INCOMPATIBILITY OF MOTHER AND FOETUS WITH RESPECT TO THE ISO-AGGLUTINOGEN A AND ITS ANTIBODY

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1. INTRODUCTION

Recent research on the Rh antigen discovered by Landsteiner and Wiener (1940) has provided conclusive evidence pointing to the occurrence of maternal iso-immunization—i.e. the production of antibodies by the mother in response to the presence of corpuscular antigens in the foetus. Maternal iso-immunization throws new light on the occurrence of neonatal haemolytic anaemias, of stillbirths, and of abortions in sibships containing members so affected. The importance of its contribution to neonatal and foetal death naturally prompts us to ask to what extent: (a) other foetal antigens evoke the production of maternal antibodies; (b) other maternal antibodies may penetrate the placental barrier with lethal or semi-lethal consequences to the foetus or newly born.

These issues had in fact provoked attention before work on the *Rhesus* antigen clarified current views. Diamond, Blackfan, and Baty (1932) and independently Parsons, Hawksley, and Gittins (1933) suggested that hydrops foetalis, icterus gravis neonatorum, and anaemia of the newborn are different manifestations of the same disease, which they called erythroblastosis foetalis. As the name suggests, its characteristic feature is excess of immature red blood cells in the blood of the foetus. The reason for this remained unexplained until 1940. In 1939 Levine and Stetson described an unusual case of intra-group agglutination in which a woman just delivered of a macerated stillborn foetus was transfused with blood from her husband of the same iso-agglutino-gen group (O). Shortly after this she showed all symptoms of incompatible transfusion. Other tests subsequently proved that her serum agglutinated her husband's cells, though she was later transfused with blood from donors of group O whose cells did not agglutinate in this way. Tests on a number of group O donors, to find a miscible blood, showed that only 20% were indeed compatible. Levine and Stetson made further tests, and showed that such agglutination was due to an irregular iso-agglutinin independent of the factors M, N, or P, but were unable to obtain a

sufficient quantity of the serum to test the incidence in groups A, B, etc. These examples of immediate reactions following post-partum transfusion have been recorded several times and are not easily explicable on the hypothesis that the woman had developed antibodies to an unusual agglutinin in blood from a previous transfusion. Levine and his co-workers—Levine, Burnham, Katzin, and Vogel (1941), Levine (1943)—suggested that the foetus inherits in such cases from the father a dominant gene responsible for the production of an antigen which passes through the placenta and stimulates the mother to produce antibodies also placenta-diffusible and hence of evoking haemolysis in the foetus. The case mentioned above and those reported by Levine and Katzin (1940) were shown to be due to the antigen which reacts to serum of rabbits immunized to blood cells of the *Rhesus* monkey.

Implicit in this interpretation is a postulate, the importance of which was first emphasized by Haldane (1942)—viz. that the placentae of *some*, but not all, women are permeable to antigens and some antibodies. This exposes a vista of new possibilities. Prior to this demonstration of iso-immunization by the Rh antigen as a major circumstance contributory to erythroblastosis foetalis, several writers—Hirsfeld (1928) in particular—had suggested that blood-group incompatibility between mother and foetus with respect to the A-B-O series might be the cause of foetal death and severe jaundice. Hirsfeld used the term “heterospecific pregnancies” to denote such cases of incompatibility and endeavoured to demonstrate the fact by determining relative birth-weights. After the general adoption of Bernstein’s triple allelomorph theory of the inheritance of the A-B-O groups, Hirsfeld neglected to follow up this suggestion, though he had in fact observed (1925) that there are less A children from matings A father by O mother than from the reciprocal matings. Nearly twenty years elapsed before the elucidation of the Rh antigen prompted Levine (1943) to a preliminary review of seven published studies on the A-B-O group.

Levine’s review is suggestive, but by no means conclusive, with respect to the thesis advanced—that is to say, the possibility that the antibody for the antigen A, present in the serum of a mother of group O, may diffuse through the placenta into the circulation of a foetus which itself belongs to group A. The consequences need not be identical with those characteristic of erythroblastosis foetalis, but may be none the less disastrous to the foetus on that account. Accordingly, certain phenotypic mother–child combinations with respect to the A-B-O grouping might be associated with high foetal or neonatal mortality. For instance, there should then be a deficiency of A offspring of unions between a father A and a mother O, but no corresponding deficiency with respect to A offspring of the reciprocal mating. This was in essence the test which Levine applied. The object of the present enquiry is to explore the significance of disparities of this sort more extensively.

The table Levine has published summarizes 8 studies. Of these he claims that 7 “confirm the concept of selective foetal death by iso-immunization”. We

have not been able to obtain access to Hirszfeld's or to Clausen's studies in present circumstances, and one of Levine's samples is based on his own unpublished results. Reference to the original sources of the remainder does not in all cases confirm Levine's figures. A recalculation of the data based on the relations shown on p. 4 appears in Table I, in which Levine's figures are in parentheses. The gene frequencies used were obtained by successive approximation in three stages—first from the square root formulae (p. 4), then from the Bernstein modifications, and finally from the quartic maximum likelihood equations. It is noteworthy that results of only three studies yield satisfactory confirmation of Levine's hypothesis. One is inconclusive and the other (Vuori) points decisively to the opposite conclusion. Vuori's result may conceivably be due to a clerical error, since such errors are not entirely absent from his tables.

TABLE I

		% A	Δ	n	t	P	N
Hirszfeld (1928) (15 authors)	O × A	(65·1)					
	A × O	(56·0)					
Hirszfeld and Hirszfeld (1928)	O × A	66·18 (63·1)	-4·095	68	1·045	0·3	} 730
	A × O	57·63 (60·5)	1·491	59	0·392	0·7	
Landsteiner and Levine (1928)	O × A	60·56 (60·6)	-5·15	142	0·881	0·4	} 1,047
	A × O	45·3 (44·8)	13·62	117	2·522	0·01	
Wiener and Vaisberg (1931) (whites only)	O × A	59·02 (54·8)	-1·936	122	0·355	0·72	} 807
	A × O	46·99 (52·5)	8·667	83	1·901	0·06	
Clausen (1934)	O × A	(66·3)					
	A × O	(62·0)					
Vuori (1930)	O × A	48·67 (46·7)	14·718	150	2·398	0·02	} 1,774
	A × O	64·24 (63·8)	-8·698	151	1·472	0·15	
Landsteiner and Wiener (1941) (whites only)	O × A	68·42 (68·4)	-5·23	56	1·480	0·17	} 342
	A × O	25·00 (25·0)	6·85	20	3·527	0·003	
Levine and Landsteiner (unpublished data)	O × A	(77·1)					
	A × O	(45·2)					

% A = Percentage of A offspring from mating class (figures in brackets from Levine, 1943).

Δ = Deficiency in observed A offspring over calculated.

n = Number of offspring in mating class.

t = Ratio of Δ to standard deviation of mating class.

P = Probability that observed difference is due to chance.

N = Total number of persons (parents and offspring) in whole investigation.

2. COMPOSITION OF DATA FOR THIS ENQUIRY

In what follows the convention adopted is to denote the male partner as the first and the female as the second—i.e. O.A means the mating of a father group O

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with a mother group A, and A.O means the mating of a father of group A with a mother of group O. A preliminary analysis exhibited in Table III refers to studies already tabulated in Lattes (1932), who does not separately record offspring of O.A and A.O. This table refers only to studies based on 1,000 or more parents and children and excludes earlier and therefore less reliable samples even if so large. Since we cannot here compare deficiency with respect to the expectation of A offspring in the reciprocal matings mentioned we can only apply a cruder test, that is to say, determine whether the observed proportion of A offspring is in fact deficient. The calculation of expectation is based on Bernstein's method with the usual symbols, viz.:

- (i) (A), (B), (O) for the phenotypic frequencies;
- (ii) a, b, r, for the corresponding gene frequencies, connected by the relation:

$$a = 1 - \sqrt{(O) + (B)}$$

$$b = 1 - \sqrt{(O) + (A)}$$

$$r = \sqrt{(O)}$$

On the assumption that all phenotypes are equally viable, the expectations of offspring of the several possible unions in a population mating at random are as shown in Table II.

TABLE II

Mating	O	A	B	AB
O × O	1	0	0	0
O × A	$\frac{r}{a+2r}$	$\frac{a+r}{a+2r}$	0	0
O × B	$\frac{r}{b+2r}$	0	$\frac{b+r}{b+2r}$	0
O × AB	0	$\frac{1}{2}$	$\frac{1}{2}$	0
A × A	$\frac{r^2}{(a+2r)^2}$	$\frac{(a+r)(a+3r)}{(a+2r)^2}$	0	0
A × B	$\frac{r^2}{(a+2r)(b+2r)}$	$\frac{r(a+r)}{(a+2r)(b+2r)}$	$\frac{r(b+r)}{(a+2r)(b+2r)}$	$\frac{(a+r)(b+r)}{(a+2r)(b+2r)}$
A × AB	0	$\frac{a+2r}{2a+4r}$	$\frac{r}{2a+4r}$	$\frac{a+r}{2a+4r}$
B × B	$\frac{r^2}{(b+2r)^2}$	0	$\frac{(b+r)(b+3r)}{(b+2r)^2}$	0
B × AB	0	$\frac{r}{2b+4r}$	$\frac{b+2r}{2b+4r}$	$\frac{b+r}{2b+4r}$
AB × AB	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$

It should be emphasized that expectations calculated on this basis imply the qualification stated above—i.e. equal viability of the phenotypes. Hence if the phenotype A were in fact slightly more viable than O, failure to detect a deficiency of individuals of group A would not necessarily disprove the hypothesis under examination. This is one possible explanation of the lack of any clear indications in support of the hypothesis furnished by the figures in Table III.

TABLE III

Author	N	% Deficiency of A	n
Snyder	1,095	— 14.3	264
Furuhata	3,962	6.5	471
Sievers	1,394	6.1	218
Thomson	1,303	1.0	171
Hazelhorst	3,024	3.0	349
Landsteiner and Levine ..	1,017	— 3.0	230
Kossovitch	966	— 1.6	217
Vuori	1,777	3.4	301
Ichida	1,681	8.0	233
Wiener and Vaisberg ..	1,007	3.8	223

N=Total number of persons (parents and children) in whole investigations.

n=Number of children in mating class (O×A).

Of the 10 studies here exhibited, 7 provide evidence of deficiency with respect to offspring of group A—a fact which would be suggestive were it not for the magnitude of the excess of Snyder's careful study. It is difficult to assess the importance of this discrepancy with justice. Although Snyder carried out his serological tests with the utmost circumspection, analysis of his figures by the χ^2 test shows that they do not refer to a homogeneous population mating at random.

Since evidence provided by examination of the correspondence between observed and expected numbers of A offspring in a mixed assembly of parents of whom one belongs to group O and one to group A fails to provide the decisive test of the hypothesis of iso-immunization as applied to the A-B-O grouping, it remains to examine two possibilities: (a) whether the proportion of A offspring in O.A matings exceeds that of the reciprocal union; (b) whether the proportion of A offspring in A.O as opposed to O.A matings falls off with increasing birth rank in such a way as to indicate an increasing titre of anti-A in the maternal circulation as the result of previous production of the antibody in response to the presence of an A foetus *in utero*.

It is clear that a conclusive answer to the question raised by Levine calls either for re-investigation by recourse to a large pool of new material, or for examination of available data separable into sibships of matings O.A and A.O. An *ad hoc* investigation would be protracted and laborious. In the absence of new evidence any examination of available documentary evidence which conforms to the requirements stated should restrict itself to the subject matter of more recent inquiries

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undertaken with due regard to the pitfalls which beset pioneer work, and based on samples which we may plausibly ascribe to a homogeneous population mating at random. Accordingly, we have arbitrarily rejected all data published before 1927 and selected from accessible literature after that date only such accounts as conform to other requirements stated above, rejecting those that do not provide explicit family data on such relevant issues as order of birth. Of about forty studies examined only 12, as listed below, satisfy all these requirements.

Sample No.	No. of Families	No. of Children	Author
1	150	430	Hirszfeld and Hirszfeld (1927)
2	166	715	Landsteiner and Levine (1928)
3	118	571	Wiener and Vaisberg (1931)
4	68	215	Schiff and Sasaki (1932)
5	100	219	Hyman (1935)
6	56	205	Zieve, Wiener, and Fries (1936)
7	50	157	Matta (1937)
8	112	433	Dahr (1940)
9	58	226	Landsteiner and Wiener (1941)
10	235	595	Taylor and Prior (1938); Race, Ikin, Taylor, and Prior (1942)
11	40	138	Wiener and Sonn (1943)
12	86	235	Wiener, Sonn, and Belkin (1944)

The first investigation listed—that of the Hirszfelds—was undertaken to explore a possible relationship between the A-B-O blood groups and immunity to diphtheria and scarlet fever by recourse to the tests of Schick and Dick. It is a very complete sample, since it gives the age, blood group, and sex of parents and children for each family. Landsteiner and Levine (2) were investigating the heredity of the M and N groups in the U.S.A. They also give the blood group and sex of parents and children in order of birth, as do Wiener and Vaisberg (U.S.A.) in a similar study with the same end in view. Both of these studies include coloured families—20 in the first and 13 in the second—though not distinguished as such by Landsteiner and Levine. Wiener and Vaisberg do so, and we have rejected these in our analysis of their data. Schiff and Sasaki (Berlin) again give sex and blood group of parents and children, together with M N type, and the “secretor” property. Hyman (U.S.A.), studying the inheritance of the M and N factors, gives blood group and sex throughout, while Zieve, Wiener, and Fries (U.S.A.) give, in a very detailed family study on the inheritance and linkage relations of allergic disease,

for each person the age, sex, blood group, M N type, whether or not allergic, and occasionally the secretor type. Matta, working in Glasgow, examined a number of families serologically, and lists his results separately for each family, distinguishing the sex of the parents but not of the children. His book gives the results of another study done in Cairo. Unfortunately, this is incomplete from the point of view of this investigation. Not infrequently it cites only one parent. Examination of the residual data by the χ^2 test showed that the sample was not random. Hence it was necessary to reject it. Dahr (Germany) was investigating the heredity of the factor P, and gave at the same time the A-B-O groups and M N types, with sex of parents and children. This was the latest German sample available. The English sample (10) was collected to investigate the distribution and heredity of both the A₁-A₂ and the M N types. It gives the sex of parents and children. The latest three samples of the first group, Landsteiner and Wiener (9), Wiener and Sonn (11) and Wiener, Sonn, and Belkin (12), all from the U.S.A., were undertaken to investigate the genetics of the Rh factor and its subgroups, but record A-B-O and M N determinations together with the sex of parents and children. In our analysis below, coloured families have been omitted from the two of these samples which contained them.

Table IV gives the percentage distribution of the blood groups of parents and children in these studies (P=parents, C=children). With due regard to such variations as arise from small sample size, the group as a whole is fairly homogeneous; and the distribution of the children closely resembles that of their parents—an indication of genetic equilibrium in the population.

TABLE IV
% Distribution of A-B-O Blood Groups

Sample No.	No. of Parents	No. of Children	O		A		B		AB	
			P	C	P	C	P	C	P	C
1	300	430	36.67	36.51	44.00	43.26	11.33	12.09	8.00	8.14
2	332	715	45.18	48.39	35.84	34.82	15.36	12.17	3.61	4.62
3	236	571	43.64	46.41	37.71	36.43	13.56	12.61	5.08	4.55
4	136	215	44.12	36.28	37.50	48.37	11.76	11.16	6.62	4.19
5	200	219	46.00	45.66	41.00	39.27	8.50	8.22	4.50	6.85
6	112	205	36.61	40.98	45.54	44.39	16.07	9.76	1.79	4.88
7	100	157	46.00	40.76	40.00	43.31	9.00	10.19	5.00	5.73
8	224	433	44.20	41.11	41.96	44.57	9.38	11.55	4.46	2.77
9	116	226	38.79	38.94	41.38	44.25	12.07	12.39	7.76	4.42
10	470	595	45.11	47.39	41.91	37.65	10.64	10.42	2.34	4.54
11	80	138	38.75	37.68	43.75	47.10	11.25	13.04	6.25	2.17
12	172	235	44.19	41.28	36.63	44.68	11.05	11.06	8.14	2.98

Tables V and VI respectively summarize the numbers of families and children in each of the 16 mating classes. The total number of families extracted from these

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12 investigations is 1,239, and the total number of children 4,139. Although these figures result from a summary of samples having different individual distributions, and cannot therefore be regarded strictly as from a homogeneous sample, reciprocal mating groups may legitimately be compared, since for each sample, as for the sum, the expectation is equal in each group. Thus the most significant departures from equality occur in the reciprocal groups O.A and A.O, and in O.B and B.O,

TABLE V
No. of Families

Sample No.	O.O	O.A	A.O	O.B	B.O	O.AB	A.B.O	A.A	A.B	B.A	A.AB	A.B.A	B.B	B.AB	AB.B	AB.AB	Total
1	22	23	22	11	4	4	2	30	9	9	6	3	0	1	0	4	150
2	32	32	25	11	12	3	3	18	12	10	0	4	3	0	0	1	166
3	24	23	18	3	8	1	2	15	5	7	5	1	3	2	1	0	118
4	13	16	13	0	3	1	1	4	4	5	4	1	1	1	1	0	68
5	21	21	20	5	2	0	2	14	4	6	2	1	0	0	0	2	100
6	9	7	11	0	5	0	0	12	4	3	0	2	3	0	0	0	56
7	11	7	8	2	5	1	1	11	1	1	1	0	0	0	0	1	50
8	23	24	18	3	4	3	1	22	3	3	1	1	2	2	2	0	112
9	8	12	6	3	5	0	3	10	4	1	2	3	0	1	0	0	58
10	45	52	46	9	12	1	2	37	9	11	4	1	3	1	2	0	235
11	4	11	6	3	1	2	0	6	3	0	2	1	1	0	0	0	40
12	13	16	16	4	5	3	6	10	5	3	1	2	1	0	0	1	86
TOTAL	225	244	209	54	66	19	23	189	63	59	28	20	17	8	6	9	1,239

TABLE VI
No. of Children

Sample No.	O.O	O.A	A.O	O.B	B.O	O.AB	A.B.O	A.A	A.B	B.A	A.AB	A.B.A	B.B	B.AB	AB.B	AB.AB	Total
1	65	68	59	24	12	13	3	94	24	28	19	8	0	4	0	9	430
2	136	142	117	49	49	10	13	78	47	43	0	17	10	0	0	4	715
3	121	122	83	10	40	6	11	60	25	35	25	6	12	10	5	0	571
4	35	54	45	0	7	2	5	13	7	21	14	3	3	4	2	0	215
5	46	40	41	12	4	0	5	35	8	19	3	2	0	0	0	4	219
6	42	34	40	0	13	0	0	39	15	7	0	6	9	0	0	0	205
7	38	18	20	7	14	2	7	33	2	5	5	0	0	0	0	6	157
8	86	81	85	9	18	12	5	79	13	14	4	5	12	6	4	0	433
9	28	56	20	12	19	0	7	45	9	3	5	20	0	2	0	0	226
10	128	132	106	28	34	2	2	77	23	29	12	3	12	4	3	0	595
11	14	36	21	11	7	6	0	20	11	0	6	3	3	0	0	0	138
12	40	52	49	9	12	3	14	26	12	6	3	7	1	0	0	1	235
TOTAL	779	835	686	171	229	56	72	599	196	210	96	80	62	30	14	24	4,139

where the observed numbers of children are respectively 835: 686, and 171: 229. If we regard the mean as the expected number of children, and calculate the probability of observed deviations therefrom we get:

$$\text{O.A } t=3.82, n=1,521, P<0.001$$

$$\text{O.B } t=2.9, n=400, P=0.0035$$

There is thus a highly significant deficiency of A.O compared with O.A children (and families). As will appear later, this is almost entirely attributable to selective loss of A children from matings A.O. For the matings O.B and B.O, there is a deficiency of O.B families and children. This too is largely attributable to selective loss of B children. Evidently, the mechanism of this loss is not comparable to that of A children in A.O families.

3. GENETIC ANALYSIS OF THE SAMPLES

Of matings which are relevant to the end in view, only A.O and O.A need concern us. On the assumption that such incompatibility militates against the viability of children of group A by mothers of group O the hypothesis of mother-foetus incompatibility with respect to the A antigen is susceptible of more than one line of attack. At the same time it implies: (a) that the net fertility of matings A.O is less than that of O.A; (b) that the observed offspring of group A in matings A.O falls short of expectation to a greater extent than in matings O.A.

We may examine the data in Table VII from either viewpoint. To say that (a) is correct means that the ratio of the total number of offspring whose parents are A.O to the total number of offspring whose parents are O.A is less than unity. This statement is of course independent of whether we are considering individual homogeneous samples or a congeries of such samples. That is to say, it is equally applicable to the grand total of data such as those in Tables VII and VIII, as to those of the individual studies there exhibited; and the cogency of a conclusion derived from the summation of the data is not diminished by uncertainty with respect to the significance of the individual items, since the magnitude of the effect may be such as to be manifest only of a gross sample larger than our individual subsamples. The same considerations apply *mutatis mutandis* to a second implication of (a). If the fertility of A.O is less than that of O.A matings, there will be more childless unions of the type A.O than of the type O.A. Hence the total number of families with parents O.A should significantly exceed those with parents A.O.

As regards the number of families respectively with parents A.O and O.A Table VII shows that the number of those with a male parent of group A is deficient in 9 out of the 12 samples, and excessive in two only. For the entire group of 453 families the observed proportions with one parent of group O and one of group A we have:

$$\text{O.A } 53.9 \pm 2.3\%$$

$$\text{A.O } 46.1 \pm 2.3\%$$

$$\text{Difference } 7.8 \pm 3.3\%$$

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Thus the deficiency with respect to A.O families is more than twice its standard error. The discrepancy between the total numbers of children in the two groups is even more striking. The deficiency of A.O children is over 5 times its standard error, and is therefore highly significant. The actual proportions of children of one or other mating in the two classes are:

$$\begin{aligned}\text{O.A.} & 54.9 \pm 1.3\% \\ \text{A.O.} & 45.1 \pm 1.3\% \\ \text{Difference} & 9.8 \pm 1.8\%\end{aligned}$$

TABLE VII

Sample No.	No. of Families		No. of Children		Mean No. of Children per family	
	O.A.	A.O.	O.A.	A.O.	O.A.	A.O.
1	23	22	68	59	2.96	2.68
2	32	25	142	117	4.44	4.68
3	23	18	122	83	5.30	4.61
4	16	13	54	45	3.38	3.46
5	21	20	40	41	1.90	2.05
6	7	11	34	40	4.86	3.64
7	7	8	18	20	2.57	2.50
8	24	18	81	85	3.38	4.72
9	12	6	56	20	4.67	3.33
10	52	46	132	106	2.54	2.31
11	11	6	36	21	3.27	3.50
12	16	16	52	49	3.23	3.06
TOTAL	244	209	835	686	3.42	3.28

TABLE VIII

Sample	O.A. A.O. Fams.	O.A. A.O. Chil.	O.A.	A/O ratio Exp.	A.O.
1	1.05	1.15	1.96	1.48	1.36
2	1.28	1.21	1.54	1.34	0.83
3	1.28	1.47	1.44	1.36	0.88
4	1.23	1.20	1.45	1.36	4.00
5	1.05	0.98	1.22	1.37	1.54
6	0.64	0.85	1.00	1.50	2.64
7	0.88	0.90	8.00	1.38	1.86
8	1.33	0.95	1.89	1.40	1.36
9	2.00	2.80	2.30	1.45	0.33
10	1.13	1.25	1.44	1.39	1.72
11	1.83	1.71	1.40	1.46	1.33
12	1.00	1.06	1.89	1.35	0.96
TOTAL	1.17	1.22	1.62		1.28

The right-hand half of Table VIII shows data relevant to (b) above. On the assumption of equal viability and random mating, the expected A/O ratio of children is the same for sibships with parents A.O and O.A, viz.:

$$\frac{(a+r)}{r} = \sqrt{1 + \frac{(A)}{(O)}}$$

For the O.A group, 9 samples out of 12 have sibs belonging to group A in excess of expectation and for the A.O group only 5. The proportion of A offspring in O.A families exceeds that in A.O families in 8 of the samples. Of itself, this is not conclusive; but we can probe the issue further by an examination of the data in other ways which permit us to pool the data of the 12 samples. We may first ask how far the net excess of A children in O.A families contributes to the excess of the total number of offspring in such families. The relevant figures are as follows:

Parents	No. of Children		Total
	O	A	
O.A ..	320	515	835
A.O ..	301	385	686

The net excess of A children in O.A families is thus $515 - 385 = 130$. The excess of total children is $835 - 686 = 149$. So the deficiency of A children in A.O families tallies closely with the lower fertility of such families.

We can also select from our data a class of families which we are entitled to pool. If the parent of group A is known to be heterozygous in virtue of having one or more children of group O, the expected ratio of children of group A to children of group O in matings of either type under discussion does not depend on the gene frequencies a and r . Accordingly such samples are on all fours, and we can combine our data to test for the discrepancy between the observed and expected A/O ratio. If the two phenotypes are equally viable, the expected ratio is unity. Since the probability of getting a child of group O from matings O.A or A.O when the parent of group A is heterozygous is 0.5, it is quite possible for all children of such parents to be of group A or all of group O. If all are of group A we have no means of deciding whether the parent of group A is himself or herself heterozygous. This is of course an outstanding statistical problem of human genetics arising from the small size of the human family. Two well-known methods, respectively called the "sib" and the "familial", have been devised for factorial analysis of human data with due regard to its relevance, especially in connection with familial traits—i.e. such as

turn up commonly among several sibs of parents who are themselves normal, hence putatively both heterozygous. For the particular class of matings involving one recessive and one heterozygous parent an alternative procedure is open. If p and q respectively stand for the probabilities that an individual will be of the recessive and dominant phenotype, the respective probabilities that a sibship of s members will be exclusively composed of one or the other are p^s and q^s . For unions of one heterozygous and one homozygous parent, $p=\frac{1}{2}=q$, and $p^s=q^s$. In other words, sibships with only recessive offspring and sibships with offspring of only the dominant phenotype will occur with equal frequency in such matings. In making the judgment that the parent of the dominant phenotype is heterozygous we have to exclude from our sample all sibships exclusively composed of the dominant phenotype. The preceding considerations show that we can compensate for this act of selection by also excluding from our sample the corresponding number of all sibships exclusively composed of the recessive phenotype. The theoretical expectation is then what it would be if our sample were complete—i.e. $A=\frac{1}{2}=O$.

The entire group of 12 samples yielded in all 249 sibships with: (a) parents O.A or A.O; (b) at least one sib of group A and at least one sib of group O. We thus have 249 sibships with one parent of group O and one manifestly heterozygous parent of group A excluding sibships composed exclusively of individuals of group O. These families were as follows:

			No. of families	Percentage
O.A	..	..	135	54.2 ± 3.2
A.O	..	..	114	45.8 ± 3.2
Total	..	..	249	100
Difference	..		21	8.4 ± 4.5

The composition of these families is as follows:

Parents			O	A	Total	% of A
O.A	..	..	272	278	550	50.5 ± 2.1
A.O	..	..	254	209	463	45.1 ± 2.3

The corresponding figures for all O.A and A.O families in the 12 samples are:

	O	A	Total	% of A
O.A	320	515	835	61.7 ± 1.7
A.O	301	385	686	56.1 ± 1.9

All the figures cited with respect to this selected group of families of which the A parent is heterozygous thus tally with the conclusion that A offspring of A.O unions are less viable than A offspring of O.A unions.

4. INFLUENCE OF BIRTH RANK

By analogy with what is known of the *Rhesus* antigen, the hypothesis that the placenta of some mothers permit diffusion into the foetal circulation of the antibody for the A antigen normally present in the serum of an O mother suggests another possibility—viz. that the antigen A diffuses through the placenta of some women, and hence provokes the production of an increasing titre of the corresponding serum agglutinin (anti-A). If so, the viability of A should diminish with increasing birth rank in unions of the type A.O. Table IX presents the relevant raw data, respectively pooled from all families in the 12 samples and from such families as have a heterozygous parent of group A with at least one offspring of the same group. Table X gives the A/O ratio. For the selected group this should be 1.0 irrespective of birth rank, if both phenotypes are equally viable. It is noteworthy that the A/O ratio: (a) exhibits no tendency to diminish with increasing birth

TABLE IX
Offspring of groups O and A by Birth Rank

(a) All families:

Birth Rank	1		2		3		4		5		6		7		8		9		10		All	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
O × A	100	144	84	131	59	100	33	60	18	39	14	18	7	14	2	6	2	2	1	1	320	515
A × O	73	136	85	96	50	69	38	44	30	24	14	9	7	3	2	2	1	2	1	0	301	385

(b) Families with one heterozygous parent of group A with at least one offspring of group A:

	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
O × A	74	61	67	68	56	59	32	39	17	27	14	11	7	8	2	4	2	1	1	0	272	278
A × O	49	65	69	45	45	46	36	28	30	14	14	5	7	2	2	2	1	1	1	0	254	209

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rank in families with parents O.A; (b) is greatest for first births in families with parents A.O, being progressively and consistently less for successive birth ranks from 3 onwards.

TABLE X
Ratio A/O Children by Birth Rank

(a) All Families:

Birth Rank	1	2	3	4	5	Over 5
O.A	1.44	1.56	1.96	1.81	2.17	1.58
A.O	1.86	1.13	1.38	1.16	0.80	0.64

(b) Families with one heterozygous parent of group A with at least one offspring of group A:

O.A	0.82	1.00	1.05	1.21	1.59	0.92
A.O	1.33	0.65	1.02	0.78	0.47	0.44

For the families as a whole A/O is necessarily greater than unity, since its theoretical value is:

$$\frac{a+r}{r} = 1 + \frac{a}{r}$$

For O.A families of the selected group of the bottom half of the table, the values of this ratio fluctuate indiscriminately around 1.0 as we should expect. In the A.O families of this group it conspicuously exceeds unity only for first births.

The A/O ratio (0.65) for children of birth rank 2 is noticeably a close approximation to the reciprocal of the corresponding ratio (1.33) for birth rank 1 in the group of families with a heterozygous father having at least one offspring of group A. This relation is theoretically deducible as a consequence of the method of selecting such families for observation. Two-child families so delimited may be first O, then A, or *vice versa*. If we denote the coefficient of selection against group A for first and second pregnancies by s_1 and s_2 respectively, the proportion of survivors belonging to group A and hence of complete 2-child families of the two types specified (here denoted O: A and A: O) will be:

$$\text{O: A} : 1-s_2$$

$$\text{A: O} : 1-s_1$$

In the first birth rank the ratio A/O for *completed* 2-child families delimited as above will therefore be: $(1-s_1) \div (1-s_2)$, and the corresponding ratio for the second birth rank will be $(1-s_2) \div (1-s_1)$.

From the finite difference equation for selection of completed c -child families, it appears that the A/O ratio falls off less steeply as c increases, if we choose our families in accordance with the limitation stated above. As compared with children of higher birth rank, children of birth rank 2 are heavily weighted by

2-child families which are much more numerous than larger families in our group. This offers an explanation of what is a seemingly anomalous feature of Table X—i.e. the fact that the A O ratio is lower for the second than for the third birth

FIG. 1.—Ratio of A O children by birth rank for families with a heterozygous parent of group A having at least one child of group A. Shaded: parents O.A. Black: parents A.O.

rank. The fact that the A O ratio exceeds unity for the first birth rank in the selected group of families with a heterozygous male parent is also in accordance with expectation, because the method of choice excludes 1-child fraternities from the pool. We have seen that the ratio A O for birth rank 1 in a 2-child family is $(1-s_1) \div (1-s_2)$. By hypothesis s_2 exceeds s_1 , hence $(1-s_1) > (1-s_2)$ and $(1-s_1) \div (1-s_2) > 1.0$.

5. DISCUSSION

The data examined in what has gone before consistently point to the existence of incompatibility between mothers of group O and foetuses of group A. Such incompatibility is already known to exist with respect to the Rh antigens of an Rh-positive foetus and the antibodies they may evoke in the circulation of an Rh-negative mother. This prompts us to explore the respective contributions of such incompatibilities to foetal death. The data surveyed point to a net deficiency of 25% A children in A.O. matings. For a population with the genotypic frequencies definitive of the one here discussed, this would signify a foetal death rate of 8% of A children or 3% of *all* conceptions. This makes no allowance for corresponding incompatibilities with respect to the B antigen and its antibody. The figure cited is greatly in excess of present estimates of the contribution of the Rh antigens to foetal and neonatal death—i.e. about 0.5%. Our conclusion is thus contrary to the prevailing belief (see Levine, Burnham, Katzin, and Vogel, 1941; Levine, 1943; Polayes, 1945) that the mortality contribution of incompatibility with respect to the iso-agglutinin groups is trivial: but it is fully consistent

with the view that such incompatibilities do not significantly contribute to the incidence of erythroblastosis foetalis.

FIG. 2.—Superimposed histograms showing pooled numbers of children of group A in the twelve samples from families consisting only of children of group A respectively with parents A.O (black) and O.A (excess shaded).

6. SUMMARY

1. Levine's theory of the pathogenesis of haemolytic disease of the newborn by iso-immunization of the mother to the Rh factor on foetal erythrocytes suggests that there may be comparable intra-uterine incompatibilities of mother and foetus in particular with regard to the A-B-O groups of Landsteiner.

2. With this end in view, twelve recently published heredity studies have been selected for large size of sample, reliability of technique, and full family data.

3. The mating class A.O (father A, mother O) was chosen for special examination as the largest relevant type of union in most populations.

4. A deficiency both of families with parents A.O and of children in such families as compared with those having parents O.A (father O, mother A) has been shown to be almost entirely attributable to selective loss of children of group A.

5. Examination of the A/O ratio among children of successive birth ranks revealed a steady decline with increasing birth rank in families with parents A.O, suggestive of either: (a) an increasing titre of anti-A in the maternal circulation; or (b) increasing placental permeability with increasing maternal age.

6. The data examined and the normal presence of iso-agglutinins in blood of mothers in group O as prescribed by Landsteiner's reciprocal rule are consistent with the view that A-B-O iso-immunization acts early in foetal life to produce abortion or miscarriage, giving rise to few cases of erythroblastosis.

7. Not all families A.O are equally affected—i.e. other circumstances, such as differential placental permeability, appear to be involved.

8. A provisional estimate based upon plausible assumptions suggests that A-B-O iso-immunization is responsible for the loss of about 25% of the A children expected from marriages $A \times O$, or about 3% of all conceptions.

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