The Action of Selection on the Principal Rh Alleles

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INTRODUCTION

HALDANE (1942), and later others (e.g., Strandskov, 1945; Stern, 1949), have pointed out that natural selection would be expected to affect the frequencies of the Rh blood group genes because of the occurrence of erythroblastosis fetalis. This pathological and often fatal condition results from the isoimmunization of a pregnant woman who lacks an Rh (or Hr) antigen which the fetus carries on its red blood cells. Although at least ten such antigens are now known (C, C^w, C^u, c^v, c, D, D^u, d, E, e), yet nearly 90 per cent of erythroblastosis fetalis results from isoimmunization by D (Rh_o). A certain proportion of offspring perishes from the destruction of erythrocytes by the antibodies produced by the mother. If D is the antigen concerned, the genotype of the mother must be dd for this to happen, and every such offspring killed must be heterozygous (Dd), since the mother herself can supply the offspring only with a d gene. This simultaneous elimination of a D and a d gene from the population works to depress the frequency of the rarer allele. To illustrate, a loss of one gene of each sort from a group of ten genes, 6 of which are D and 4 of which are d, leaves the ratio of $D:d$ at 5:3 or 37.5% d, instead of 40% d as before. In a population of many D and d genes, the same differential would work against the rarer allele, but of course more slowly.

The d gene has a frequency of 39.5 per cent in the white North American population; D, of 60.5 per cent (calculated from 12,140 individuals typed at the Baltimore Rh laboratory, 1945–1946; Sacks, *et al.*, 1947; see also Rife, 1948; Ottensooser & Pasqualin, 1949). The frequency of d in England (40.4%) and in other western European countries is not very different (Race, *et al.*, 1948). Selection would therefore be expected with the passage of time to lower the frequency of d (Rh negative) and to raise that of D (Rh positive).

An assumption implicit in these considerations seems to have been overlooked, namely, the assumption that the Rh-negative women actually produce fewer living children than the Rh-positive women. If, for any reason whatever, the Rh-negative women were to equal the Rh-positive women in number of living children, and assuming that a certain number of deaths from erythroblastosis fetalis actually occurs, this would mean that the Rh-negative women had replaced defunct Dd children by normal dd children. The net effect would

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be to increase the gene frequency of *d*, rather than to decrease it. The possibility that this might occur has, in fact, been suggested by Fisher (see Race, 1944) and Spencer (1947).

In earlier times, or wherever the pattern of family size is large, the situation just sketched would probably be impossible. As the number of pregnancies per woman increased, Rh-negative women would fall farther and farther behind the Rh-positive women in the number of surviving children. On the other hand, there prevails today in the Western world a pattern of very small family size, and such a pattern should make it no longer difficult for Rh-negative sensitized women either to reach the typical family size before losing babies through erythroblastosis fetalis, or else to replace the losses of *Dd* infants by unaffected *dd* children. Only in the case of Rh-negative immunized women married to homozygous *DD* men would the latter course be virtually impossible. Yet even these women would in most cases be able to achieve the typical family size. Thus, the net reproductive rate of cities of 100,000 or over in the Middle Atlantic States of the U.S.A. is stated to be 0.75, which means that on the average each woman in such a community has only 1.5 children who reach maturity. Since it has been found (Glass, 1949) that 54.4% of the Rh-negative women eventually immunized are not yet sensitized in their second pregnancy, 32.9% not yet in their third, and 19.3% not even in their fourth, it appears clear that relatively few Rh-negative women would experience any difficulty, due to erythroblastosis fetalis, in having two or three children.

DIFFERENTIAL REPRODUCTIVE RATES OF RH-POSITIVE AND RH-NEGATIVE WOMEN

This reasoning suggested that an actual comparison of progeny size of Rh-positive and Rh-negative women should be made. For statistical purposes it would of course be ideal if the study could have been limited to women whose reproductive period was ended; but this would have introduced variability due to mortality occurring among their children. Also, the available data would not permit this. Instead, all Rh-negative women within the child-bearing age who were typed by the Baltimore Rh Typing Laboratory during a two-year period were included. The control group was composed of a considerably larger number of Rh-positive women selected at random from the cases also typed within the same two-year period. For any women who returned to the laboratory within the given period because of a second pregnancy, only the initial record was used, since it seemed highly probable that a far larger proportion of Rh-negative women than of Rh-positive women would in fact return. That is, all Rh-negative women except those married to Rh-negative husbands might be expected to return for retesting if newly pregnant, whereas the Rh-positive women, having once been typed and assured that they would experience no

encounter with erythroblastosis fetalis, would not come back. By the pro-
cedure chosen, the danger of bias through unequal sampling of the two groups
of women was avoided.

Because with the passage of time the proportion of primigravidas typed at
the laboratory has increased and conversely the proportion of multigravidas
has decreased, only those data collected during the early operation of the
laboratory might be expected to yield a random sample of the women through-
out their reproductive period. The records used were therefore those of the
period August, 1945 to August, 1947, the first two years of operation of the
laboratory. The unique quality of this sample should be emphasized. No future
collection of data at the Baltimore Rh Typing Laboratory nor any other within
the same locality can be expected to provide a true cross section in time of
the female population within the reproductive period, inasmuch as future

TABLE 1. PREGNANCIES AND LIVING CHILDREN OF RH-POSITIVE AND RH-NEGATIVE WOMEN

GROUP OBSERVED	NUMBER OF WOMEN	PREGNANCIES	LIVING CHILDREN
Whites: total	8,421	15,406	11,856
Rh+	5,048	8,971	6,954
Rh−: total	3,373	6,435	4,902
non-sensitized	2,946	5,385	4,210
sensitized	427	1,050	692
Negroes: total	2,723	7,720	6,444
Rh+	2,148	6,153	5,182
Rh−: total	575	1,567	1,262
non-sensitized	508	1,326	1,066
sensitized	67	241	196

typing will be limited more and more to primigravidas and become more and
more biased through the inclusion of a higher proportion of multigravida Rh-
negative women than of multigravida Rh-positive women. That this predicted
trend is actually taking place is shown by the fact that in the latest published
report of the Baltimore Rh Laboratory (Sacks, *et al.*, 1949), the proportion
of Rh-negative white women typed during the entire period of 3 years and 3
months from the initiation of the laboratory has risen to 17.48 per cent, in a
total of 43,165 white persons. Unless, therefore, some other laboratory can
provide a sample of comparable magnitude from a previously untyped pop-
ulation, the present study will have to stand alone.

Because of the generally recognized differences in family size between ne-
groes and whites and also because of the significantly different proportions of
Rh-positive and Rh-negative persons in these racial groups (see Potter, 1947;
Ottensooser & Pasqualin, 1949; Sacks, *et al.*, 1949), the data for negroes and
whites have been kept separate. However, the negro group is in each category

that which was found at random within the total group of all women for the period studied and the Rh type in question. Table 1 summarizes the raw data. Table 2 compares pregnancies per woman, living children per woman, and living children per pregnancy. Among both white and negro women, the mean number of living children per pregnancy is less for the Rh-negative women than for the Rh-positive women. This is in accord with the expectation based on the knowledge that erythroblastosis fetalis does account for the death of some children of Rh-negative mothers. The differential for the negro women (0.037) is greater than for the white (0.013), a fact which reflects the poorer obstetrical and pediatric care received as a rule by women and infants of the lower economic groups, to which most negroes in Baltimore belong, and the

TABLE 2. MEAN NUMBERS OF PREGNANCIES AND LIVING CHILDREN OF RH-POSITIVE AND RH-NEGATIVE WOMEN (WITH STANDARD ERRORS OF MEANS)

GROUP OBSERVED	MEAN NUMBER PREGNANCIES	MEAN NUMBER LIVING CHILDREN (PER WOMAN)	MEAN NUMBER LIVING CHILDREN (PER PREGNANCY)
Whites			
Rh+	1.777 ± 0.017 †	1.378 ± 0.016 ‡	0.775
Rh−: total	1.908 ± 0.023	1.454 ± 0.020	0.762
non-sensitized	1.83	1.43	0.78
sensitized	2.46	1.62	0.66
Negroes			
Rh+	2.865 ± 0.048 ¶	2.412 ± 0.042 §	0.842
Rh−: total	2.725 ± 0.085	2.195 ± 0.074	0.805
non-sensitized	2.61	2.11	0.81
sensitized	3.6	2.92	0.814

† $t = 4.65$, $P < 0.001$　　　　　　　　‡ $t = 3.05$, $P < 0.01$
¶ $t = 1.35$, $0.10 < P < 0.20$　　　　§ $t = 2.41$, $0.01 < P < 0.02$

consequently higher mortality among the children when erythroblastosis fetalis does occur, as has been previously reported (Sacks, *et al.*, 1949). The mean number of pregnancies is also lower among the Rh-negative negro woman than among the Rh-positive negro women, although the small difference is not significant for a sample of this size. The data for the negro women therefore conform to the principle described by Haldane, and one may conclude that in this element of the population the *d* gene is indeed being eliminated by selection directed against the heterozygote.

For the white women, the situation is reversed. The mean number of living children per woman is greater for the Rh-negative women than for the Rh-positive women (difference significant at the 1% level). This is clearly due to the marked difference in the mean number of pregnancies per woman in the two groups, a difference significant at the 0.1% level, and especially attrib-

utable to the high number of pregnancies per woman in the sensitized Rh-negative women. Among the white women, therefore, the *d* gene is not being reduced in frequency, but would appear to be on the increase at the expense of its *D* allele. Since the difference in mean number of living children and the difference in mean number of pregnancies per woman are clearly far higher in the negro women than in the white women, it seems logical to relate the complete reversal in the action of selection upon the heterozygote to size of family. Where family size is large (negro group), selection against the heterozygote operates so as to reduce the frequency of the rarer allele. But where family size is small (white group), compensating factors may shift the differential rates of reproduction so as to favor the rarer allele.

DISCUSSION

A question at once arises from the data just presented, namely, why do the Rh-negative white women in fact outclass the Rh-positive white women in number of pregnancies and consequently in number of living children? One suspected source of error may be ruled out. If there were different frequencies of the Rh-positive and Rh-negative types in different economic classes of the white population, such that relatively more Rh-positive individuals occurred in the higher economic groups and more Rh-negative individuals in the lower economic groups, then the well-known higher reproductive rate of the latter would produce a spurious increase in family size in the Rh-negative group. It was impossible to check this for the precise sample studied, for no information regarding the economic status of the cases had been recorded. But a sample from a later period of the laboratory's operation was available through a study made by Dr. Christopher Tietze of 3215 women, primiparas excluded. The husbands of the women typed were classified into six occupational categories (professional, business, skilled workers, semi-skilled workers, laborers, and "others"). In all of these classes the Rh-positive and Rh-negative types were in good agreement with expectation, the largest deviation being in the category of "other" professions than the five specific classes. $\chi^2 = 8.77$; D.F. $= 5$; $0.20 > P > 0.10$. It therefore seems unlikely that there is any real difference in the *Rh* (*D*) frequencies in different economic levels of the white population studied.

The remaining plausible explanation would appear to be that the loss of children through erythroblastosis fetalis, either as stillbirths or as postnatal fatalities, may serve to motivate parents, particularly the mothers, to replace them. As Fisher (see Race, 1944) and Spencer (1947) have suggested, families tend to fill up to a certain average size for a given population or stratum within a population. It may even be that this increased motivation to child-bearing might lead to over-compensation. That is, such women might end

up by having more living children than those who have not lost children. For any group in which the average size of family is small, this would be relatively easy.

There is some evidence apart from the present study that such a situation may occur. In a study of acholuric jaundice, Race (1942) found that persons with this semi-dominant condition, which causes significant losses of infants, nevertheless "had more live children than their normal sibs." Twenty-one acholuric men and women had 37 living offspring in spite of having lost 18 offspring through postnatal death or miscarriage. Their 15 normal sibs had 19 living offspring and had lost none. The excess of living children over and above those of their non-affected sibs was more characteristic of females with acholuric jaundice than of the males, as the following calculation of the number of living children per parent shows:

acholuric males.. 2.1
normal males.. 2.0
acholuric females... 1.45
normal females... 0.78

The data, of course, are not extensive enough to warrant the attribution of statistical significance to the difference. It may well depend upon the inclusion by chance of a group of non-acholuric women of unusually low fecundity. Nevertheless, the facts show that it is not impossible for women with such a handicap to produce in spite of it more living children than their normal sibs.

A further example of higher fertility on the part of individuals who lose children through the action of natural selection has recently been published by Silvestroni, et al. (1950). In the district of Ferrara, Italy, where the frequency of microcytemia (thalassemia) is over 10%, the gene appears to be maintained in the population by the higher fertility of $Mm \times Mm$ matings, in spite of the lethality of the homozygous (MM) condition. The data reported were as follows:

MATING	NUMBER MATINGS	AVERAGE NUMBER CHILDREN	AVERAGE NUMBER LIVING CHILDREN
$mm \times mm$	1,170	3.60 ± 0.087	3.14
$Mm \times mm$	269	3.62 ± 0.17	3.03
$Mm \times Mm$	19	5.89 ± 0.96	3.26

Because of the small numbers of recorded matings involving heterozygotes, the statistical significance of the difference is questionable, but it parallels the phenomenon reported by Race for acholuric jaundice and the data on erythroblastosis fetalis reported here. This evidence therefore tends to support the explanation applied to the data of the present study, namely, that the Rh-negative women who lose offspring through erythroblastosis fetalis experience

an increased motivation to have additional children, as Fisher suggested (see Race, 1944). This leads them, consciously or unconsciously, to "fill up their families" to that size characteristic of their group or stratum in the population, insofar as this is possible. If the standard size of family in their element of the population is low, they may over-compensate for their losses and as a group actually exceed the standard size of family. This is what seems to be happening among the white women of Baltimore.

Let us now consider the influence of the differential reproduction rates upon the gene frequencies of D and d. It is a matter of record that the average size of family (number of living children) in the population of the United States has decreased rather steadily since 1810 (see Lorimer & Osborn, 1934, p. 17), declining by somewhat more than one-half in the last hundred years. Consequently the prevailing pattern of family size in Baltimore and other large cities did not reach that level which permits the Rh-negative women readily to exceed the Rh-positive women in differential reproduction rate, until a generation or two ago. Moreover, this pattern of low family size prevails only in urban communities with a population of 26,000 or more (National Resources Committee, 1938; 16th Census of the U. S., 1944). In rural areas and smaller cities and towns, where the net reproductive rate is still 1.0 or above (approximately equivalent to 2 living children per family), the Rh-negative women perhaps do not equal the Rh-positive women in the reproductive rate. The situation found to exist at present in the Baltimore white population is unlikely to have had, until now, any marked influence upon Rh gene frequencies in the population of the United States, even if the same differential be assumed to prevail in other large urban centers. Nevertheless, the differential is so great that, if maintained, it should have a considerable effect on the Rh gene frequencies in the future, especially if the net reproductive rate in rural areas and of all elements of the population continues to approach that of the white urban portion of the population. This will be clear from the following considerations. Let us assume, to be conservative, that Rh-negative women merely equal Rh-positive women in reproductive rate. Then the displacement of Rh-positive by Rh-negative genes in the population must equal the number of heterozygous fetuses and infants eliminated by erythroblastosis fetalis and replaced by Rh-negative children. This frequency may be estimated in two independent ways.

First, the frequency of fatal erythroblastosis fetalis may be calculated from clinical records. The Baltimore Rh Laboratory has reported a general incidence of 1 case of erythroblastosis fetalis per 170 deliveries in a total of 28,551 deliveries, in their 2-year survey (August, 1945 to August, 1947, unpublished). In 53.8 per cent of 119 patients, the condition proved fatal. The combination of these statistics gives a minimum value of 3.2 deaths per 1000 births. Potter (1947) has given the incidence of hemolytic disease at the Chicago Lying-in

Hospital in 1944 as 1 in every 150 births, and Diamond in 1945 also reported an incidence of 1 in 150 births. Potter also gave a figure for the mortality of 64.8 per cent (1/392:1/252). From these statistics a figure of 4.3 deaths per 1000 births may be calculated.

These values for the mortality from erythroblastosis agree very well with that assumed by Haldane (1942) as the basis of his calculations of selection against the *Rh* heterozygotes,[1] namely, $k = 0.05$. For close to 10 per cent of all births are *Rhrh* heterozygotes born to *rhrh* mothers: 0.366 *RhRh*; 0.478 *Rhrh*; 0.156 *rhrh* genotypes in the American white population, giving $0.156[0.366 + \frac{1}{2}(0.478)] = 0.0944$. Hence k, the mortality from erythroblastosis fetalis among these *Rhrh* heterozygotes born to *rhrh* mothers, must be approximately ten times as high as it is in the general population. It must be kept in mind that the frequency of a fatal conclusion was even higher in the period before effective transfusion for saving the erythroblastotic infant had come into general practice, just as it has become even lower since the period studied. Our figures at the Baltimore Rh Laboratory indicate that the present mortality rate from erythroblastosis fetalis is almost exactly 33.3 per cent of those affected. This would mean about 2 deaths from this cause per 1000 births.

The second method of determining the frequency of loss is to take the difference in the mean number of living children per pregnancy for the Rh-positive and Rh-negative groups of women. These data are given in table 2. The mean number of living children per pregnancy is smaller in Rh-negative white women by a value of 13 children per 1000 pregnancies. (For the Rh-negative negro women it is smaller by a value of 37 children per 1000 pregnancies.) This value is somewhat larger than one per cent, but perhaps not significantly so. Comparing the two independent reckonings, the loss from erythroblastosis fetalis may be estimated as about 1.0 per cent. This value is double that assumed by Haldane. Replacement of Rh-positive by Rh-negative genes at any such rate as this would require but few generations to bring the two genes to an equal frequency, particularly since, as the frequency of the Rh-negative type in the population rose, the frequency of hemolytic disease would increase, and consequently the replacement of Rh-positive by Rh-negative types would be accelerated. The process of replacement would theoretically continue to accelerate, as Haldane (1942, p. 337, fig. 2) has shown, until the gene frequency of d has become 0.8202, i.e., until the population consists of 67.3 per cent Rh-negative and 32.7 per cent Rh-positive persons. Before this point has been reached, the frequency of erythroblastosis fetalis would already have

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[1] Throughout this note the term 'Rh-positive' has been used in the usual sense of "clinically Rh-positive," i.e., cells containing antigen D or Rh_o. Also, when referring back to early papers, such as Haldane's (1942), the original gene symbol *Rh* is retained; it is, of course, equivalent to D in the present Fisher-Race nomenclature or to *any one* of the alleles producing antigen D in Wiener's symbolism, i.e., R^1, R^2, R^0, or R^z.

reached its maximum, when the gene frequency had reached the values 0.667 d and 0.333 D. The height of this maximum depends only on (1) the frequency of d, and (2) the frequency of loss of Rh heterozygotes (Dd) and their replacement. If the present mortality from erythroblastosis fetalis is indeed around one per cent of all births, the current trend leads to a prediction of an increase of about 50 per cent in its incidence. That is, it will rise to 1.48 per cent; or 15 deaths per 1000 births. Only thereafter could it be expected to decrease. On the other hand, if we take into account the reduction in mortality from erythroblastosis fetalis likely to ensure from the full application of medical measures, k should be reduced to about one-half of its former value. In other words, the efforts of medical science to save erythroblastotic infants by measures known at present are just about effective enough to counteract the predicted rise in mortality because of the increase in frequency of the rh gene in the population. Like an animal running on a treadmill, our strenuous efforts will just maintain the status quo.

Thus we may conclude that, although the prevailing situation can throw little light on the past history of the Rh gene freqeuencies, it promises to have considerable importance for the future, unless in some way the current trend be reversed.

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SUMMARY

If the action of selection against Rh heterozygotes is as described by Haldane, it must reduce the gene frequency of that allele which is less common in the population, namely, rh. This should be evident in a lower number of living children per Rh-negative mother than per Rh-positive mother. For if the number of children is the same in these two classes, it signifies that losses of erythroblastotic heterozygotes have been equalled by births of additional viable children, i.e., $rhrh$ homozygotes. The net result would be to increase the gene frequency of rh at the expense of Rh.

Number of living children has been tabulated for the two classes of women in the Baltimore population, and for whites and negroes respectively. Among the negro women, who have on the average a larger family size than white women, the relations are as predicted by Haldane: mean number of living children of Rh-positive women, 2.41; of Rh-negative women, 2.20. Among the white women the relationship is the reverse: Rh-positive women, 1.37 living children; Rh-negative women, 1.453 (difference significant at 1 per cent level). This difference is accounted for by a significantly higher number of pregnan-

cies (8 per cent increase) among the Rh-negative women. It follows that for the white population studies, selection is actually increasing the gene frequency of *rh*.

This situation, which does not prevail in the negro population studied, is most to be expected where effective control over family size exists and in those groups within which a pattern of very small size of family has been established. It is not likely to have existed for more than a generation or two in the past, although Fisher thinks otherwise; but it may reasonably be expected to become more prevalent in the future. The frequency of fatal erythroblastosis fetalis may consequently be expected to increase, if untreated, to a maximum of about 15 per 1000 births (1.5 per cent) in such urban U. S. populations as that studied, or in the general population to the extent that the pattern of small family size becomes more universal.

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