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NATURE OF THE DETERMINANTS OF RHESUS ISOIMMUNIZATION

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The circumstance necessary for the development of haemolytic disease of the newborn, the marriage of an Rh(D)-positive man to an Rh(D)-negative woman, is found in about 14 per cent. of all marriages, but only a small proportion of these result in an affected infant. This is because only about one woman of every twenty at risk develops immunity to her Rh-positive infants, for once immunity has developed all subsequent Rh-positive babies will be affected. It is not known why one mother should develop antibodies when nineteen fail to do so. ABO incompatibility between baby and mother reduces the risk still further, but as two-thirds of foetuses are compatible, this provides only a part explanation and has little bearing upon the main issue.

A first approach to the problem may be made in the following terms. We may ask whether the determining factors are present when the marriage is contracted and are intrinsic to the combination; or whether they are related to individual pregnancies, independently of the specific constitution of the marriage. If the first be true, then a prediction of the likelihood of haemolytic disease may eventually prove possible at the time of the marriage, and in the meantime failure of immunization in early pregnancies may be taken as reassurance for later ones. If the second be true, then no such prediction is possible, nor can early failure of immunization be regarded as more than an initial escape. Possibly both factors operate.

Fortunately, a study of the frequency-distribution of cases of haemolytic disease and particularly of "first affected" infants according to birth order, offers an approach to this question.

If predetermined factors were operating, this distribution would show a relative crowding of "first affected" infants in early pregnancies. If entirely predetermined, all the "first affected" infants of homozygous fathers would be second babies. If no predetermined factors operated, we could expect a relatively greater dispersion among later pregnancies. Different distributions can thus be expected according to whether one hypothesis or another is set up. These distributions are calculable and may be compared with the observed distribution.

MATERIAL

During the periods July, 1947, to June, 1951, and January, 1953, to December, 1956, we recognized 920 "first affected" infants in Northumberland and Durham. For the purposes of the present study we think it reasonable to exclude sixteen first babies, and the remaining 904 were distributed according to their birth order as shown in Table I. We have omitted the period July, 1952, to December, 1952, because our records in this period included only treated cases, and these may be selected by birth order. On the other hand, we believe that the 904 cases in Table I are unselected. They were detected almost entirely upon the basis of routine

TABLE I

BIRTH ORDER OF 904 "FIRST AFFECTED" BABIES

Birth Order ..	2	3	4	5	6+7	8 or Later	Total
No. of Children	392	261	121	74	33	23	904
Percentage of Total ..	43·4	28·9	13·4	8·2	3·7	2·5	100·1

antenatal antibody tests in Rh-negative women, or because a positive Coombs' test was found on the cord blood collected routinely from the infants of all Rh-negative women. Only about 10 per cent. were first suspected upon clinical grounds. ABO haemolytic disease has been excluded and a positive Coombs' test has been taken as defining the limits of the disease. By considering only "first affected" babies, the effect of a diseased child on family size has been avoided.

The proportion of cases ascertained in Northumberland and Durham during the above periods increased in the early years, but for the past 5 years has remained constant at five to six affected infants per 1,000 births. We calculate that over the whole period we have recognized at least 90 per cent. of all cases.

We therefore regard Table I as reliable. The 904 cases include stillbirths (8 per cent.) and the birth orders are calculated upon the basis of all previous children, stillborn, dead, and surviving, but excluding abortions. As we consider only "first affected" babies, we may presume that the death rate and stillbirth rate among previous children is the same as in the total population.

Comparison with the Hypothetical Distributions

For comparison we propose a model in which the risk of an Rh(D)-negative women developing immunity is resolved into two parts: first, her initial susceptibility; second, her probability (p) of becoming sensitized to Rh-antigen at any given pregnancy. Of the two components, only p is relevant to the determination of the birth order distribution. We suppose, for the time being, that initial susceptibility is an all-or-none phenomenon and divides the population into two distinct parts, that the value of p is constant in the susceptible population and in successive pregnancies, and that sensitization is followed automatically by immunity to the next Rh(D)-positive foetus.

With a homozygous husband, there is a probability (p) that the first pregnancy will sensitize the wife, and a probability ($1 - p$) of its not doing so. At the second pregnancy, there is a probability p of an affected baby, a probability $p(1 - p)$ of sensitization, and a probability $(1 - p)^2$ of remaining unsensitized. Proceeding further, it can be shown that the probability that a baby of birth order "n" will be "first affected" is $p(1 - p)^{n-2}$. When p equals 1, the series is 0, 1, 0, 0, 0 . . . . etc. When p approaches zero, the series is p(0, 1, 1, 1, 1 . . . . etc.).

With a heterozygous husband, there is a probability $p/2$ of sensitization at the first pregnancy, and a probability $p/4$ that the second child will be affected. Also at the second pregnancy, there is a probability $\frac{p}{2}\left(1 - \frac{p}{2}\right)$ of sensitization, and a probability $\left(1 - \frac{p}{2}\right)^2$ of remaining unsensitized. Proceeding as in the last case, it can be shown that the probability of an affected baby is zero at the first pregnancy, and $\frac{x}{2} + \frac{p}{4}\left(1 - \frac{p}{2}\right)^{n-2}$ at subsequent pregnancies, where x is the value calculated for the immediately preceding pregnancy. When p equals 1, the series is $0, \frac{1}{4}, \frac{2}{8}, \frac{3}{16}, \frac{4}{32}$ . . . . etc., tending towards zero. When p approaches zero, the series is $p\left(0, \frac{1}{4}, \frac{3}{8}, \frac{7}{16}, \frac{15}{32} \ldots . \text{etc.}\right)$ tending towards $\frac{p}{2}$.

We have computed the probabilities that children of different birth orders will be the "first affected" in a sibship at different levels of p, and have combined the values for D-homozygous and D-heterozygous husbands in the proportions of their occurrence in the general population, that is, 418 homozygous to 582 heterozygous (Mollison, Mourant, and Race, 1952). We have applied the results to the birth order distribution for the Northern Region of England in 1951, as shown in Table II, and have reduced the results to the percentage distributions which are given in Table III (Registrar-General, 1953). This year, 1951, was chosen because it is the latest in

Table II

LEGITIMATE MATERNITIES, BY BIRTH ORDER

England and Wales, Northern Region, 1951

Birth Order	Number of Babies
1	18,787
2	15,685
3	8,548
4	4,163
5	2,220
6	1,152
7	671
8	410
9	285
10	178
11+	212
Total	52,311

which the Registrar-General gave regional data, and because, judging from the national figures from 1947, it seems reasonably representative of the period as a whole. The secular changes of birth order distribution during the period 1947 to 1956 are relatively small when we ignore, as we do in computing the distributions, the proportion of first births, and when we need to know only the proportion of second to third and later births. The "Northern Region" is greater than the area we have covered, but in the years up to 1949, when figures for North I (Northumberland and Durham) and the Northern Region (Northumberland, Durham, Cumberland, Westmorland and North Riding of Yorkshire) were published together, the birth order differences between the two were trivial. Like the Registrar-General, we have included both live and stillbirths in assessing birth order; the only difference is that he limits his data to legitimate maternities.

TABLE III

PROPORTIONATE DISTRIBUTIONS, ACCORDING TO BIRTH ORDER, OF "FIRST AFFECTED" BABIES

Values expected at different levels of probability of sensitization (p)

Birth Order	p				Observed
	Very Small	0·05	0·1	0·2	
2	43·3	44·9	47·0	51·0	43·4
3	25·5	26·6	26·7	26·6	28·9
4	13·7	13·2	12·7	11·7	13·4
5	7·5	6·9	6·5	5·5	8·2
6+7	6·3	5·5	4·8	3·7	3·7
8 or Later	3·8	3·0	2·3	1·5	2·5
Total	100·1	100·1	100·0	100·0	100·1
χ^2	18·0	10·5	11·4	31·7	—

Note: χ^2 is estimated by the application of the calculated proportions to 904 children and comparision with the observed distribution.

It is clear from Table III that only the distributions in the region of $p = 0{\cdot}05$ are compatible with the observed distribution. This estimate refers only to the susceptible part of the population. In the whole population of this district, we know the incidence of haemolytic disease of the newborn to be about 5 per thousand births, and in our experience 64 per cent. of these (3·2 per 1,000 births) are "first affected" babies. Of every thousand births, 140 are to the D-negative wives of D-negative husbands. When we applied the calculated risks of babies being "first affected" to 140 babies distributed as in Table II, we found that the observed incidence of 3·2 per 1,000 births was best matched upon the supposition that $p = 0{\cdot}06$.

This estimate of p in the whole population of D-negative mothers is no less than our estimate of p in the susceptible part of this population. If the terms of our model are accepted, it follows that all, or almost all, D-negative women, are susceptible.

The Validity of the Model.—This conclusion is conditional upon the validity of our model and we must therefore reconsider its premises. We presumed, in constructing it, that p was constant throughout the susceptible population, but we know that this is not strictly so. The probability of sensitization is influenced, for example, by the ABO compatibility of mother and foetus (Mollison, Mourant, and Race, 1952), and it has been shown that R_2 is a more powerful antigen than R_1 (Murray, 1957). A large proportion of these effects is randomly determined after marriage, but, part at least, is predetermined. If, however, the distribution of p is wider than we have presumed, this can only strengthen our general conclusion, for over-representation of mothers in the upper part of its range must have moved the observed distribution of births to the left. Indeed, the tolerance between the observed distribution and that for the lowest possible values of p is so narrow as to suggest that the range of p must be within close limits.

We also presumed, in constructing the model, that p operated at the sensitization level, and that, once sensitized, a mother would always become immunized to the next Rh-positive child; but there are at least two other possibilities.

The first is the converse of our model, namely that initial sensitization is universal and subsequent immunization proportional. This could occur, for example, if the necessary correspondence between successive babies were not simply that they should both be Rh-positive, but that they should correspond exactly in some combination of several (unknown) factors.

The second main possibility is that both initial sensitization and subsequent immunization depend upon proportional risks. This, for example, would be the situation if the accidental release of foetal blood into the maternal circulation were necessary in each of two pregnancies. In this example, both risks might be nearly equal.

At the cost of increased complexity, we may extend the generality of our model to cover both of these possibilities, as well as the original one. This is done by resolving p into two values: a probability of sensitization (s) at an early pregnancy, and a probability of immunization to an effective level (t) at a

later one. It is specified therefore that st equals p as used in the original model.

The probability that a baby of given birth order will be a "first affected" baby is calculable, for homozygous fathers, as follows:

Pregnancy 1 . . . 0
Pregnancy 2 . . . st
Pregnancy 3 . . . $st[(1-s)+(1-t)]$
Pregnancy 4 . . . $st[(1-s)^2+(1-s)(1-t)+(1-t)^2]$
Pregnancy 5 . . . $st[(1-s)^3+(1-s)^2(1-t)+(1-s)(1-t)^2+(1-t)^3]$
Pregnancy 6 . . . $st[(1-s)^4+(1-s)^3(1-t)+(1-s)^2(1-t)^2+(1-s)(1-t)^3+(1-t)^4]$
Pregnancy n . . . $st[(1-s)^{n-2}(1-t)^0+(1-s)^{n-3}(1-t)^1 \ldots\ldots+(1-s)^0(1-t)^{n-2}]$.

For heterozygous fathers the sequence is similar, but s is replaced by $\frac{s}{2}$, and t by $\frac{t}{2}$:

Pregnancy 1 . . . 0

Pregnancy 2 . . . $\frac{st}{4}$

Pregnancy 3 . . . $\frac{st}{4}\left[\left(1-\frac{s}{2}\right)+\left(1-\frac{t}{2}\right)\right]$ . . . etc.

It is simply shown that, as t approaches 1 (and s approaches the p of the original model), these expressions approximate to those we have already used. It is clear also, from the symmetry of the above terms, that the expressions derived for our original model cover also the first alternative possibility, namely that of universal sensitization and proportional immunization; the effect of s approaching 1 is the same as the effect of t approaching 1. It remains only to consider, then, the supposition that both s and t are proportional.

The greatest departure from the original model occurs when s equals t. This is also the likely consequence of the specific example (*i.e.* two leakages of foetal blood) which we quoted. In this event, the general model for homozygous fathers reduces to:

Pregnancy 1 . . . 0
Pregnancy 2 . . . p
Pregnancy 3 . . . $2p(1-\sqrt{p})$
Pregnancy 4 . . . $3p(1-\sqrt{p})^2$
Pregnancy 5 . . . $4p(1-\sqrt{p})^3$
Pregnancy n . . . $(n-1)p(1-\sqrt{p})^{n-2}$ . . where $p=st$.

For heterozygotes, p is replaced by $\frac{p}{4}$, thus:

Pregnancy 2 . . . $\frac{p}{4}$

Pregnancy 3 . . . $\frac{2p}{4}\left(1-\sqrt{\frac{p}{4}}\right)$

Pregnancy 4 . . . $\frac{3p}{4}\left(1-\sqrt{\frac{p}{4}}\right)^2$

Pregnancy n . . . $\frac{(n-1)p}{4}\left(1-\sqrt{\frac{p}{4}}\right)^{n-2}$.

These values differ considerably from those of our earlier hypotheses and we have therefore recalculated the expected distributions for various values of p (*i.e.* st). These are compared with the observed data in Table IV.

TABLE IV

PROPORTIONATE DISTRIBUTIONS, ACCORDING TO BIRTH ORDER, OF "FIRST AFFECTED" BABIES

Values expected when the probability of sensitization (s) and immunization (t) are equal, at different levels of their product (p)

Birth Order	p				Observed
	0·05	0·1	0·15	0·2	
2	32·5	37·6	41·5	44·5	43·4
3	28·6	29·7	29·9	29·6	28·9
4	16·9	15·9	14·7	13·6	13·4
5	9·7	8·3	7·2	6·2	8·2
6+7	8·1	6·1	4·9	4·8	3·7
8 or Later	4·2	2·3	1·8	1·4	2·5
Total	100·0	99·9	100·0	100·1	100·1
χ^2	69·2	21·3	8·8	18·4	—

The best fit upon this hypothesis is when p is approximately 0·15, that is, $s=t=0·39$. This value, if applied to all relevant marriages, would give an incidence of 8·6 "first affected" infants per thousand births, compared with the observed incidence of 3·2 per 1,000 births. But if only 37 per cent. of D-negative women were susceptible, we could postulate that two events, each with a probability of 0·39 and occurring at two separate pregnancies, were necessary for the development of immunity.

This then is a second and alternative explanation of the facts.

DISCUSSION

Our conclusions take the form of two main alternatives.

1 (*a*) The ability to develop Rh antibodies is not

predetermined, and any Rh-negative woman may produce them.

(*b*) Whether or not immunity develops is determined after marriage, and the process is triggered by a single event, the risk of whose occurrence is about 1:17. This is probably related to one of the pregnancies, but our method does not distinguish which pregnancy this is.

2 (*a*) The ability to develop Rh antibodies is predetermined and only a proportion of women can produce them.

(*b*) In the susceptible women, the process is triggered by a series of two events in different pregnancies.

(*c*) If we presume that the two events have an equal probability of occurrence, the risk of each is about 0·39, and the proportion of D-negative women who are susceptible is about 37 per cent.

These alternatives represent only the limits of a continuous series of possibilities where, beginning at the second hypothesis, the proportion of susceptible women rises as the probabilities of the two events become unequal in either direction, and the first hypothesis constitutes the limit.

The fact that both universal susceptibility and the elimination of one of the two events occur at the same point on this scale adds to the plausibility of the first hypothesis. But the ultimate distinction between the two alternatives will rest upon other data. Unfortunately, those so far published seem contradictory. Gerrard and Waterhouse (1953) studied the sisters of immunized women, but found no evidence of the genetic determinants postulated by Wiener (1946). Owen, Wood, Foord, Sturgeon, and Baldwin (1954) produced data in support of their ingenious suggestion of a predetermining factor based upon immunological tolerance, but the data of Booth, Dunsford, Grant, and Murray (1953) seem incompatible. Nevanlinna (1953), who, like ourselves, studied the birth order distribution, supported Wiener's genetic hypothesis. This was because of a relatively increased proportion of early births in his data, a difference for which we have no explanation.

Our first approach to this problem has also resolved itself into a paradox. On the one hand it is plain that we have failed to exclude the possibility of predetermined susceptibility, but on the other hand universal susceptibility is seen to be possible, and failure of immunization at early pregnancies is therefore no reassurance for later ones.

SUMMARY

The nature of the determinants of Rhesus isoimmunization must affect the birth order distribution of "first affected" babies, and the expected values are calculated upon a series of hypotheses.

The observed values correspond with one or other of two main hypotheses. The first supposes that all women are susceptible and that the main determinant is a single event, occuring after marriage, and that the risk of this event is about one in seventeen. The second supposes that only a proportion of women are susceptible and that for them the disease is determined by two events in different pregnancies, each with a relatively high risk of occurrence.

In either event, failure to develop antibodies in early pregnancies is no reassurance for later ones.

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