Selection at the ABO Locus

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BRUES (1954) argued on anthropological grounds that the ABO blood group polymorphism is maintained by natural selection, since not more than one-fifth of the possible range of variation is occupied by populations now living. Considering the great variability of gene frequencies among local populations of the same ethnic origin, it seems reasonable to suppose that only selective factors could restrict variation among more distantly related groups. Accepting the evidence for elimination of A-incompatible zygotes (Waterhouse and Hogben, 1947), Brues was able to construct a system of selection coefficients, favoring compatible AO, BO, and AB zygotes, which gives an equilibrium at the mean world ABO frequencies and is stable over the range of observed variations in frequency.

This ingenious approach takes advantage of the fact that ABO distributions are known with considerable precision. However, by this method we cannot hope to distinguish between differential fertility and mortality, between genetic drift and variable selection, among alternative systems of selection coefficients which could give the same equilibrium, or even among systems with the same relative, but different absolute, values of the selection coefficients. Brues was aware of these disadvantages, but she drew attention also to the difficulties inherent in a direct study of ABO selection. By immunological methods it has not been possible to distinguish between AA and AO, or BB and BO, so that selective differences between homozygotes and heterozygotes are obscured. Technical errors may be of the same magnitude as selection effects, which are therefore "likely, even if detected, to be challenged for a long time in the immunological field". Under modern conditions of greatly relaxed selection, "the mortality causes involved may be totally different from those which decimated the young and adolescent in prehistoric times". Finally, the operation of selection may be so complex, involving slight and perhaps compensatory shifts in fertility and viability, as to defy direct analysis.

Since publication of this provocative paper by Brues, several developments have made the direct study of ABO selection appear more feasible. Levine (1959) and Cohen and Glass (1959) demonstrated that A- or B-incompatibility has a protective effect in RH hemolytic disease, apparently by reducing the length of time errant fetal cells persist in the maternal circulation. The ABO blood groups

are associated with resistance to other diseases (Roberts, 1957), type O suffering from an increased risk of duodenal ulcer but enjoying some protection from stomach cancer. Matsunaga and Itoh (1958) have published data which strongly support the hypothesis of ABO maternal-fetal incompatibility as a major cause of fetal death. Finally, methods have been devised which permit a more rigorous and efficient analysis of segregation data in man than has hitherto seemed possible (Morton, 1959). These procedures have been incorporated into a program for the IBM 650 electronic computer, called SEGRAN. [This program, written by Nancy S. Jones, is available from the Department of Medical Genetics, University of Wisconsin.] Stimulated by these developments, we have analysed 2836 families from 38 European and American studies (see references) and 2578 father-mother-child trios (Johnstone, 1954). Japanese material will be treated in detail in a later report as a further test of hypotheses elaborated here (Chung, Matsunaga, and Morton, in preparation).

EQUILIBRIUM OF A AND O GENES

The authors of our family studies excluded sterile matings, abortions, and untested children. Often family size was not complete at the time of testing, and reproductive compensation for fetal deaths may have obscured selection effects. We have no assurance that modern fertility patterns are the same as those under which the populations approached equilibrium or that selection effects are homogeneous among populations.

With these reservations, we may examine fertility differentials within studies, excluding matings that involve the B gene in either parent. Tabe 1 shows that there is no evidence for differences in net fertility between A and O parents, except that A-incompatible matings, in which the father possessed the A antigen and the mother lacked it, are significantly less fertile than other matings. The mean reduction in fertility associated with an A-incompartible mating is estimated to be .1545/2.9566, or 5.2 per cent. Matsunaga and Itoh (1958), who have published the most extensive study on ABO groups and fertility, found that the number of living children per mating was reduced 16.3 per cent in A- and B-incompatible matings ($\chi_1^2 = 25.8$). Their data are especially valuable because they include childless couples and are from a population with "scarcely any effective control over family size", under conditions that were more rigorous than now prevail (Haga, 1959).

We were not able to confirm the report by Matsunaga and Itoh of reduced fertility of O fathers among compatible matings. In our data O fathers are

TABLE 1. MULTIPLE REGRESSION ANALYSIS OF NUMBER OF TESTED CHILDREN PER FAMILY WITHIN STUDIES BY PHENOTYPE OF PARENT (A OR O)

Source	Effect
A—incompatibility	$-.1545 \pm .0640$
A vs. O fathers after fitting above	$-.0240 \pm .0734$
A vs. O mothers after fitting above	$.0531 \pm .0690$
Mean of compatible matings	2.9566

TABLE 2. MULTIPLE REGRESSION ANALYSIS OF NUMBER OF TESTED CHILDREN PER FAMILY WITHIN STUDIES BY GENOTYPE OF CHILD (AA, AO, OR O)

Source	Effect
A—incompatibility	$-.2577 \pm .1109$
AO after fitting above	$.0989 \pm .1292$
AA after fitting above	$-.1606 \pm .2273$
Mean of O genotype	2.9806

slightly, but not significantly, more fertile than A fathers. However, the standard error of the difference is large.

An alternative approach to fertility effects is based, not on parental phenotypes, but on the progeny genotypes. In a randomly mating population, the probability that a parent of phenotype A- be homozygous AA is $h = p/(p + 2r)$, where p is the frequency of gene A and r is the frequency of gene O (Morton, 1959). For each study we estimate h from a large population sample (McArthur and Penrose, 1951, and later authors), weight by the number of tested children, and take the mean, h = .1672. In A × O matings, the expected proportion of O children is $(1 - h)/2 = .416$, and in A × A matings the expected proportions of AO and OO children are about $(1 - h^2)/2 = .486$ and $(1 - h)^2/4 = .173$, respectively. Using the expected proportions as independent variates, we can perform a multiple regression analysis within studies by genotype of child (table 2). The effect of A-incompatibility is still clearly significant, amounting to an estimated reduction in relative fertility of .2577/2.9806, or 8.6 per cent, in matings of AA males with OO females and half of this, or 4.3 per cent, in $AO \times OO$ matings. This is equivalent to a selection coefficient against incompatible zygotes of .086 or more, depending on the extent of reproductive compensation for dead children. There is no significant effect on fertility of the AO and AA genotypes in compatible matings. However, the observed deviations are in the direction that would tend to maintain a balanced polymorphism, viz., increased fertility for AO children compared with AA and OO children.

Fertility differentials provide only a lower limit for selection because of the likelihood of compensation for early deaths. However, mortality at any stage before or after birth should be reflected by the segregation ratio. Let p be the expected proportion (segregation frequency) of O children in $AO \times OO$ matings. Then for an A × O mating the probabilities for r children of type O in a sibship of size s are

$$P(r = 0) = h + (1 - h)(1 - p)^s$$

$$P(r > 0) = (1 - h) \binom{s}{r} p^r (1 - p)^{s-r}$$

where h is the probability that the A parent is homozygous. In A × A matings the corresponding probabilities are

$$P(r = 0) = h(2 - h) + (1 - h)^2 (1 - p)^s$$

$$P(r > 0) = (1 - h)^2 \binom{s}{r} p^r (1 - p)^{s-r}$$

TABLE 3. SEGREGATION ANALYSIS OF A, O MATINGS

Father	Mother	U_p	K_{pp}	U_h	K_{hh}
A	O	−20.798	5135.08	17.311	2095.20
O	A	−209.946	5847.88	82.706	2362.54
A	A	−11.799	5723.43	—	—

Maximum likelihood scores have been developed for this situation (Morton and Chung, 1959). The scores (U) and their variances (K) are given in table 3, using p = $\frac{1}{2}$ for backcrosses and $\frac{1}{4}$ for intercrosses, and h = .1672.

As anticipated on the hypothesis of maternal-fetal incompatibility, the segregation frequency of O is significantly lower in O$\circ$ × A φ matings than in $\circ$ × O φ matings (χ^2 = 2.773, P = .048 by a one-tailed test). However, instead of an excess of O children in A$\circ$ × O φ matings, there is a deficiency in the O$\circ$ × A φ type (χ^2 = 7.537, P = .006), which is evidently not due to error in estimating the proportion of matings which cannot segregate, since the deviations for h are nonsignificant. Possible explanations for the excess of A children include heterozygote advantage, extramarital conceptions, meiotic drive, and false positive blood tests.

Discrimination among these alternatives is afforded by analysis of the relations among sibship size, parity (birth order), and the segregation frequency. Table 4 gives the segregation scores by sibship size for the 38 family studies, omitting Johnstone's trios, which include only one child per family. There is no significant association between sibship size and the scores for h in either compatible or incompatible matings, nor between sibship size and the scores for p in compatible matings. However, in the incompatible matings (A$\circ$ × O φ) there is a highly significant increase of O children with sibship size, as would be expected on the hypothesis of increasing risk for maternal immunization. The regression of the segregation frequency is .0274 ± .0083, and since the average parity in a sibship of size s is (s + 1)/2, this corresponds to a regression on parity of .0548 ± .0166.

Confirmation of this result may be sought in the regression of the segregation frequency on parity within sibship size for the 24 studies which reported parity. This regression is .0433 ± .0166, in good agreement with the evidence from sibship size. Since the two sources of information are independent, they may be pooled, giving .0490 ± .0117 as the combined estimate. The corresponding estimate for compatible matings is −.0270 ± .0142, which when pooled with the information from sibship size gives −.0202 ± .0100. Thus the segregation frequency of O not only increases with parity in incompatible matings, but decreases in compatible ones.

We cannot explain the excess of A children in compatible matings or the divergence between reciprocal crosses in terms of blood typing errors, which are usually failures to detect weak A reactions rather than false positives (Wiener, 1943). Similarly, extramarital children may be ruled out as a cause of these results. Let v be the frequency of unrecognized extramarital children. Among births to A mothers with O husbands, the frequency of O children is

$$(1 - h) \{(1 - v)/2 + vr/2(p + r)\} = (1 - h)\{\tfrac{1}{2} - .14325v\},$$

TABLE 4. RECIPROCAL A × O MATINGS BY SIBSHIP SIZE (s)

$p = \frac{1}{2}$, $h = .16720$

s	U_p	K_{pp}	U_h	K_{hh}
	O♂ × A♀ (compatible)			
1	−10.183	308.24	6.114	111.11
2	20.640	704.31	3.026	326.26
3	−7.673	909.82	−.058	458.25
4	−60.186	812.46	8.524	385.97
5	−26.122	547.36	10.301	228.77
6	−2.599	328.48	−1.624	118.06
7	−34.098	486.00	1.234	150.89
8	−12.305	180.83	−1.361	49.12
9	4.000	58.92	−2.402	14.20
10	−0.194	98.97	10.692	21.42
Total	−128.720	4435.37	34.446	1864.05
Regression on s	−.0067 ± .0070		.0053 ± .0122	
	A♂ × O♀ (incompatible)			
1	−14.183	302.53	8.515	109.05
2	−20.234	704.31	15.820	326.26
3	−47.255	753.04	−11.451	379.53
4	−4.892	603.93	−6.998	287.53
5	−8.122	637.62	3.096	265.43
6	35.668	364.98	9.301	131.18
7	13.476	220.91	−5.095	68.59
8	4.000	51.67	−2.402	14.03
9	15.827	88.38	3.510	21.30
10	12.000	32.99	−1.201	7.14
Total	−13.715	3760.35	13.096	1610.04
Regression on s	.0274 ± .0083		−.0071 ± .0142	

or an apparent segregation frequency of $\frac{1}{2} - .14325v$. Among births to O mothers with A husbands, the frequency of O children is $(1 - h)(1 - v)/2 + rv/(p + r) = (1 - h)\{\frac{1}{2} + .35675v\}$, or an apparent segregation frequency of $\frac{1}{2} + .35675v$. If the excess of A children in compatible matings were due to extramarital conceptions, their frequency would have to satisfy the relation $v = -U_p/.14325K_{pp} = .2506$, which is incredibly high. If the regression of segregation frequency on parity were due to a trend in the frequency of extramarital children, this would have to amount to $.0490/.35675 = .1374$ in incompatible matings, and $.0202/.14325 = .1410$ in compatible matings, per unit of parity. This is not only an absurdly large effect of parity on extramarital conception, but it is also contrary to the observation that the frequency of extramarital children is highest in the first-born (Johnstone, 1954; Edwards, 1957).

Meiotic drive (Sandler and Novitski, 1957) cannot explain the deficiency of A children from incompatible matings, since fertility is significantly reduced. However, it is not possible to rule out meiotic drive as the cause of A excess in compatible matings. Production by AO mothers of an excess of functional A eggs, increasing with parity, could formally explain the results, although the great age of ABO polymorphism in primates is inconsistent with the expectation that instances of meiotic drive should have a short evolutionary history, as sensitive

TABLE 5. SEGREGATION ANALYSIS OF ABO MATINGS

Type	Segregants		Compatible matings		Incompatible matings	
	T	t	U_p	K_{pp}	U_p	K_{pp}
A-compatibility						
A × O	AO	O	−209.946	5847.88	−20.798	5135.08
A × B	AO	O	6.202	1146.53	32.068	629.90
	AB	BO	49.266	762.70	23.700	793.43
A × AB*	AB	BO	13.230	573.05	—	—
AB × B	AO, AB	BO, BB	−12.000	352.00	0	136.00
A × A	AA, AO	O	−11.799	5723.43	—	—
B-compatibility						
B × O	BO	O	21.833	1427.34	−7.682	1622.04
B × A	AB, BO	AO, O	−54.554	1748.03	12.523	1734.50
B × AB†	AB	AO	5.651	215.93	—	—
AB × A	AB, BO	AA, AO	−8.000	912.00	46.000	852.00
B × B	BB, BO	O	−20.426	680.13	—	—
A-, B-compatibility						
AB × O	AO	BO	22.000	572.00	22.000	788.00
AB × AB	AA	BB	0	56.00	—	—
	AA, BB	AB	8.000	128.00	—	—

* Exclude AA, AO progeny.
† Exclude BB, BO progeny.

chromosomes are replaced with insensitive ones (Hiraizumi, Sandler, and Crow, 1960). The critical observations would be provided by $AA \, \male \times AO \, \female$ matings, which should give an excess of AO children under heterozygote advantage, but an excess of AA children if meiotic drive is the cause. Unforturnately, the dominance of the A gene precludes such a test. However, table 5 gives evidence against meiotic drive, since reciprocal A × B matings show no deficit of the O gene from AO and BO mothers.

Assuming tentatively that the excess of A in compatible matings is due to heterozygote advantage, rather than meiotic drive, this heterosis must increase with parity and act at a very early fetal stage, since it is not reflected by differential fertility. Maternal-fetal incompatibility acts at a later stage, since it is reflected not only by reduced fertility, but also by an increase in recognized fetal deaths (Matsunaga and Itoh, 1958). Let x denote the fitness of AO incompatible zygotes and y the fitness of AA and OO zygotes, relative to a fitness of unity for compatible AO zygotes up to the time of blood typing. In $A \, \male \times O \, \female$ matings the segregation frequency of O is $p = y/(x + y)$, in the reciprocal mating it is $y/(1 + y)$, and in A × A matings it is $y/2(1 + y)$. Scores for p may be transformed into scores for x and y by taking $U_x = U_p(dp/dx)$, $U_y = U_p(dp/dy)$, $K_{xx} = K_{pp}(dp/dx)^2$, $K_{yy} = K_{pp}(dp/dy)^2$, and $K_{xy} = K_{pp}(dp/dx)(dp/dy)$. Setting x = y = 1, we obtain

$$U = \begin{pmatrix} 5.1995 \\ -59.1609 \end{pmatrix}, \qquad K = \begin{pmatrix} 320.9425 & -320.9425 \\ -320.9425 & 775.8636 \end{pmatrix},$$

from which x = .89758 ± .07290 and y = .88138 ± .04689.

TABLE 6. MATRIX M FOR A, O EQUILIBRIUM

Mating		Genotype			Fertility
♂	♀	AA	AO	O	
AA	AA	1	0	0	1
	AO	.46848	.53152	0	1
	O	0	.91354	0	0.91354
AO	AA	.46848	.53152	0	1
	AO	.23424	.53152	.23424	1
	O	0	.48274	.47403	0.95677
O	AA	0	1	0	1
	AO	0	.53152	.46848	1
	O	0	0	1	1

Substituting these estimates of x and y, we may construct a matrix M of the products of the segregation frequencies and mating type fertilities, in which the rows represent mating types and the columns are genotypes (table 6). If there are n alleles, the number of genotypes is $r = n(n + 1)/2$, and M has r^2 rows and r columns. The sum of terms in any row equals the estimated relative fertility of that mating type, and the inner product of the vector of mating type frequencies and any column, divided by the sum of these products, is the estimated frequency of the corresponding genotype in the next generation. We suppose that mating is random by zygotes, so that the product of the genotype frequencies gives the mating type frequencies.

A program, called EQUIGEN, has been written for the IBM 650 electronic computer to form successive generations for any initial vector of genotype frequencies G and matrix M. This program, prepared by Nancy S. Jones, was used to verify that the current selection intensities operating on the MN locus would lead to a stable equilibrium near the present gene frequencies (Morton and Chung, 1959). Applied to the A, O model of table 6, EQUIGEN demonstrates that a stable equilibrium exists at frequencies of .22419 for AA, .51767 for AO, and .25814 for OO, at which point the gene frequency of O is .51698 and the heterozygote is 1.0365 times as frequent as under random mating without selection.

This is a higher frequency of the A gene than is found in most populations. Very likely the model is too simple, especially in assuming equal fitness for AA and OO zygotes. A lower fitness for AA would stabilize the gene frequencies at their observed value. Unfortunately, the amount of information about the fitness of the AA genotype is very small, only 22.36 units, and the difference between AA and OO is not significant ($\chi^2 = 1.160$).

Equilibrium of the A, B, O genes

Caucasian samples yield a small amount of information about B, the rarest of the ABO factors. In matings involving only the B and O genes the regression of number of tested children on expected frequency of B-incompatibility within studies is $-.0397 \pm .1563$, which is not significantly different from zero, on the one hand, or the demonstrated A-incompatibility effect on the other. There is considerable evidence that A_1-incompatibility is most serious, B less serious,

TABLE 7. SUMMARY OF SEGREGATION ANALYSIS OF ABO MATINGS

Mating type	O mother		Regression on parity	A or B mother		Regression on parity
	U_p	K_{pp}		U_p	K_{pp}	
A-incompatible	−20.798	5135.08	.0490 ± .0117	55.768	1559.33	−.0023 ± .0220
B-incompatible	−7.682	1622.04	.0407 ± .0205	58.523	2586.50	.0066 ± .0150
Compatible test-cross	—	—	—	−236.465	10169.78	−.0165 ± .0082

and A_2 least serious with respect to ABO hemolytic disease in the children of O mothers (Levine, 1955; Munk-Andersen, 1957; Zuelzer and Kaplan, 1954). However, it may be misleading to reason from hemolytic disease in livebirths to effects on fetal deaths. In particular, the demonstrated increase in early fetal deaths with ABO incompatibility may be due to tissue necrosis rather than red cell destruction (McNeil et al., 1954; Matsunaga and Itoh, 1958). The pooled regression on incompatibility over all matings within studies is −.1898 ± .0949, or a relative reduction in fertility of 6.3 ± 3.2 per cent.

The segregation analysis of all mating types is given in table 5. For B parents, h was calculated from population gene frequencies as $q/(q + 2r)$, where q is the frequency of gene B and r is the frequency of gene O, and the mean value of h is .0669. The data are summarized in table 7, together with the regressions of segregation frequency on parity, obtained as for the A, O matings by pooling the regressions from half-sibship size and from parity within sibship size, which are homogeneous.

In children of O mothers the decline in frequency of A and B children with parity is highly significant. There is no indication that the B-incompatibility effect is appreciably less than for A-incompatibility. We interpret the parity regression as due to differential mortality of incompatible fetuses and infants as the result of isoimmunization of the mother by previous pregnancies.

The evidence from A and B mothers is altogether different. The regression of segregation frequency on parity in incompatible matings is .0038 ± .0124, which is not significantly different from zero but is significantly less than the parity effect of .0470 ± .0102 for incompatible children from O mothers ($\chi^2 = 7.27$, P < .01). Thus isoimmunization of A or B mothers by incompatible pregnancies appears to have only a negligible selective effect. This is consistent with the fact that ABO hemolytic disease of the newborn is practically restricted to O mothers (Levine, 1955; Munk-Andersen, 1957).

Although there is no evidence for isoimmunization of A and B mothers by incompatible pregnancies, there is a deficiency of incompatible children which is barely significant when compared with genetic expectation ($\chi^2 = 3.15$, P = .04 by a one-tailed test) and is highly significant when compared with compatible matings of A and B mothers ($\chi^2 = 7.61$, P < .01). We interpret this deficiency of incompatible children, in the absence of a parity effect, as due to differential mortality of incompatible fetuses and infants by normal or hetero-immune maternal antibodies, such as those elicited by vaccines and bacterial

TABLE 8. STUDIES ON MORTALITY AND ABO INCOMPATIBILITY

McNeil et al. (1954)		Matsunaga and Itoh (1958)		Haga (1959)		Reed and Kelly (1958)
Mating	Aborter women	Abortions	Stillbirths and postnatal deaths	Miscarriages	Stillbirths	Miscarriages and stillbirths
Compatible	36/233	273/2639	258/2366	28/1628	23/1628	56/317
	.155	.103	.109	.017	.014	.177
Incompatible, O	38/129	148/911	142/763	23/596	14/596	27/111
mother	.295	.162	.186	.039	.023	.243
Incompatible, A	11/42	147/1017	150/870	25/695	26/695	13/51
or B mother	.262	.145	.172	.036	.037	.255

antigens. The existence of a heteroimmune mechanism is also suggested by the fact that primiparae make up half of the described cases of hemolytic disease of the newborn (Levine, 1955), although isoimmune sensitization of the mother apparently occurs in the postpartum period (Zuelzer and Kaplan, 1954). A possible difference in the immune response of O versus A and B mothers is supported by the difference in sedimentation constants for anti-B from A_1 and O persons (Filitti-Wurmser et al., 1952) and the crossreactivity of antibody from O persons (Rosenfield, 1953). It is not known whether the seasonal variation in A and B antibody titers is heteroimmune in origin (Shaw and Stone, 1958), or whether it affects the incidence of icterus praecox (Levine, 1955).

Three other studies support our conclusion that maternal-fetal incompatibility is an important cause of mortality in pregnancies to A and B mothers, despite the absence of evidence for isoimmunization or hemolytic disease of the newborn (table 8). McNeil et al. (1954) found a highly significant excess of women with two or more abortions among incompatible matings (χ^2 = 10.35, P < .002). This excess remains significant when incompatible matings of type O women are excluded (χ^2 = 2.90, P = .045 by a one-tailed test). Matsunaga and Itoh (1958) found a highly significant increase in abortions and perinatal and postnatal deaths in incompatible pregnancies to A and B mothers, compared with compatible pregnancies (χ^2 = 12.19 and 23.18, respectively, P < .001). Haga (1959) reported on a Japanese population under a more favorable environment. In his material the mortality rates and incompatibility effects are much reduced, and there is no significant relation between incompatibility and postnatal deaths. However, both miscarriages and stillbirths are significantly increased in incompatible pregnancies to A and B mothers (χ^2 = 7.70 and 12.79, respectively, P < .01). Other studies which indicate a significant effect of ABO incompatibility on fetal death, but either are smaller or do not separate different types of incompatible matings, were published by Levine (1943), Wiener et al. (1949), Grubb and Sjostedt (1955), Matsunaga (1955), and Reed and Kelly (1958). There is no evidence which suggests that mortality is less for incompatible children of A or B than of O mothers.

It is possible to develop an approximate model for isoimmunization of O mothers. Suppose that an incompatible child has c occasions, on the average, to

sensitize the mother so that subsequent incompatible fetuses will be eliminated pre- or post-natally. Then in a testcross the mean number of occasions for sensitization per pregnancy is $c/2$, and the probability that a pregnancy will not sensitize is $e^{-c/2}$. Therefore, the probability that the mother was not sensitized by pregnancies preceding the nth pregnancy is $e^{-c(n-1)/2}$. The natural logarithm of the ratio of compatible to incompatible survivors is

$$y = \ln(p/q) = c(n-1)/2 + \text{constant}.$$

To estimate c we have the equation

$$dy/dn = c/2 = (dy/dp)(dp/dn).$$

Since the regression of segregation frequency on parity is $dp/dn = .0470$, and $dy/dp = 1/pq \cong 4$, we have

$$c = 8(.0470) = .376.$$

Thus an incompatible child has an appreciable probability of sensitizing the mother. The probability of incomplete sensitization, permitting survival of a subsequent incompatible pregnancy, may well be higher. The means of sensitization is poorly understood, but it seems important that maternal isoantibodies do not increase appreciably in titer up to the delivery of normal live births, but increase postpartum if the infant is both A- or B-incompatible and an ABO secretor (Zuelzer and Kaplan, 1954). It is not known whether an early fetal death is capable of sensitizing the mother.

We turn now to another aspect of the data in table 7, the excess of heterozygotes in compatible matings. This amounts to $.0233 \pm .0099$, with a regression on parity of $.0165 \pm .0082$. We interpret this as for the A, O data, to indicate that heterozygotes are favored in compatible matings, their advantage increasing with parity. There is no evidence to implicate or exclude immune mechanisms in this selection, which is similar in magnitude to MN advantage, although the latter does not increase with parity (Morton and Chung, 1959). In both cases differential mortality must occur as early fetal deaths, since it is not reflected in abortion histories (Matsunaga and Itoh, 1958).

As the first step in developing a genetic model for ABO selection, the segregation frequencies in table 5 were regressed on variables which represented the progeny genotypes, their magnitude indicating the proportions expected and their sign whether that genotype contributes to the segregation frequency p or to $1 - p$. Compatible and incompatible progeny were represented by different variables. This analysis showed that there was no significant difference among the viabilities of AO, AB, and BO zygotes either in compatible or incompatible matings, nor among AA, BB, and OO. However, there was a significant difference between compatible heterozygotes on the one hand, and homozygotes and incompatible heterozygotes on the other.

Accordingly the following model was examined. Let x denote the fitness of incompatible heterozygotes and y the fitness of homozygotes, relative to the fitness of compatible heterozygotes. Maximum likelihood analysis, carried out

in the same way as for the A, O genes, gives x = .8935 ± .0483, y = .9065 ± .0366. These values lead to an unstable equilibrium for the A and B genes, the rarer of the two being eliminated from the population. Since a tri-allele equilibrium appears to have persisted for a long time, we are led to seek a model that will be consistent with the segregation data but which will provide a stable equilibrium. Perhaps the simplest and most likely model is that AA and BB homozygotes are at a disadvantage compared to other genotypes. Let x be the fitness of incompatible heterozygotes, y the fitness of AA and BB, and z the fitness of OO.

We find

$$x = .9062 \pm .0460$$

$$y = .9091 \pm .1252$$

$$z = .9179 \pm .0346$$

The estimated value of y is not significantly less than 1, and it still does not provide a stable polymorphism, but a slightly reduced value of y will do so. If y were .85, and x and z as estimated, a stable equilibrium would be established at phenotype frequencies of .0898 A, .0898 B, .0044 AB, and .8161 O. Slight changes in selection coefficients would produce an equilibrium closer to world blood type frequencies, but our sample is too small to justify further manipulations. However, since both x and z are significant, the genetic evidence strongly supports the assumption of Brues (1954) that ABO polymorphism is maintained because heterozygote advantage in compatible matings more than compensates for selection against heterozygotes in incompatible matings.

DISCUSSION

In the absence of a large body of family data, some investigators have sought for evidence of ABO selection in mother-child pairs (Kirk et al., 1955; Reed, 1956). The difficulties in analysis of this material may be illustrated by the large sample of Kirk et al. The number of AB mothers is 583 and the number of AB children only 436. This difference is highly significant (x^2 = 21.21, P < .0001) and suggests misclassification of AB infants. The proportion misclassified is about (583 − 436)/583 = .25, which is identical with the frequency of A_2B among AB persons. It would seem that a large proportion of A_2B infants had been mistyped, a common error (Wiener, 1943). If there is no selection and if mating is at random, the number of O children from A and B mothers would be expected to equal the number of A and B children from O mothers. However, there is a significant deficiency of the incompatible class (x^2 = 5.97, P < .02). When our selection model is fitted, with allowance for AB misclassification, there is a deficiency of B children from B mothers and of A children from A mothers, and an excess of O children from O mothers and of A children from B and AB mothers, the overall fit being poor (x_{10}^2 = 40.06, P < .001). Whether these discrepancies are due to differential fertility, assortative mating, classification errors, or differences in parity or viability cannot be determined from mother-

child pairs, which are a poor substitute for family data. A properly designed family study of this size would not only elucidate the selection mechanisms we have observed, but would probably lead to recognition of other mechanisms that are obscured in our relatively small sample.

Attempts to use genetic evidence on ABO selection began with Hirszfeld and Zborowski (1925), who noticed a lower incidence of A children from incompatible than from compatible A $\times$ O matings. This was confirmed by Levine (1943) and by Waterhouse and Hogben (1947), whose analysis was severely criticized by Bennett and Brandt (1954). The latter authors introduced maximum likelihood scores and found a highly significant deficiency of incompatible children. However, they also observed significant heterogeneity among their 12 samples of incompatible matings, although not among compatible ones. Not realizing that this is to be anticipated from differences among studies in parity and other factors affecting isoimmunization and selection, they tested the main effect against this heterogeneity mean square as error, and found that the deficiency of incompatible children was now only barely significant (t = 1.93, P = .04 by a one-tailed test). Their estimate of the segregation frequency of O children in incompatible matings is .580, based on 1125 units of information. Our study comprises 5135 units of information about this mating type. The difference in precision is partly due to the larger body of data on which our analysis is based, partly to the extremely low efficiency of Bennett and Brandt's analysis. They exclude matings with only A or only O children. This double truncation leaves only $4s(2^{s-1} - s)/(2^{s-1} - 1)$ units of information in a scorable sibship of size s with p = $\frac{1}{2}$, and only $1 - (\frac{1}{2})^{s-1}$ of testcross sibships are scorable. From the same material an efficient analysis gives 4s units of information, so the relative efficiency of Bennett and Brandt's method is $1 - s/2^{s-1}$, which is 0 for sibships of size 1 or 2, only $\frac{1}{4}$ for sibships of size 3, and $\frac{1}{2}$ for sibships of size 4, approaching full efficiency in large sibships.

Several investigators have used fertility as a measure of ABO selection (Reed and Ahronheim, 1959; Bennett and Walker, 1956). Because of compensation for early fetal deaths and confounding of information if both parents are not typed, fertility can be an insensitive indicator of selection, and no clear or consistent pattern has emerged from these studies. Our material and the large sample of Matsunaga and Itoh (1958), where both parents were typed, show clear effects of incompatibility on fertility, but see Chung, Matsunaga, and Morton (in preparation) for evidence of heterogeneity in the fertility effect among populations.

There is now considerable evidence for an association between ABO blood groups and various diseases (Sheppard, 1959). So far these correlations are weak and, except for a recent report of an association with rheumatic carditis (Clarke et al., 1960), involve diseases that, because of their rarity or late onset, probably exert little selection pressure on the ABO system. As a working hypothesis we propose that the major effect of ABO selection is on early fetal and postnatal stages and that the reported associations between blood groups and adult diseases are at most second order selective effects.

The concept of the genetic load has been introduced to measure the deviation of the fitness of a population from its maximum value due to mutation, segregation, meiotic drive, gametic selection, maternal-fetal incompatibility, or other mechanisms (Morton, Crow, and Muller, 1956; Crow, 1958). Loss of fitness may be expressed as specific types of mortality, sterility, or morbidity. The only mode of selection acting on the ABO locus which causes recognizable mortality is maternal-fetal incompatibility. In randomly mating populations, the frequency of incompatible zygotes is $p(1 - p)^2 + q(1 - q)^2 = .2109$, based on the estimated gene frequencies given by MacArthur and Penrose (1951) for the U. S. population ($p = .249$, $q = .084$, $r = .667$). The frequency of incompatible matings is $pq(p + 2r)(2 - q) + pq(q + 2r)(2 - p) + r^2(1 - r^2) = .3623$. Thus the proportion of incompatible children in incompatible matings is $.2109/.3623 = .5821$, of whom $1 - .9062 = .0938$ are estimated from the segregation analysis to be eliminated by maternal-fetal incompatibility. This is in good agreement with our estimate of a relative fertility loss of 6.3 per cent, suggesting that there is little reproductive compensation for dead children, at least in incomplete families. If these eliminations occur as recognizable abortions or perinatal deaths, we would expect the incidence of such deaths to be increased by $(.0938)(.5821) = .0546$ in incompatible matings. This is less than the mortality difference reported by Matsunaga and Itoh (1958), but greater than the mortality difference found by Haga (1959). To determine whether all the mortality due to ABO incompatibility occurs at recognizable stages, we require a very large study of mortality and ABO segregation in the same population. Unfortunately, such a study has not yet been made.

The expressed genetic loads due to mutation and segregation increase linearly with inbreeding. Maternal-fetal incompatibility shares with gametic selection and meiotic drive the interesting property of decreasing with inbreeding and therefore makes a negative contribution to the total genetic load measured in consanguineous marriages (Crow and Morton, 1960). The incompatibility load when the mother is not inbred is $(1 - 2F)L$, where L is the incompatibility load in a randomly mating population and F is the inbreeding coefficient of the zygote. Doubling a gamete to make it completely homozygous actually reduces the incompatibility load to zero, but if we assume, as is true for the mutation and segregation loads, that the expressed load is $A + BF$ and the total load is $A + B$ (Morton, Crow, and Muller, 1956), then the incompatibility load for the common kinds of inbreeding appears to contribute L to A and $-2L$ to B, or $-L$ to the total load. The fact that B/A for many types of mortality is large and positive demonstrates that the incompatibility load is a negligible part of the total genetic load as measured from inbreeding. However, the incompatibility load may be an appreciable part of the expressed genetic load in a randomly mating population. For the ABO locus, we estimate

$$L = (.2109)(.0938) = .0198$$

Thus the effect of ABO incompatibility is to kill about 2 per cent of all zygotes. Clearly there cannot be many loci with as great an incompatibility effect as

this, or they would account for all of the genetic load expressed as stillbirths and neonatal deaths in a randomly mating population.

The contribution of ABO incompatibility to the inbreeding load B is $-2L = -.0396$. The negative contribution of all types of maternal-fetal incompatibility to the inbreeding load may account in part for the observation that inbreeding of the fetus has less effect on miscarriages and stillbirths than on postnatal and very early prenatal deaths (Slatis, Reis, and Hoene, 1958; Mares, Menge, Tyler, and Casida, 1960).

In conclusion, we would like to make a few remarks about the validity of our material and the design of further studies. We consider the existence of a large effect of maternal-fetal incompatibility on pre-natal and perinatal deaths as firmly established both by segregation analysis and pregnancy histories. The evidence for parity effects and heterozygote advantage in compatible matings is less secure, not only because of heterogeneity in our material but also because no other body of data has yet been assembled to provide an independent test of these effects. In an area fraught with many pitfalls, some of them unsuspected, it would be well to regard the evidence for the effects other than incompatibility as only preliminary.

There should be no need to stress the importance to medicine and human biology of ABO maternal-fetal incompatibility as a major identifiable cause of mortality, which because of the advanced state of knowledge of immunogenetics can be studied, understood, and perhaps prevented. A problem of this significance deserves much more searching inquiry than it has received so far, combining the advantages of genetic methods with pregnancy histories and immunological tests. The errors of anamnestic reports of fetal wastage are eliminated in segregation analysis, which reflects differential mortality at early stages of development more precisely than the most accurate fetal death registry.

The sample size in an adequate study must be of the order of tens of thousands of pregnancies, studied for at least the ABO and Lewis antigens and ABO secretion of the child, both parents, and other sibs if possible. Due attention should be given to the obstetrical history preceding each birth, especially with regard to abortions. The frequency of genetic exceptions ("paternity exclusions") should be studied as a function of parity, as a guide to interpreting parity effects on segregation frequencies. Attention should be paid to possible residual effects of ABO incompatibility, such as icterus praecox, cerebral palsy, and mental defect (Wiener et al., 1949). It is important that these studies be carried out under both rigorous and modern environments to determine not only the present effects of ABO blood groups but also the selection pressures that established the polymorphism under primitive conditions.

Not many years ago, it was generally believed in this country that the major genetic polymorphisms are nonadaptive, or else controlled by extremely weak selection pressures. Now we recognize that they are often maintained by intense selection which is amenable to detailed analysis. From a passive role as neutral markers of prehistoric migrations, the genetic polymorphisms have taken their proper place as major selective agents. Yet we know only a little about the

selective forces involved and their interactions. In the unraveling of this tangled skein, the methods of segregation analysis can play an important part.

SUMMARY

Segregation analysis has been applied to Caucasian family data on ABO blood groups. Maternal-fetal incompatibility significantly reduces fertility by 6.3 ± 3.2 per cent and causes elimination of 9.4 ± 4.6 per cent of incompatible zygotes. Thus there is no evidence in these incomplete families of reproductive compensation for dead children. Abortion studies show that many of these deaths (amounting to 2 per cent of all zygotes) occur at recognizable stages of development, but the published material on fetal loss is inadequate to specify the distribution of mortality with any precision. The frequency of incompatible children of O mothers declines with parity while the frequency of incompatible children of A and B mothers does not. In both cases there is a significant overall deficiency of incompatible children compared with compatible matings. These data suggest that isoimmunization by fetal antigens is a major factor in the outcome of pregnancies to O mothers, but that heteroimmune mechanisms are more important for A and B mothers. Published studies of abortions and perinatal deaths support the conclusion that the overall effect of maternal-fetal incompatibility on mortality is sensibly the same for O, A, and B mothers, despite the fact that clinical hemolytic disease of the newborn is almost restricted to children of O mothers.

In compatible matings there is a significant excess of heterozygotes, increasing with parity. However, this is not supported by abortion data, so that it seems necessary to invoke either early fetal death of homozygotes, meiotic drive, or some unrecognized bias in our material. Extramarital children do not provide a likely explanation, because their frequency would have to be very high and increase with parity at an astounding rate. Available evidence actually indicates a maximun frequency of extramarital children in first births. Meiotic drive is unlikely because reciprocal A $\times$ B matings show no deficit of O gametes from AO and BO mothers.

The literature on prenatal ABO selection is critically reviewed. The contribution of maternal-fetal incompatibility to the genetic load is discussed, and it is concluded that few loci can be as important causes of fetal death as the ABO system. The requirements for a definitive study of ABO effects are considered.

On this evidence, we propose as a working hypothesis that the principal mechanisms of selection which maintain the ABO polymorphism act during fetal and early postnatal stages, and that the reported associations between blood groups and adult disease are at most second order selective effects.

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