Selective Survival in Dizygotic Twins in Relation to the ABO Blood Groups*

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SELECTIVE PROCESSES affecting fetal survival have important implications for human genetics, and have a particular bearing upon the interpretation of twin evidence. It is the possible importance of early selection in dizygotic twins in relation to the ABO blood groups which constitutes the subject of this paper.

An early observation on the fetal development of twins, which has been the subject of constant review and reinterpretation, concerns the fusion of twin placentae, and the consequent occurrence of vascular communications between the placentae. It was originally held that a fused or single placenta, and a single chorion, were both indisputable evidence, and a prerequisite for proof of monozygotic twinning in all placental mammals from armadillo to man. It is now known that at least one third of all human monozygotic twins may have separate chorions as well as separate placentae (Essen-Moller 1941, Corner 1955). While a single chorion is not known to occur in dizygotic twinning, except secondarily, as a result of fusion and partial absorption, (Arey 1922), a single placenta may be found. In dizygotic twin cattle it has long been known that fusion of the placentae may be accompanied by vascular communications between the placental vessels of the twin fetuses. In 1916 Lillie explained the 'free-martin' in dizygotic cattle twinning on the basis of fusion and vascular communication between placentae; in 1945 Owen described the 'red-blood-cell chimera' in dizygotic cattle twins, as a result of this same phenomenon. In spite of these findings, it was generally considered that in man actual vascular communications between the placentae occurred only in monozygotic twins, (Price 1950, Wenner 1952), until Dunsford, et al, in 1953, discovered the first red-blood-cell chimera in a human twin. This discovery suggested that vascular communications between placentae, not only can, but do occur in human dizygotic twins. It has recently been confirmed (Booth, et al 1957; Nicholas, et al 1957). Because of the common occurrence of red-blood-cell chimeras in dizygotic cattle twins, and their apparent rarity in human twins, it is now assumed that vascular communications between human dizygotic twin placentae is a rare event. However, with the placental, developmental, and serological differences which exist between man and cattle (Hancock 1954), there is no reason for assuming that the consequences of this event would be entirely comparable in man and cattle. It is possible that in man the more common consequence might be early fetal death rather than the occurrence of a blood group chimera.

In man, a possible relationship between early fetal death and the blood group

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factors has been frequently suggested. With discovery of the role of maternal-fetal Rh incompatibility in the pathogenesis of erythroblastosis (Levine, Katzin and Burnham 1941), an extensive literature has developed, indicating that maternal-fetal blood incompatibility in the ABO system may be a cause of sterility or early fetal death, (Levine 1943; Fisher, as quoted by Race and Sanger 1950). That fetal death due to incompatibility could occur in very early development is known, for the AB agglutinogens are present in detectable strength in the red blood cells of the month old fetus (Kemp 1930; Wiener 1943). It is therefore possible that an exchange of incompatible blood between twin fetuses, as well as maternal-fetal exchange, could result in fetal death and abortion well before the end of the third month of development. There is little likelihood that a pregnancy terminated before this time would be recorded in the obstetrical record as having been that of a multiple conception. Even if but one of the twin fetuses was to die prior to this time, as a result of either maternal, or co-twin blood incompatibility, and the pregnancy continued to term, absorption of one twin fetus might easily obscure the original twin status of the surviving infant (Kindred 1944). The assumed frequency of multiple ovulation in the human female, and consequently of multiple conception, is based in large part upon the observed frequency of dizygotic twinning. Obviously then, a selective survival for dizygotic twins taking place within the first two, or even three, months of fetal life might not be revealed by data commonly available in the obstetrical record. If an appreciable early loss of the products of multiple ovulation is in fact occurring, this would have important implications, not only for our theories concerning human twinning and the methods employed in twin analysis, but would also add to our understanding of human ovulation and human fertility as well.

If an early loss of dizygotic twins is occurring due to ABO incompatibility, it should be found that dizygotic twins differ less in their blood group factors than would otherwise be expected. This hypothesis can be tested by comparing the intra-pair blood group differences of dizygotic twins with those of single born siblings, or by calculating the theoretical expectancies, utilizing population gene frequencies. Schiff and von Verschuer (1933) in utilizing the twin study method for verifying the inheritance of the ABO blood group system noted that the number of dizygotic twin pairs discordant in the ABO system was less than expected. A similar observation has been reported by Gedda (1951). Beginning in 1954, a serological study of twins and their available siblings was undertaken specifically to test the hypothesis of early fetal death in dizygotic twins as a result of blood incompatibility.

THE STUDY SAMPLE

The first prerequisite for a study of blood group factors in relation to survival in twins is to obtain a sample of twin subjects and their siblings unselected on the basis of any prior knowledge as to their blood group differences. The subjects obtained for this study came from two different sources, unselected as to their zygosity, and free from any discernible bias as to their blood group factors. The larger of the two groups of twins, which will be referred to as the adult series, are twin pairs and their

available siblings who have been under extensive study for several years (Osborne 1956), and will be reported in greater detail elsewhere. This series consists of 131 pairs of Caucasian twins drawn from a variety of sources in New York City, unselected as to sex and zygosity, ranging in age from 18 to 55 years, and obtained for the purpose of establishing a population of adult twin subjects in good general health. Twenty-nine siblings were available and willing to participate in the study, and have received examinations comparable to those given the twin propositi.

The second group of twins, which will be referred to as the juvenile series, consists of all the twins available for study at the ages of 4 to 8 years who were born in Sloane Hospital between 1946 and 1952, to women included in a large cooperative study, known as the Fetal Life Study, carried out under the Departments of Obstetrics and Pediatrics (McIntosh et al 1954). In this cooperative study, all of the expectant mothers admitted to Sloane Antepartum Clinic with a duration of pregnancy of four months or less, on their first visit, were included in the study. After exclusion of the women transferred or discharged from the clinic for various reasons, (McIntosh, et al 1954), there were a total of 5964 pregnancies, of which 84 were twin pregnancies. In 1955, the parents of twins in this population were contacted by the original study group, and all of the pairs with both members still surviving and available for study, were brought into the pediatric clinic for blood tests and zygosity diagnosis. A total of 100 twins, of which there were 48 complete pairs and 18 single born siblings were studied for this population. No selection factor which would bias this sample could be detected for the twins who had either left the hospital area, or failed to respond for this study. It is therefore considered that this juvenile series constitutes as unbiased and complete an ascertainment of twins from an essentially normal reproductive population as it is possible to obtain for the purposes of the present investigation.

A total of 179 twin pairs were obtained from these two samples, of which 94 pairs are dizygotic: 60 pairs in the adult series, and 34 pairs in the juvenile series. 47 single born sibs of the twins were also studied: 29 in the adult series, and 18 in the juvenile series.

The blood determinations, for both the adult and juvenile series, were done by professional blood laboratories; all determinations were done at least in duplicate and independently, to assure the highest degree of typing accuracy practicable. The diagnosis of zygosity in all of the like sex twins has been based upon proving dizygosity, first, by a proven difference in a blood group factor, and then by adding other reliable criteria such as eye color, hair color, ear form, and digital hair; only accepting differences which left no question as to the diagnosis of dizygosity. These criteria, as well as the method of diagnosis, have been discussed (Osborne 1956), and will be presented in detail in a forthcoming publication. The advantage of this method of diagnosis, in general, and particularly in the present study, is that it provides a strict test of the hypothesis, with a known direction of possible error in diagnosis. The only mistake in diagnosis which can occur with this method, is the misclassification of an unusually similar pair of dizygotic twins as monozygotic. By placing the first emphasis in the diagnosis upon a proven difference in a blood factor, it is possible that some bias has been introduced for the purposes of the present

study. This bias, however, would tend to increase, rather than decrease, the average intra-pair difference in the dizygotic twins for their blood group factors, and would make the test of the present hypothesis a more conservative one.

RESULTS

In table 1 the dizygotic twin pairs are listed separately for the adult and juvenile series, according to the ABO blood types. The male and female like-sexed pairs have been combined, as there were no apparent sex differences in either series. In the adult dizygotic twins 70.0 per cent were found to be concordant in their ABO blood types, and in the juveniles 91.3 per cent are concordant.

The number of concordant twin pairs that would be expected to occur in these two populations are compared to the observed in table 2. The juvenile series is a racially mixed population, (white and negro), consequently, it has been necessary to handle the white and negro twin pairs separately. Because of the obvious difference between the juvenile and adult series in age, as well as in the per cent concordant for the ABO system as seen in table 1, the juvenile whites have not been combined with the white adult series. The gene frequencies used for calculating the expected

TABLE 1. ABO BLOOD GROUPS OF DIZYGOTIC TWIN PAIRS

	Adult		Total		Juvenile		Total	
	Like Sex	Unlike Sex	n	%	Like Sex	Unlike Sex	n	%
O-O	13	8	21	35.0	13 (8)*	9 (6)	22	64.8
A-A	11	3	14	23.3	1 (1)	6 (2)	7	20.7
B-B	2	0	2	3.4	1 (1)	0	1	2.9
AB-AB	4	1	5	8.3	1	0	1	2.9
Concordant	30	12	42	70.0	16	15	31	91.3
O-A	7	1	8	13.3	1 (1)	0	1	2.9
O-B	2	3	5	8.3	0	1 (1)	1	2.9
O-AB	0	1	1	1.7	0	0	0	0
A-B	3	0	3	5.0	0	0	0	0
A-AB	0	1	1	1.7	0	0	0	0
B-AB	0	0	0	0	0	1	1	2.9
Discordant	12	6	18	30.0	1	2	3	8.7
Total	42	18	60		17	17	34	

* Numbers in parenthesis designate the number of negro twin pairs out of the Total Juvenile series in the particular category.

TABLE 2. DIZYGOTIC TWIN PAIRS CONCORDANT FOR ABO

	N	Observed	Expected	Variance	χ^2	P
Juvenile White	14	13	9.093	3.187	4.790*	.03
Juvenile Negro	20	18	12.328	4.729	6.803*	.01
Adult	60	42	38.970	13.659	1.659	.20

* With one degree of freedom.

TABLE 3. ABO CONCORDANCIES AND DISCORDANCIES AMONG SIB-SIB AND TWIN-SIB PAIRINGS

	MZ				DZ				
	Sib-Sib		MZ-Sib		Sib-Sib		DZ-Sib		
	Conc.	Disc.	Conc.	Disc.	Conc.	Disc.	Conc.	Disc.	Total
Adult	8	10	8	7	3	4	11	4	55
Juvenile White	3	0	0	3	0	0	2	0	8
Juvenile Negro	0	0	0	0	4	4	6	4	12
Total	11	10	8	10	7	8	19	8	75
Per Cent	52.4		44.4		46.7		70.4		

proportion of discordant negro twin pairs are the New York City figures of Landsteiner and Levine (Moore 1955). The phenotype frequencies given by Landsteiner and Levine agree very closely with those of the study population; since the calculated gene frequencies would be more reliable from the larger data, these were used, ($p = .198$, $q = .142$, $r = .660$). The gene frequencies used for whites are the combined New York City and North Carolina frequencies of Landsteiner, Levine, Weiner, and Boyd, ($p = .248$, $q = .078$, $r = .674$), as given by Moore (1955). These frequencies appeared to fit the white data of this study. Using these gene frequencies, the probabilities of different mating types were calculated, and the resulting probability that both members of a dizygotic twin pair would be concordant in their ABO blood groups was obtained. From this the expected number of concordant twin pairs was calculated for comparison to the observed as presented in table 2. Chi-square was calculated by treating the twin pairs as families of two,

$$\chi^2 = (\text{observed} - \text{expected})^2/\text{variance}. \qquad \text{(Smith 1956).}$$

The variance is $(c \times n)(1 - c)$, where c equals the probability of concordant twin pairs, and n is the total number of dizygotic pairs. The excess of concordant pairs in the juvenile white has a probability value of 0.03, and in the juvenile negro it is less than 0.01, while for the adult series the excess of concordant pairs is not statistically significant. In the negroes it is primarily type O concordant pairs which contribute to the observed excess. If the type O pairs are taken separately, by the same method as above, $\chi^2 = 15.280$, $P = <.001$. In the juvenile white it is similarly the Type O pairs which contribute the most to the concordancy excess. In the adults it is again an excess of type O, with a relatively great excess of AB–AB pairs, and an actual deficiency of A–A and B–B pairs.

The significant excess of dizygotic pairs concordant for ABO blood groups among both the white and negro juvenile twins, plus the excess of concordant pairs in the adult series, even though not significant in the latter, strongly support a hypothesis of selective survival in dizygotic twins in relation to the ABO system. The similarity in the concordancy pattern between the adult and juvenile series, in the presence of the marked difference of concordancy ratio in the two series, suggests that some factors other than the ABO blood groups alone may be associated with survival in dizygotic twins. Aside from age, which by definition separate the adult and juvenile series, there are important factors peculiar to each group which will be discussed presently.

The concordance-discordance data for the sibling pairs are presented in table 3. While the number of sibling pairs are not adequate for statistical treatment, they are presented here as they lend some support to certain other observations and should be of interest for comparison with the data of other studies. The sibling pairs of the monozygotic and dizygotic twins are presented separately. Sibling pairs were obtained by pairing the single born sibs of the twin propositi. If there was only one single born sibling of a twin pair, obviously no sibling (sib-sib) pair could be formed, but this sib is paired with one of his twin born sibs to form a MZ–Sib, or DZ–Sib pair, as the case may be. Two single born siblings of a twin pair provided one Sib–Sib pair, and two MZ–Sib or DZ–Sib pairings, only one member of a twin pair taken at random being used for such pairings. The monozygotic Sib–Sib, MZ–Sib and dizygotic Sib–Sib pairings very nearly agree in the percent of concordant pairs, while the proportion of concordant pairs in the DZ–Sib comparison is much higher, closely resembling the value observed for dizygotic twin pairs. The high DZ–Sib concordancy ratio, and its similarity to the dizygotic twin pair value, rather than to the other sib comparisons, would seem to suggest that some factor other than vascular communications between the twin placentae may be responsible for the excessive dizygotic twin concordancies.

DISCUSSION

In 1933 Schiff and Von Verschuer reported a study of 446 twin pairs, 202 monozygotic, and 244 dizygotic, for whom they obtained ABO blood groups. These authors noted a deficiency of like sex dizygotics and of unlike sex twin pairs discordant for the ABO blood groups. Of the 244 dizygotic pairs, 156 or 63.9 per cent were found to be concordant for ABO. According to their calculations, the theoretical expectancy was only 45.6 per cent (Schiff and Von Verschuer 1933). Their theoretical value was apparently obtained empirically, and while it agrees exactly with the observed value for sibling pairs in the present data, this theoretical value is less than would be calculated from German gene frequencies using the methods of the present study. Gedda (1951) reported findings similar to those of Schiff and Von Verschuer and those of the current study. In 39 pairs of dizygotic twins, 64 per cent were found to be concordant in their ABO types, while only 58 per cent of 93 sib pairs were concordant. The blood group combinations in Gedda's data are not available, but Schiff and Verschuer's data show, as in the present study, that the twins concordant for Type O account for most of the observed excess of concordant pairs. The similarity of the findings of the present study with those of the two earlier studies could well be fortuitous, but it must be considered to give added support to a hypothesis of selective survival. The fact that other reports of this nature are lacking is not surprising, for the blood grouping of large numbers of twins has been almost exclusively done for the sole purpose of diagnosing zygosity. In such studies unlike sex pairs are not blood grouped and the statistical treatment of the blood data is initially based upon an assumption that no selective factor will interfere with realization of the mathematically expected segregation ratios. That selective survival in dizygotic twins may be taking place, in relation to the ABO blood groups, would now be difficult to question, particularly in the light of the significant excess of concordant pairs

in both the white and negro juveniles who represent unbiased twin samples of a normal reproductive population. The relative excess of concordant pairs in the juvenile as compared to the adult series, however, suggests something other than a simple blood incompatibility factor, and may actually provide an opportunity for investigating other factors which may be associated with selective survival in relation to the blood groups.

Important differences exist between the adult and juvenile twin series which may have an important bearing upon the comparative findings in the two populations. In the adult series it was not only necessary for both members of every twin pair to survive into adult life, instead of only to the age of 4 to 8 years, but it was also necessary for both members of each adult pair to present a reasonable state of health to be considered eligible for study. For the juvenile series, on the other hand, survival and availability until the age of 4 to 8 years were the sole selection criteria. If severely impaired health at an early age is positively correlated with adult health, which would appear to be a reasonable assumption, many of the juvenile twins would fail to qualify as adults for the adult twin study population. The medical histories of the juvenile twins were independently evaluated by the authors, and a conservative count was made of the twins definitely presenting a sufficient impairment of health to preclude their possible consideration, even at their present age, for a study comparable to the adult series. [Examples of twin members considered to be in poor health are: 1. a Mongolian Idiot; 2. a celiac, requiring constant medical care, for Acute U.R.I. conditions, (including: bilateral otitis media, bronchitis, spasmodic croup, tonsilitis, and pharyngitis)—from 9 mos. of age until the time studied at 4.8 yrs.; 3. a child with a systolic murmur detectable at 5 wks. of age and persisting to the present, cerebral palsy spastic diplegia, and having autistic behavior; 4. a child having episodes of seizures and convulsions from 2 mos. of age to the present, mentally retarded, and having poor motor coordination.] On the basis of the medical record thirty per cent of the juvenile twin pairs studied (40 per cent of the negro, and 50 per cent of the white), were considered unsuited for a study comparable to that of the adult group. Most interestingly, these pairs in inferior health included one male and one female monozygotic pair, one male and one female like sex dizygotic twin pair, and 11 out of the total of 17 unlike sex pairs in this population. In the unstudied pairs, 28.6 per cent were similarly classified as being in poor health. It is apparent that the adult twin series, and perhaps the dizygotics in particular, represent pairs selected by health, and survival, from a vastly larger initial twin population than the juvenile series. If health and survival correlate with favorableness of prenatal development, and if it can be further assumed that unfavorable prenatal development is associated with conditions which increase the likelihood of either inter-twin or maternal-fetal blood exchange, then it would be expected that twins selected for good health, in this case the adult twins, would have a relatively larger proportion of pairs discordant for their blood factors. The possible importance of this selection factor in the present study may be supported by the suggestive findings of Bennett and Walker (1956). In studying fertility and the blood groups in East Anglian blood donors, it was found that for the newly born child, the chance of death under 10 years of age was significantly greater ($\chi^2 = 10.4$, $P = 0.001$) when the father is of

group O, Rh positive. In populations where approximately one half of the fathers would be of type O, 85 per cent of which would be Rh positive, a larger proportion of the type O concordant dizygotic twin pairs would be expected to have type O, Rh positive fathers. The present data indicated that concordant pairs, particularly if they are concordant for type O, are favoured presumably in prenatal life. If there were a differential loss of type O twins in the first 10 years of life, then by adult life, the initial excess of type O concordant pairs would be in part lost, resulting, as we have observed, in the relatively smaller proportions of concordant pairs in the adult series.

A second difference between the adult and juvenile twin series concerns that of twin birth rank. In the adult series 36.7 per cent of the dizygotic twins were the result of the first pregnancy, while for the juvenile series only 23.5 per cent represented the first pregnancy. The same relative proportions hold for the monozygotic twin pairs, being 40.9 per cent and 21.4 per cent respectively. These differences in parity between the two twin samples, while not statistically significant, suggest a difference in reproductive history, and the observed differences in the proportion of twin pairs concordant for the ABO blood groups may reflect a prenatal affect associated with maternal age and parity. A differential fertility with maternal age and the ABO blood groups has been shown by Kirk, Kirk, and Stenhouse (1953) with the fertility of type O women increasing at the age of the highest frequency of dizygotic twinning. It may well be that there is an excess of type O women having dizygotic twins which could in part account for the observed excess of type O concordant twin pairs. If maternal and parity factors relate to both the ABO blood groups and to dizygotic twinning then in twin populations characterized by different parity orders contrasting concordancy ratios could attain, as has been observed in the present study. Certainly the possibility of a maternal and parity affect is suggested; this is presently under investigation by the authors.

There would appear to be little doubt but that in certain types of twin populations, at least, some selective factors may be operating to significantly alter the theoretically expected segregation ratios in the ABO blood group system. Whether this is due to early fetal death as a result of a transplacental exchange of incompatible blood, or vascular communications between dizygotic twin placentae can not be determined. Mechanisms other than antigenic incompatibility, such as dispermic fertilization of a single ovum, could also result in similar findings. However, since there is no evidence at the present time for such twinning mechanisms, and since antigenic incompatibility has been established as a factor in fetal survival, this constitutes the more likely hypothesis. Until such time as this problem can be more fully evaluated, the present findings clearly imply the necessity for caution in employing calculated probabilities for a correct diagnosis of zygosity. Perhaps most importantly, the present study brings twin evidence to the support of the hypothesis of a relationship between the ABO blood group system, fertility, and early fetal death, with the implication that the phenomenon of multiple ovulation and conception is more frequent than has been suspected, and may consequently be intimately associated with the general problem of human fertility.

SUMMARY

Evidence has been presented which supports a hypothesis for a selective survival in dizygotic twins in relation to the ABO blood group system, by demonstrating an excess of dizygotic twin pairs concordant in the ABO blood groups. The possible association of this selective mechanism to maternal age and parity are discussed, and some of its implications are presented.

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