSZFELD, L. 1928. Weitere Untersuchungen über de Vererbung der Empfänglichkeit für Infektionskrankheiten. *Zschr. Immunforsch.* 54: 81-104.
- HIRSZFELD, L. 1928. *Konstitutionsserologie und Blutgruppen forschung*. Berlin: J. Springer. 1928.
- HIRSZFELD, L. AND ZBOROWSKI, H. 1925. Gruppenspezifische Beziehungen zwischen Mutter und Frucht und elektive Durchlässigkeit der Placenta. *Klin. Wschr.* 4: 1152-1157.
- JOHNSTONE, J. M. 1954a. Heterospecific pregnancy. *Brit. J. Prev. Soc. M.* 8: 117-123.
- JOHNSTONE, J. M. 1954b. Sex ratio and the ABO blood group system. *Brit. J. Prev. Soc. M.* 8: 124-127.
- KIRK, R. L., KIRK, M. AND STENHOUSE, N. S. 1953. Differential fertility between women of blood groups O and A. *Brit. J. Prev. Soc. M.* 1: 1-8.
- KIRK, R. L., SHIELD, J. W., STENHOUSE, N. S., BRYCE, L. M. AND JAKOBOWICZ, R. 1955. A further study of A-B-O blood groups and differential fertility among women in two Australian maternity hospitals. *Brit. J. Prev. Soc. M.* 9: 104-111.
- LEVINE, P. 1943. Serological factors as possible causes of spontaneous abortions. *J. Hered.* 34: 71-82.
- LEVINE, P. 1948a. The mechanism of transplacental isoimmunization. *Blood* 3: 404-413.
- LEVINE, P. 1958. The influence of the ABO system on Rh hemolytic disease. *Human Biol.* 30: 14-28.
- LEVINE, P., VOGEL, P., AND ROSENFELD, R. E. 1953. Hemolytic disease of the newborn. *Adv. Pediat.* 6: 97-156.
- LI, C. C. 1955. *Population Genetics*. University of Chicago Press.
- LOGHEM, J. J., VAN AND SPAANDER, J. 1948. L'influence de l'incompatibilité du système ABO sur l'antagonisme Rh. *Rev. hémat.* 3: 276-286.
- LUCIA, S. P. AND HUNT, M. L. 1950a. The significance of ABO compatibility and its relationship to the intensity of Rh immunization. *Blood* 5: 767-772.
- LUCIA, S. P. AND HUNT, M. L. 1950b. Hemolytic disease of the newborn. I. An analysis of a sample of an obstetric population. *J. Laborat. Clin. M.* 35: 24-27.

- LUCIA, S. P. AND HUNT, M. L. 1950c. Hemolytic disease of the newborn. II. An analysis of maternal antipartum factors in a sample of sensitized Rh negative women in relation to the clinical condition of the child. *J. Laborat. Clin. M.* 35: 28-34.
- MALONE, R. H. 1949. ABO incompatibility between husband and wife and its relation to Rh immunization. *Brit. J. Social M.* 4: 228-229.
- MATSUNAGA, E. 1955. Intra-uterine selection by the ABO incompatibility of mother and foetus. *Am. J. Human Genet.* 7: 66-71.
- MATSUNAGA, E. 1956. Selektion durch Unverträglichkeit im ABO-Blutgruppen-system zwischen Mutter und Fetus. *Blut Zschr. Gesamte Blutforschung II*: 188-198.
- MATSUNAGA, E. AND ITOH, E. 1958. Blood groups and fertility in a Japanese population (with special reference to intra-uterine selection due to maternal-foetal incompatibility). *Ann. Human Genet.* 22: 111-131.
- MATSUNAGA, E. 1959. Selection in ABO polymorphism in Japanese populations. *J. Med. Educ.* 34: 405-413.
- NEVANLINNA, H. R. 1953. Factors affecting maternal Rh immunization. *Annules Med. Exp. et Biol. Fenniae*, vol. 31, suppl. 2.
- NEVANLINNA, H. R. AND VAINIO, T. 1956. The influence of mother-child ABO-incompatibility on Rh immunization. *Vox Sang.* 1: 26-36.
- OVERSTREET, E. W., TRENT, H. F., HUNT, M. AND LUCIA, S. P. 1947. Does Rh-isoimmunization cause early abortion. *Am. J. Obst.* 54: 235-241.
- OWEN, R. D., WOOD, H. R., FOORD, A. G., STURGEON, P. AND BALDWIN, L. G. 1954. Evidence for actively acquired tolerance to Rh antigens. *Proc. Nat. Acad. Sc.* 40: 420-424.
- RACE, R. R. 1952. Part I, The Rh groups. In, *The Rh Blood Groups and their Clinical Effects*. Medical Research Council Memor. No. 27, London: Her Majesty's Stationery Office.
- RACE, R. R., TAYLOR, G. L., CAPPELL, D. F. AND McFARLANE, M. M. 1943. The Rh factor and erythroblastosis fetalis. An investigation of 50 families. *Brit. M. J.* 2: 289-292.
- REED, T. E. 1956. Tests of models representing selection in mother child data on ABO blood groups. *Am. J. Human Genet.* 8: 257-268.
- REED, T. E. AND KELLY, E. L. 1958. The completed reproductive performances of 161 couples selected before marriage and classified by ABO blood groups. *Ann. Human Genet.* 22: 165-181.
- REEPMAKER, J. 1956. *ABO Antagonisme en Morbus Haemolyticus Neonatorum*. H. E. Stenfert Kroese, N. V. Leiden.
- SANGHVI, L. D. 1951. ABO blood groups and sex-ratio at birth in man. *Nature* 168: 1077.
- SNEDECOR, G. W. 1950. *Statistical Methods*, 4th ed., Iowa State College Press.
- SPEISER, P. AND JANCIK, E. W. 1953. Über den immunobiologischen Antagonismus zwischen den Klessichen Blutgruppen und den Blutfaktoren beim Zustandkommen eines Factoren bedingten morbus hemolyticus neonatorum. *Klin. Wschr.* 31: 995-997.
- STERN, K., DAVIDSOHN, I. AND MASAITIS, L. 1956. Experimental studies on Rh immunization. *Am. J. Clin. Path.* 26: 833-843.
- STRUTHERS, D. 1951. ABO groups of infants and children dying in the west of Scotland. *Brit. J. Social M.* 4: 223-226.
- WATERHOUSE, J. A. H. AND HOGGEN, L. 1947. Incompatibility of mother and foetus with respect to the iso-agglutinin A and its antibody. *Brit. J. Social. M.* 1: 1-17.
- WIENER, A. S. 1942. The Rh factor and racial origins. *Science* 96: 407-408.
- WIENER, A. S. 1945. Competition of antigens in iso-immunization by pregnancy. *Proc. Soc. Exp. Biol.* 58: 133-135.
- WIENER, A. S., NAPPI, R. AND GORDON, E. B. 1953. Studies in Rh sensitization. VI. ABO blood groups and Rh subtypes in sensitized and nonsensitized Rh negative pregnant women. *Blood* 8: 1024-1028.
- WOOLF, B. 1955. On estimating the relation between blood group and disease. *Ann. Human Genet.* 19: 251-253.

TABLE I. ABO BLOOD GROUP DISTRIBUTION OF WHITE AND NEGRO MOTHERS¹ OF BALTIMORE

	O						A						B						AB						Total Number		
	White			Negro			White			Negro			White			Negro			White			Negro			White	Negro	
	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±	No.	Per Cent	±
Rh NS Series	1109	43.5	± 1.0	185	47.6	± 2.5	1048	41.1	± 1.0	108	27.8	± 2.3	296	11.7	± 0.6	83	21.3	± 2.1	99	3.9	± 0.4	13	3.3	± 0.9	2552	389	2941
Rh S Series	564	40.5	± 1.3	68	47.6	± 4.2	651	46.8	± 1.3	48	33.6	± 4.0	125	9.0	± 0.8	18	12.6	± 2.8	52	3.7	± 0.5	9	6.3	± 2.0	1392	143	1535
Glass and Li	45.2			47.6			39.5			28.3			11.2			20.0			4.2			4.1					

Significance Tests: Comparison of observed ABO distribution with expectancy based on distribution of Glass and Li:

Negro

$$\begin{aligned} \text{Rh NS Series} \quad \chi^2_3 &= 4.235, .30 > p > .20 & \chi^2_3 &= 0.948, .90 > p > .80 \\ \text{Rh S Series} \quad \chi^2_3 &= 32.279, p < .001 & \chi^2_3 &= 6.947, .10 > p > .05 \end{aligned}$$

White

¹ All mothers tabulated in mother-offspring combinations and/or mating categories for whom race and blood group are known.

TABLE II. ABO BLOOD GROUP DISTRIBUTION OF PARENTS INCLUDED IN MATINGS OF Rh NS AND Rh S SERIES

	O			A			B			AB			Total Off-spring
	No. Parents	% Total Parents	No. of Off-spring	No. Parents	% Total Parents	No. of Off-spring	No. Parents	% Total Parents	No. of Off-spring	No. Parents	% Total Parents	No. of Off-spring	
Rh NS Series													
Mothers	2313	43.58 ± 1.03	2242	923	39.90 ± 1.02	1978	204	12.71 ± 0.69	658	88	3.80 ± 0.40	186	5064
Fathers	2313	42.37 ± 1.03	2133	940	40.64 ± 1.02	2053	291	12.58 ± 0.69	653	102	4.40 ± 0.43	225	5064
Total Parents	4626	42.97 ± 0.73		1863	40.27 ± 0.72		585	12.64 ± 0.49		190	4.11 ± 0.29		
Expectancy		45.3			38.7			11.8			4.2		
Rh S Series													
Mothers	1383	41.50 ± 1.32	1716	620	44.83 ± 1.34	1872	133	9.62 ± 0.79	397	56	4.04 ± 0.53	175	4160
Fathers	1383	48.95 ± 1.34	2016	544	39.33 ± 1.31	1652	128	9.26 ± 0.78	398	34	2.46 ± 0.42	94	4160
Total Parents	2766	45.23 ± 0.95		1164	42.08 ± 0.94		261	9.44 ± 0.56		90	3.25 ± 0.34		
Expectancy		45.3			38.6			11.9			4.2		

TABLE III. ABO BLOOD GROUP DISTRIBUTION OF OFFSPRING OF MATINGS OF Rh NS AND Rh S SERIES

Offspring	Grand Total	O Offspring		A Offspring		B Offspring		AB Offspring		Unknown Blood Group	
		No.	% Known Blood Group	No.	% Known Blood Group	No.	% Known Blood Group	No.	% Known Blood Group	No.	% Total Blood Group
Rh NS Series											
Females	2385	621	44.87 ± 1.34	528	38.15 ± 1.31	1177	12.79 ± 0.90	58	4.19 ± 0.54	1001	41.97 ± 1.01
Males	2457	663	45.23 ± 1.30	555	37.86 ± 1.27	190	12.96 ± 0.88	58	3.96 ± 0.51	991	40.33 ± 0.99
Male + Female	4842	1284	45.05 ± 0.93	1083	38.00 ± 0.91	387	12.88 ± 0.63	116	4.07 ± 0.37	1992	41.14 ± 0.71
Sex Unknown	222	12	32.43 ± 7.70	14	37.84 ± 7.98	6	16.22 ± 6.06	5	13.51 ± 5.62	185	83.33 ± 2.50
Total Offspring	5064	1296	44.89 ± 0.93	1097	38.00 ± 0.90	373	12.92 ± 0.63	121	4.19 ± 0.37	2177	42.99 ± 0.70
Expectancy		45.3		38.7		11.8		4.2			
Rh S Series											
Females	1740	637	50.64 ± 1.41	476	37.84 ± 1.37	115	9.14 ± 0.81	30	2.38 ± 0.43	482	27.70 ± 1.07
Males	1938	694	50.55 ± 1.35	521	37.95 ± 1.72	121	8.81 ± 0.59	37	2.69 ± 0.44	565	29.15 ± 1.03
Male + Female	3678	1331	50.59 ± 0.97	997	37.89 ± 0.95	236	8.97 ± 0.56	67	2.55 ± 0.31	1047	28.47 ± 0.74
Sex Unknown	482	73	49.66 ± 4.12	51	34.69 ± 3.93	16	10.88 ± 2.57	7	4.76 ± 1.76	335	69.50 ± 2.10
Total Offspring	4160	1404	50.54 ± 0.95	1048	37.72 ± 0.92	252	9.07 ± 0.54	74	2.66 ± 0.31	1382	33.22 ± 0.73
Expectancy		45.3		38.6		11.9		4.2			

Expectancy based on U. S. population blood group frequencies (Glass and Li) and racial distribution of series.

TABLE IV. MEAN MATERNAL AGES BY ABO BLOOD GROUP OF MOTHERS

Series	O		A		B		AB		Total	
	No.	Mean Age ±S.E.	No.	Mean Age ±S.E.	No.	Mean Age ±S.E.	No.	Mean Age ±S.E.	No.	Mean Age ±S.E.
Rh NS	1294	27.045 ±0.155	1156	27.393 ±0.171	379	27.223 ±0.305	112	27.888 ±0.586	2941	27.237 ±0.106
Rh S	612	28.423 ±0.229	685	28.870 ±0.224	136	28.118 ±0.484	60	29.025 ±0.743	1493	28.625 ±0.149
T-tests: Rh NS vs. Rh S Series		t = 4.976 P < .01		t = 5.241 P < .01		t = 1.566 NSD		t = 1.201 NSD		t = 7.601 P < .01

Significance Tests:

Most deviant pair within series:
Other deviations within series:

Rh NS Series

O vs. AB t = 1.390 NSD
O vs. A t = 1.505 NSD

Rh S Series

B vs. AB t = 1.023 NSD
O vs. A t = 1.395 NSD
O vs. AB t = 0.778 NSD

TABLE V. DISTRIBUTION OF PARENT-OFFSPRING BLOOD GROUP COMBINATIONS

Offspring	Series	Ascertainment	Mothers					Fathers				
			O	A	B	AB	Total	O	A	B	AB	Total
O	Rh NS	Single	876	365	133		1374	654	286	88		1028
		Multiple	1027	432	148		1607	805	348	114		1267
	Rh S	Single	462	183	52		697	383	170	39		592
		Multiple	1024	395	97		1516	880	354	90		1324
A	Rh NS	Single	344	695	49	54	1142	254	544	34	35	867
		Multiple	407	828	57	64	1356	306	695	42	42	1085
	Rh S	Single	74	395	7	23	499	148	261	15	20	444
		Multiple	204	850	20	50	1124	324	629	33	31	1017
B	Rh NS	Single	146	62	152	46	406	76	44	125	50	295
		Multiple	169	69	178	60	476	90	59	162	54	365
	Rh S	Single	21	13	56	20	110	37	14	39	6	96
		Multiple	43	32	152	50	277	94	31	101	15	241
AB	Rh NS	Single		50	46	19	115		45	38	11	94
		Multiple		59	59	20	138		54	46	11	111
	Rh S	Single		13	5	11	29		14	7	5	26
		Multiple		31	25	25	81		29	18	16	63
Total	Rh NS	Single	1366	1172	380	119	3037	984	919	285	96	2284
		Multiple	1603	1388	442	144	3577	1201	1156	364	107	2828
	Rh S	Single	557	604	120	54	1335	568	459	100	31	1158
		Multiple	1271	1308	294	125	2998	1298	1043	242	62	2645