Further Factors Influencing Rh-immunization: II

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In a previous paper dealing with Rh haemolytic disease (Renkonen and Timonen, 1967) the large numbers of boys (M/F 1.60) and twins (3.0%) observed among the firstborn children preceding the affected child were discussed. These observations suggest that the Rh-sensitized mothers were exceptionally tolerant of their foetuses, suggesting, perhaps, that the passage of foetal cells through placental barriers into the maternal circulation was exceptionally easy. Further information on the role of tolerance in Rh-immunization would be gained from a study of the ABO groups of mother and child. These groups are known to play a role in histocompatibility (Ceppellini *et al.*, 1966; Kuhns *et al.*, 1966) and also to interfere with Rh-immunization (Levine, 1943). When the red cells of the foetus are agglutinated by maternal isoagglutinins, the mother is less likely to be immunized than those mothers whose ABO groups are compatible with those of their foetuses. From the point of view of histocompatibility two types of mother-child combination, both usually considered to be compatible, are especially interesting. (1) Mother and child belonging to the same ABO group. (2) Mother and child belonging to

different ABO groups, where the child's red cells are not agglutinated by the mother's serum.

Finnish Series

The series of Finnish Rh-immunized mothers and their first affected children (Renkonen and Timonen, 1967) included 585 mother-child pairs whose ABO groups are known (Table I). The series of normal mother-child combinations (Table II) are derived from disputed cases (982) of paternity, and from a study of transferrin groups (598). When a mother has had several children, the mother-child combinations are included in the series with each of her children, as in the multiple ascertainment series of Cohen (1960). Chessboard tables, mother versus child, were drawn up, and the observed number of children was compared with the expected numbers which were calculated in two ways (1) in accordance with Bernstein's theory, using the gene frequencies (Streng, 1935), and the number of children born to mothers in each ABO group (Table III); (2) the second set of expected figures was obtained by the algebraic method used by Murray, Knox, and Walker (1965); for this purpose the corresponding chessboard with normal mother-child combinations was reduced to the size of the series of immunized mothers. The observed and expected numbers of children are given in Table IV. There was, indeed, a surplus (more than the expected number) of mother-child combinations where both belonged to the same ABO group (Group I):

+ 77.7/322.3 = + 24.1% (gene frequencies)
+ 96.4/303.6 = 31.8% (algebraic).

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TABLE I
ABO GROUPS OF MOTHERS AND THEIR FIRST AFFECTED CHILDREN
(FINLAND)

		Blood Groups of Mothers					Expected (based on gene frequencies)	Difference (observed—expected)
		O	A	B	AB	Total		
Blood groups of affected children	O	121	59	27	—	207	194	+ 13
	A	26	206	4	15	251	245	+ 6
	B	1	11	54	21	87	101	— 14
	AB	—	11	10	19	40	45	— 5
Total		148	287	95	55	585		
Expected (gene frequencies)		194	245	101	45		585	
Difference (observed—expected)		— 46	+ 42	— 6	+ 10			± 0

Gene frequencies p 0.290, q 0.134, r 0.576.

TABLE II
ABO GROUPS OF NORMAL MOTHERS AND THEIR CHILDREN
(FINLAND)

		Blood Groups of Mothers					Expected (based on gene frequencies)	Difference (observed—expected)
		O	A	B	AB	Total		
Blood groups of children	O	281	156	82	—	519	524.4	-5.2
	A	159	384	33	56	632	600.7	-28.7
	B	92	27	115	69	303	272.3	+30.7
	AB	—	46	40	40	126	122.8	+3.2
Total		532	613	270	165	1580		
Expected (gene frequencies)		524.2	660	272.3	122.8		1580	
Difference (observed—expected)		+7.8	-47.7	-2.3	+42.2			0

Gene frequencies p 0.290, q 0.134, r 0.576.

TABLE III
EXPECTED DISTRIBUTION OF CHILDREN OF O, A, B,
AND AB MOTHERS; EACH GROUP OF MOTHERS
CONSIDERED SEPARATELY

		Mothers' Blood Groups			
		O	A	B	AB
Children's blood groups, expected	O	r	$\frac{r^2}{p+2r}$	$\frac{r^2}{q+2r}$	—
	A	p	$\frac{pr+(p+r)^2}{p+2r}$	$\frac{pr}{q+2r}$	$\frac{p+r}{2}$
	B	q	$\frac{qr}{p+2r}$	$\frac{gr+(q+r)^2}{q+2r}$	$\frac{q+r}{2}$
	AB	—	$\frac{q(p+r)}{p+2r}$	$\frac{p(q+r)}{q+2r}$	$\frac{p+q}{2}$
		1.0	1.0	1.0	1.0

After Schiff (1928) and Wiener and Wexler (1958). The frequencies in their Tables have been divided by the frequencies of the respective mothers (r^2 , $2pr+p^2$, $2qr+q^2$, and $2pq$). The latter distributions have been used for calculation of the expected distribution of the totals of mothers and totals of children.

There was a deficit of 'compatible' mother-child combinations where mother and child belonged to different ABO groups, but the red cells of the children were not agglutinated by the mothers' serum (Group II):

$$-11.8/133.8 = -8.8\% \text{ (gene frequencies)}$$

$$-12.4/134.4 = -9.2\% \text{ (algebraic).}$$

There was a remarkable deficit of 'incompatible' combinations, where mother and child belonged to different ABO groups, and the red cells of the children were agglutinated by the mothers' serum (Group III):

$$-65.9/128.9 = -51.1\% \text{ (gene frequencies)}$$

$$-84.0/147.0 = -57.1\% \text{ (algebraic).}$$

Discussion

Comparison with Series from England, Netherlands, and United States. The surplus of identical mother-child combinations of group I and the deficit of 'compatible' children of group II,

TABLE IV
ABO GROUPS OF Rh-IMMUNIZED MOTHERS AND THEIR FIRST AFFECTED CHILDREN
(FINLAND)

		Blood Groups of Mothers												Total				
		O			A			B			AB							
		Observed	Exp. I*	Exp. II†	Observed	Exp. I	Exp. II	Observed	Exp. I	Exp. II	Observed	Exp. I	Exp. II	Observed	Expected I Difference (Observed—exp. I)	Expected II Difference (Observed—exp. II)		
Blood groups	O	121	85.2	104.0	59	66.0	57.8	27	24.5	30.4	—	—	—	207	194.1	+ 12.9	192.2	+ 14.8
	A	26	43.0	58.9	206	182.5	142.2	4	12.4	12.2	15	23.9	20.7	251	244.6	+ 6.4	234.0	+ 17.0
	B	1	19.8	34.1	11	15.4	10.0	54	42.9	42.6	21	19.4	25.5	87	100.8	— 13.8	112.2	— 25.2
	AB	—	—	—	11	23.1	17.0	10	15.2	14.8	19	11.7	14.8	40	45.5	— 5.5	46.6	6.6 —
Total		148	148	197.0	287	287	227.0	95	95	100.0	55	55	61.0	585	585.0		585.0	
Expected (gene frequencies)		194.1			244.6			100.8			45.5				585.0		585	
Difference (observed—expected)		— 46.1			+ 42.4			— 5.8			+ 9.5				± 0			
Difference (observed—expected) algebraic		— 49.0			+ 60.0			— 5.0			— 6.0							± 0

* Gene frequencies (p 0.290, q 0.134, r 0.576) according to Table I (and according to the r^2 ; $2pr+p^2$; $2qr+q^2$; $2pq$ for the totals of mothers and children).

† Algebraic, the difference between the figures of Tables II and III, after a reduction of the figures of Table III by multiplication by 585/1580.

TABLE V

COMPARISON OF OBSERVED AND EXPECTED RATES OF COMPATIBLE AND INCOMPATIBLE COMBINATIONS OF Rh-IMMUNIZED MOTHERS AND THEIR CHILDREN*

	England (Murray <i>et al.</i> , 1965)	The Netherlands (Reepmaker <i>et al.</i> , 1962)	U.S.A. (Cohen, 1960)	Finland (Renkonen and Timonen, 1967)
Number of mother-child combinations	1199	1607	2998	585
I: Mother and child belong to same ABO group	+11.4%	+13.8%	+11.8% (+19.2%)†	+24.1% (+31.8%)†
II: Mother and child do <i>not</i> belong to same ABO group (no agglutination of child's red cells by maternal isoagglutinins)	+11.3%	+8.1%	+3.4% (+0.3%)†	-8.8% (-9.2%)†
Difference I-II	0.7%	5.7%	8.4% (18.9%)†	32.9% (41.0%)†
III: Mother and child do <i>not</i> belong to same ABO group (agglutination of child's red cells by maternal isoagglutinins)	-53.7%	-58.9%	40.0% (-48.3%)†	-51.1% (-57.1%)†
Gene frequencies				
p	0.273	0.267	0.243	0.290
q	0.060	0.062	0.082	0.134
r	0.703	0.671	0.675	0.576

* The expected figures are calculated from gene frequencies. The sign + means that the observed numbers were higher than the expected ones. The differences are calculated as a percentage of the expected numbers.

† These expected figures are obtained by the algebraic comparison of the sensitized and non-sensitized (normal) groups.

indicates that histo-incompatibility interferes with Rh-immunization. Thus Rh-immunization is commonest in the most compatible mother-child combinations. Information from other countries about Rh-immunization should be reviewed. There are three large series, one from England (Murray *et al.*, 1965), one from the Netherlands (Reepmaker, Nijenhuis, and van Loghem, 1962), and one from the U.S.A. (Cohen, 1960), which have been restudied. The series themselves are given in the Appendix, Tables A, B, and C. In Table V, the results are summarized. The observed numbers are compared with those expected on the basis of gene frequencies, and the difference is given as a percentage of the expected number of children. In all these countries there is a surplus of combinations in which mother and child belong to the same ABO group. The surplus is high in Finland where q^* is high. Perhaps compatibility with respect to the B groups is especially important. The size of Group II (mother and child belong to different ABO groups, but no maternal isoagglutinins reacting with the child's cells are present) is variable. In England and the Netherlands there is an excess of these combinations almost equalling that of Group I, but in Finland with its high q , and to a lesser degree also in the U.S.A. there is little or no surplus in Group II, regardless of whether the expectation is based on gene frequency, or on the algebraic comparison. The critical difference be-

tween the sizes of groups I and II increases parallel with increasing q . In the series from Baltimore the difference is considerably lower than in the Finnish series, but it is still statistically significant. The frequency of q in Baltimore (0.082) is not much higher than in England (0.060), but this is not the only difference between the two. The series from Baltimore are based on multiple ascertainment, and accordingly include the important sensitizing children. Thus histocompatibility and tolerance seem to be factors favouring Rh-immunization, though comparative statistical evaluation of this heterogeneous material is difficult. In Table VI the combinations of classically incompatible mother-child pairs are analysed. Once again the frequency of q seems to be involved. When it is low as in England and the Netherlands the importance of group A as a cause for incompatibility is dominant; when it is high as in the U.S.A. and Finland the role of group B seems to be more important. However, it is hard to believe that these differences are based merely on the differences in gene frequencies, i.e. on chance. The anti-B isoagglutinins in England may be weaker than those in Finland. Differences in the strength of isoagglutinins have been noticed between German and Finnish sera (Savolainen, 1946).

AB and O Mothers. Table VII describes the AB mothers and their children. There is a tendency to sensitize AB mothers to have more AB children than expected. This is significant at

* p is the frequency of the gene I_A ; q is the frequency of gene I_B ; and r is the frequency of gene I_O .

TABLE VI
Rh-IMMUNIZED MOTHERS AND THEIR 'INCOMPATIBLE' CHILDREN, GROUP III

	England (Murray <i>et al.</i> , 1965)			Netherlands (Reepmaker <i>et al.</i> , 1962)			U.S.A. (Cohen, 1960)			Finland (Renkonen and Timonen, 1967)		
Incom- patible combina- tions Group III	Observed	97 (8.1%)			115 (7.2%)			355 (12%)			63 (18%)	
	Expected I (gene frequencies)	209.7	-112.7/209.7 = -54%	280	-165/280 = -59%	591.7	-236.7/591.7 = -40%	128.9	-65.9/128.9 = -51.1%			
	Expected II (algebraic)					687.2	-332.2/687.2 = -48%	147.0	-84.0/147.0 = -57.1%			
Incompati- bility due to child's A group	Observed	60		74		249.0		40				
	Expected I	146.0	-86/146 = -59%	192	-118/192 = -62%	380.5	-131.5/380.5 = -35%	70.6	-30.5/70.6 = -43.2%			
	Expected II					438.3	-189.3/438.3 = -43%	85.9	-45.9/85.9 = -53.4%			
Incompati- bility due to child's B group	Observed	37		41		106.0		23				
	Expected I	63.7	-26.7/63.7 = -42%	88	-47/88 = -53%	211.2	-105.2/211.2 = -50%	58.3	-35.4/58.3 = -60.7%			
	Expected II					248.9	142.9/248.9 = -57%	61.1	38.1/61.1 = -62.3%			
p	Gene frequencies		0.237		0.267		0.243		0.290			
q	Gene frequencies		0.060		0.062		0.082		0.134			
r	Gene frequencies		0.703		0.671		0.675		0.576			

the 0.05 level in the series from Finland (Table IV), but it is not visible in the series from England and the Netherlands. In the latter areas the observed (and expected) numbers of the critical AB-AB combinations are very low. The large number of immunized AB mothers, void of isoagglutinins, with AB children suggests that histocompatibility favours Rh-sensitization. In all the countries immunized AB mothers had more B children than A children as compared to the expectation. The difference is significant. Thus the presence or absence of the B group seems to be a more important factor in determining histocompatibility than A, in the same way as it was found to be a more important reason for incompatibility in the U.S.A. and Finland (Table VI). The deficit (-361) of immunized O mothers (Table IV, Appendix Tables A, B, C) is 'compensated' for by the surplus of affected O children (+332), in the same way as the surplus (+416.9) of immunized A mothers is 'compensated'

for by the deficit of affected A children (-129.1). In other words the deficit of I_0 among the immunized mothers is not real. It is compensated by an excess of heterozygote mothers of type AO.

Summary

Histocompatibility, as indicated by identical ABO groups in the mother and child, favours Rh-immunization. The excess of the observed numbers of mother-child combinations belonging to the same ABO groups was higher than that of non-identical combinations even when no maternal isoagglutinins against the child's cells were present. The difference is particularly evident in the series from Baltimore and Helsinki, with a relatively high I_B gene frequency. In some countries the group B children seem to be a more important reason for histo-incompatibility than group A. The immunized AB mothers more often had group B children than group A children. The frequency of I_0 and I_A (and accordingly of I_B) in a population seems not to be altered by Rh-immunization.

TABLE VII
Rh-IMMUNIZED AB MOTHERS AND THEIR CHILDREN

	Observed	Expected*	Difference
A children	101	119.7	-18.7/119.7 = -15.6%
B children	106	97.1	+8.9/97.1 = +9.2%
AB children	55	45.2	+9.8/55 = +21.7%

* On the basis of gene frequencies in each country.

REFERENCES

- Ceppellini, R., Curtioni, E. S., Mattiuz, P. L., Leigheb, G., Visetti, M., and Colombi, A. (1966). Survival of test skin grafts in man: effect of genetic relationship and of blood groups incompatibility. (Seventh international transplantation conference.) *Ann. N.Y. Acad. Sci.*, **129**, 421.

Cohen, B. H. (1960). ABO-Rh interaction in an Rh-incompatibly mated population. *Amer. J. hum. Genet.*, **12**, 180.

Kuhns, W. J., Rapaport, F. T., Lawrence, H. S., and Converse, J. M. (1966). Relationship of human blood group antigens and antibodies to survival of skin homografts. *Transplantation*, **4**, 250.

Levine, Ph. (1943). Serological factors as possible causes in spontaneous abortions. *J. Hered.*, **34**, 71.

Murray, S., Knox, E. G., and Walker, W. (1965). Rhesus haemolytic disease of the newborn and the ABO groups. *Vox Sang. (Basel)*, **10**, 6.

Reepmaker, J., Nijenhuis, L. E., and van Loghem, J. J. (1962). The inhibiting effect of ABO incompatibility on Rh-immunization in pregnancy: a statistical analysis of 1742 families. *Amer. J. hum. Genet.*, **14**, 185.

Renkonen, K. O., and Timonen, S. (1967). Factors influencing the immunization of Rh-negative mothers. *J. med. Genet.*, **4**, 166.

Savolainen, T. (1946). Gibt es Unterschiede zwischen den Isoagglutinintitern der einzelnen Völker? *Acta path. microbiol. scand.*, **23**, 552.

Schiff, F. (1928). Über Blutgruppenuntersuchungen an Müttern und Kindern, insbesondere Neugeborenen. *Klin. Wschr.*, **7**, 1317.

Streng, Osv. (1935). Die Blutgruppenforschung in der Anthropologie. *Acta Soc. Med. 'Duodecim'*, Ser. A., **17**, 1-313.

Wiener, A. S., and Wexler, I. B. (1958). *Heredity of the Blood Groups*. Grune and Stratton, New York and London.

Appendix

TABLE A

Rh-IMMUNIZED MOTHERS AND THEIR FIRST AFFECTED Rh INFANTS, ENGLAND (TABLE IV, MURRAY *et al.*)

		Blood groups of mothers								Total		Differ- ence
		O		A		B		AB		Ob- served	Ex- pected	
		Ob- served	Ex- pected*	Ob- served	Ex- pected	Ob- served	Ex- pected	Ob- served	Ex- pected			
Blood groups of affected children	O A B AB	452	366	183	163.6	40	34	—	—	675	593	+ 82
		49	123	343	347.7	5	11	18	16.4	415	467	- 52
		19	31	7	14.0	49	43	12	13.4	87	105	- 18
		—	—	11	18.7	6	27	5	5.2	22	34	- 12
Total		520	520	544	544	100	100	35	35.0	1199		
Expected among 1199 subjects		593		467		105		34		1199		
Difference		- 73		+ 77		- 5		+ 1				± 0

* The expected distribution of the children is calculated on the basis of gene frequencies (p 0.237, q 0.060, r 0.703) in each separate ABO group of mothers.

Group I (70.8%), mother and child belong to same ABO group.

A surplus found:

+87.1/761.9 = +11.4%.

Group II (21.1%), 'compatible' mother-child combinations, both of different ABO groups. No maternal isoagglutinins against child's red cells.

A surplus found:

+25.6/227.4 = +11.3%.

Group III (8.1%), incompatible mother-child combinations maternal isoagglutinins against child's red cells present.

A deficit found:

-112.7/209.7 = -53.7%.

TABLE B

MOTHER-CHILD COMBINATIONS IN FAMILIES WITH Rh-IMMUNIZATIONS, THE NETHERLANDS (TABLE 9, REEPMACKER *et al.*)

		Blood groups of mothers								Total		Difference
		O		A		B		AB		Observed	Ex-pected	
		Observed	Ex-pected*	Observed	Ex-pected	Observed	Ex-pected	Observed	Ex-pected			
Blood groups of children	O	506	387	253	235	52	46	—	—	811	724	+ 87
	A	53	154	564	553	8	18	18	22	643	690	- 47
	B	18	36	9	22	70	59	23	17	120	140	- 20
	AB	—	—	14	30	13	20	6	8	33	53	- 20
Total		577	577	840	840	143	143	47	47	1607		
Expected among 1607 subjects		724		690		140		53		1607		
Difference		- 147		+ 150		+ 3		- 6				± 0

* The expected distribution of the children is calculated on the basis of gene frequencies (p 0.267, q 0.062, r 0.671) in each separate ABO group of mothers.

Group I (71.3%), mother and child belong to the same ABO group.

A surplus found:

+139/1007 = 13.8

Group II (21.5%), 'compatible' mother-child combinations. Both of different ABO groups. No maternal isoagglutinins present.

A surplus found:

+26/320 = 8.1

Group III (7.2%), incompatible mother-child combinations. Maternal isoagglutinins present.

A deficit found:

-165/280 = -58.9%.

TABLE C
RH-SENSITIZED MOTHER-CHILD COMBINATIONS (MULTIPLE ASCERTAINMENT) U.S.A. (COHEN, TABLE V)

	Blood Groups of Mothers														Total				
	O				A				B				AB		Exp. I	Exp. II	Difference (observed—exp. I) (gene fr.)	Exp. II	Difference (observed—exp. II) (algebraic)
	Observed	Exp. I*	Exp. II†	Observed	Exp. I	Exp. II	Observed	Exp. I	Exp. II	Observed	Exp. I	Exp. II							
Blood groups of children { A B AB	1024	858	860.7	395	374.1	362.1	97	93.5	124.0	—	—	—	1516	1365.9	1346.8	+ 169.2			
	204	309	341.1	850	826.7	639.9	20	33.7	47.8	50	57.4	53.6	1124	1160.5	1136.4	— 12.4			
	43	104	141.6	32	45.4	57.8	152	129.0	149.2	50	47.3	50.3	277	352.0	398.9	— 121.9			
Total Expected I	1271	1271	1343.4	1308	1308.0	1163.3	294	294	370.4	125	125	120.7	2998	2998.0	2997.8	— 34.7			
	1365.9			1160.5			352.0			119.6									
Difference (observed— Exp. I, gene freq.)	— 94.9			+ 147.5			— 58.0				+ 5.5				± 0				
	— 72.4			+ 144.7			— 76.4				+ 4.3					0.2			

* Gene frequencies (p 0.243, q 0.082, r 0.675).
† Algebraic.

Group I (69.4%), mother and child belong to the same ABO group.
A surplus found:

$$+ 217/1834 = + 11.8\%$$

Observed—Exp. I

Observed

Observed—Exp. II

Observed

$$+ 330.4/1720.6 = + 19.2\%$$

Group II (19.7%), compatible mother-child combinations. Both of different ABO groups. No maternal isoagglutinins against child's red cells present.
A surplus found:

$$+ 19.7/572.3 = + 3.4\%$$

$$+ 2/590 = + 0.3\%$$

Group III (11.8%), incompatible mother-child combinations. Maternal isoagglutinins against the child's red cells present.
A deficit found:

$$- 236.7/591.7 = - 40.0\%$$

$$- 332.2/687.2 = - 48.3\%$$