Acta Genet Med Gemellol 36:275-288(1987)

Race, Zygosity, and Mortality Among Twins: Interaction of Myth and Method

C.E. Boklage
Genetics Program, East Carolina University School of Medicine Greenville, North Carolina, USA

Abstract. For epidemiological purposes, it is customary to assume that same-sex (SS) dizygotic (DZ) twin pairs are approximately equal in number to unlike-sex (OS)-DZs, the remainder of the SS pairs being monozygotic (MZ). It is also customary to consider OS-DZs to be epidemiologically representative of all DZs, which can only mean that difference in frequency of any trait between OS and SS twins is due to the MZ fraction of the SS twins. Since this is assumed as a premise, there is little value in its usual appearance as the result. The basic tenet of twin biology, that most twin excess anomalies are due to MZs, is a myth self-perpetuated by a methodological tautology, and is false, at least for mortality. In a consecutively ascertained and prospectively studied sample of 616 twin pairs, over 80% diagnosed for zygosity, it can be shown that the standard assumption mentioned above have given impossible answers. The most probable possible answer is that mortality does not differ greatly with zygosity overall, but that SSDZ mortality is much higher than that of OS twins, and probably even higher than that of MZs. Race differences in the probable answers further suggest that standard assumptions of the Weinberg method may have consistently provided false explanations for race differences in the OS fraction of twin pairs.

Key words: Twin mortality, Weinberg method, Zygosity

INTRODUCTION

With very rare exception, only large public records databases provide sample sizes sufficient for epidemiological studies concerning twins. Since, with even rarer exceptions, such

data contain no information relevant to zygosity determination beyond sex-pairing, it is customary to use the Weinberg difference method to estimate zygosity fractions. Assuming sex fractions are 50%, this requires only taking the difference between the number of SS pairs and the number of OS pairs as an estimate of the number of MZ pairs:

$$SS - OS \cong MZ$$

The logic of this approach is on its surface simple and direct. It seems reasonable to assume that the sexes of two zygotes derived by fertilization of independent oocytes are independent, in which case, sex-pairing should be binomially distributed among DZ pairs.

Tha SS male fraction of DZ pairs should be the square of the fraction male (m^2) among all such fertilizations, the SS female fraction the square of total fraction female (f^2), and the OS fraction should be twice the product of the two sex fractions, 2mf. If both sex fractions equal 0.5, $m^2 + f^2 = 2mf$, SSDZ = OS, and SS – OS = SS – SSDZ = = MZ. The correction for unequal sex fractions is mathematically trivial [MZ = SS – OS $(1/2\ mf - 1)$], but it does bring to notice an additional assumption that has in fact already been made, namely, that sex fractions are the same among twins of both zygosities. Among twins of identified zygosity, that has not seemed to be the case [11-14].

A much worse problem with this approach is that an assumption about sex-pairing distribution at fertilization is being applied to numbers observed at birth. This necessarily implies the additional assumption that all events between fertilization and birth, all prenatal mortality and all developmental anomalies, are independent of sex-pairing among DZs. This is often stated as if it were a second, separate assumption, to the effect that OS pairs can be considered epidemiologically representative of all DZs. This is only another way of saying that any difference between OS and SS pairs cannot be due to SSDZs, but most be due to MZs.

As a result, the answer to any question about zygosity differences in mortality or developmental anomaly has been assumed before the analysis is well begun. Even if the assumption should prove to be biologically correct, any answer so derived is logically unsound. In the absence of any additional information relevant to zygosity diagnosis, it is usually the case that little else could have been done.

For at least one respectable sample, with the additional information of chorionicity and/or blood typing available for most subjects, it is possible to test the consequences of applying this assumption. From those tests, it can be shown that results derived by this usual approach are impossible. Other approaches not requiring that self-fulfilling assumption yield answers that are possible, consistent, and quite different from what is commonly believed.

MATERIALS

The sample is hand is that of the Collaborative Perinatal Project of the United States National Institute of Neurological and Communicative Diseases and Stroke. Beginning in 1959, over 53,000 births were studied from the first prenatal visit, with most of the children being followed through seven years of age. The sample was consecutively ascertained, the study prospective. The characteristics of the sample have been recently reviewed [17].

The frequency of twinning in the sample does not differ in any interesting way from

that reported in the American population in the same period of time. The prenatal mortality rate among these twins in vitually identical to that reported from a multiple-source longitudinal survey of the population of metropolitan Atlanta [15]. This sample is not significantly enriched in relevant pathology.

There is one respect in which the sample might not be representative of the total United States population – the patients were primarily clinic patients in teaching hospitals. This has the effect of overrepresenting the lower socioeconomic strata of the American population, which fact may be turned to significant advantage for the present considerations. It allows racial comparisons with much-reduced concern about confoundment with socioeconomic differences.

Previous tabulations of these data, pooled over race, and in some cases over sex [3,16], did not allow analysis of interactions of individual gender with sex-pairing and zygosity, nor any testing of race differences, alone or in interaction. We reexamined records of the Collaborative Perinatal Project, and recollated the pertinent data. Most discrepancies were resolved by finding the answer to a given question recorded in an incorrect position in microfilms of the original paper records.

Mortality as defined here includes fetal death and death within the first 28 days after delivery. For some analyses we will separate deaths before and after birth.

Table 1 shows the distribution of these twin pairs and their mortality according to knowledge of their sex-pairing, chorionicity, and bloodtyping where applicable.

Table 1 - Fetal and neonatal mortality in twin pairs of the Collaborative Perinatal Project, by race, sex, and zygosity

	Deaths/individuals					
	White		Black		Total	
	Male	Female	Male	Female	Male	Female
Monochorionic (incl. monoamnionic)	24/74	7/54	10/48	15/72	34/122	22/126
MZ, dichorionic or ?C	0/18	0/22	2/36	0/56	2/54	0/78
Same-sex, known DZ	1/64	2/52	0/50	0/58	1/114	2/110
Same-sex, unknown zygosity	28/60	13/40	30/56	20/42	58/116	33/82
Opposite-sex	11/91	10/91	11/112	15/112	22/203	25/203
Total known MZ	24/92	7/76	12/84	15/128	36/176	22/204
Total known SS	53/216	22/168	42/190	35/228	95/406	57/396
Total known DZ	12/155	12/143	11/162	15/170	23/317	27/313
Total known-sex pairs	64/307	32/259	53/302	50/340	117/609	82/599
Unknown sex-pairing	5/6		16/18		21/24	
TOTAL	101/572		119/660		220/1232	

METHODS

For most of this sample, chorionicity is known. If a twin pair is monochorionic (MC), they may safely be considered MZ, regardless of any other aspect of the condition of the babies. This allows diagnosis of roughly two-thirds [cf 1] of all MZ pairs.

If a twin pair is opposite-sex, they may safely be diagnosed DZ, regardless of the

condition of the babies beyond knowing their sexes. This allows diagnosis of roughly half of all DZ pairs.

If the pair is same-sex and dichorionic (DC) or of unknown chorionicity, zygosity diagnosis can only be made by genotyping. Because this sample was collected prior to the advent of genotyping by DNA polymorphism [7,10], SS dichorionic pairs could only be genotyped if both were alive for bloodtyping. The low apparent mortality of *known* SSDZ pairs is an artifact of this methodological constraint, due to the inability to identify as DZ any SS pairs of which either members was dead.

It should be clear that the (SS-DC) pairs which cannot be diagnosed by chorionicity or unlike sex are enriched for DZ pairs; roughly three-to-one. In the era of these twin births, MZs, SSDZs and OS each represented approximately one-third of all twins according to most estimates. Since about 2/3 of MZ twins are MC [1], about 1/9 of all twins could be expected to be MZs not identifiable as such by chorionicity, and about 1/3 should be DZs not identifiable as such by sex-pairing. Thus, among the twin pairs diagnosable for zygosity only by genotyping, about 3/4 may be expected to be DZ. Most such pairs in this sample (73.8%) have been genotyped. Sixty-three per cent of the bloodtype diagnoses indicated dizygosity, consistent with the prospect that the remaining unknown-zygosity (UZ) pairs are still enriched for DZs. The most common reason for non-diagnosis was the death of one or both twins (55/99 SSUZ pairs, 36 concordant).

The methodological approach used here is fairly simple. By substracting the known members of each subgroup, living or dead, from the corresponding estimated group memberships, it can be shown that the standard approach yields mortality estimates which are impossibly high for the MZs. Alternative approaches are then demonstrated, with no aggressively circular required assumptions, which yield answers that are (bio)logically possible and mutually consistent.

RESULTS

Estimation of Sex(Pairing) × Zygosity Subgroup Mortalities Among Twins

Standard Approach, Step One

Ignoring the few pairs with unknown sex-pairing, using overall sex fractions for Weinberg method estimates, zygosity fractions (pooled over race and sex) are computed as follows:

Total male fraction, m	0.5424
Total DZs = OS/2m (1 − m)	406 pairs
SSDZs = total DZ − OS	203 pairs
MZs = total SS − SSDZs	198 pairs

Standard Approach, Step two

Assume total DZ mortality rate = OS mortality rate:	47/406 = 0.116
Estimate total DZ deaths: OS mortality rate × total DZs:	0.116 × 812 = 94
Estimate total MZ deaths: total deaths − DZ deaths:	199 − 94 = 105
Estimate MZ mortality rate: MZ deaths/MZs:	105/396 = 0.265
MZ/DZ mortality ratio:	0.265/0.116 = 2.29

So far, so good. This is the result we have generally come to expect. In this sample, however, the availability of zygosity diagnoses for most pairs allows testing of the results of the standard approach.

Of 199 deaths in the known-sex pairs, 108 occurred in individuals of identified zygosity, 91 to those of unknown zygosity. Let us compare the distribution of deaths in twins of identified zygosity to the overall estimates derived above:

	DIZYGOTICS	
	DZs	DZ deaths
Estimated	812	94
– Identified	630	50
Difference	182	44

Mortality in unidentified DZs 0.242

	MONOZYGOTICS	
	MZs	MZ deaths
Estimated	396	105
– Identified	380	58
Difference	16	47

Mortality in unidentified DZs 2.938

Among DZs, the results are clearly not consistent with the standard assumption. Mortality among unidentified SSDZs is over twice that observed among OSDZs, close to the standard estimate of total MZ mortality, and even perhaps higher than that observed for monochorionic MZs (0.226).

For MZs, the results are logically impossible. No doubt some of the "identified MZs" are in fact DZs that bloodtyping failed to identify as such. Even assuming 100% mortality of unidentified MZs, for that prospect to serve as an exit from this predicament would require failure of DZ exclusion by bloodtyping in at least 16 of the 66 pairs identified as MZ by genotyping. That efficiency of performance could be expected if only one of the markers used was informative in each family.

If all of the estimated-but-unidentified MZs died, but only once, *maximum* total MZ mortality among the known-sex pairs would be 0.187. Assigning all remaining deaths to DZs would make (*minimum*) total DZ mortality 0.154. the maximum MZ/DZ mortality ratio among the known-sex pairs is then 1.21, not much over half the ratio estimated by the standard procedure. Subtracting the OS leaves 0.192 *minimum* SSDZ mortality, still nearly twice the OS rate, and very similar to the MZ rate. Given Weinberg method estimates of zygosity fractions, and the distribution of identified zygosity, maximum MZ mortality is about the same as the corresponding minimum SSDZ mortality. You may wish to refer to these limits as we proceed.

The problem lies in the fact that, according to the Weinberg method estimates, the overwhelming majority of the undiagnosed pairs (91/99) must be DZ, whereas the usual methodology assigns over alf of their mortality to MZs.

One Alternative Approach Without Presuming MZ Excess Mortality

If the deaths occurring among twins of known sex-pairing but unknown zygosity are assigned by simple proportion, $0.92 \times 91 = 84$ SSUZ deaths would be assigned to DZs, and $0.08 \times 91 = 7$ to MZs. Adding those estimates to known occurrences yields $(58 + 7)/(380 + 16) = 0.164$ total MZ mortality rate, and $(50 + 84)/(630 + 182) = 0.165$ total DZ mortality. Dividing the DZs by sex pairing, assuming all OS pairs are identified, the

https://doi.org/10.1017/S0001566000006036 Published online by Cambridge University Press

SSDZ rate is estimated at 0.214. Overall MZ/DZ mortality ratio would be 1.025, and the SSDZ/MZ ratio would be 1.30.

Being conservative of prevailing opinion by assigning all pairs of unknown sex pairing (US), with their severe mortality, to MZs would raise estimated MZ mortality to 0.204, higher than that of total DZs, but still not exceeding that of SSDZs. These results are all possible, and within the limits estimated above.

A Second Alternative Approach

Ninety-six per cent of the estimated MZs in this sample are identified, vs only about half of the estimated SSDZs. Two thirds of the known MZs are identified by monochorionicity, observation of which is unaffected by any but the very earliest mortality, whereas the identified SSDZs had to be alive for blood-antigen genotyping. Total mortality is therefore much more closely estimated by observed mortality for MZs than for DZs. We would certainly stand on better ground estimating zygosity distribution of mortality by extrapolating from observed MZs than we have been in extrapolating from OS. The result is as follows:

$$\text{Total MZ mortality} \cong \text{observed MZ mortality} = 58/380 = 0.1526$$
$$\text{Estimated total MZ deaths} = 0.1526 \times 396 = 60$$
$$\text{Total DZ deaths} \cong \text{total deaths} - \text{MZ deaths} = 199 - 60 = 139$$
$$\text{Total DZ mortality rate} = 139/812 = 0.171$$
$$\text{divided between OS} = 47/406 = 0.116$$
$$\text{and SSDZ} = 92/406 = 0.227$$

Conservatively assigning all of the deaths in US pairs to MZs would raise estimated total MZ mortality rate to 0.196, higher than estimated for total DZs, but still not exceeding that estimated for SSDZs.

The same plausibility test applied above to the standard estimate may be applied to this one: For the total sample, 92 deaths in 406 estimated SSDZs, minus 3 deaths in 224 identified SSDZs leaves 89 deaths among 182 estimated-but-unidentified SSDZs (0.489), and 2 deaths among the 16 unidentified MZs (0.125). These answers are possible, and within the limits estimated above.

Compared to the estimate above based on uniform zygosity assignment of UZ deaths, this approach changes the assignment of only 7 (7.7% of total) UZ deaths. The consistency between results of these two estimates is satisfying. In spite of bending over backwards to be conservative of prevailing opinion, all three approaches we have taken indicate that overall MZ and DZ mortalities simply cannot differ nearly as much as usually estimated. SSDZ mortality is certainly much higher than that of OS pairs, and quite possibly even higher than that of MZs.

This "second alternative" approach may be extended, allowing us to take into account all of the available information, dividing the sample by sex and race. This is important because zygosity fractions are expected to vary by race, and mortality by sex, and because certain race-by-sex interactions are suggested. For lack of any reasonable alternative, overall zygosity fractions must still be estimated from binomial distribution of DZ sex pairing:

$$\text{OS} = \text{number of OS pairs, m} = \text{fraction male, f} = \text{fraction female}$$
$$\text{Total DZ} \cong \text{OS}/2mf$$

https://doi.org/10.1017/S0001566000006036 Published online by Cambridge University Press

$$\text{Male SSDZ pairs} \cong m^2 \times \text{total DZ} \cong m/2f \times OS$$
$$\text{Female SSDZ pairs} \cong f^2 \times \text{total DZ} \cong f/2m \times OS$$
$$\text{Male MZ pairs} = \text{SS male pairs} - m/2f \times OS$$
$$\text{Female MZ pairs} = \text{SS female pairs} - f/2m \times OS$$

It is unreasonable to continue supposing that there are no OS pairs among those of unknown sex-pairing. There is no more justification for that assumption than for supposing they are all OS; twin abortions surveyed by Uchida et al [19] were 50% OS. At least one member of each of these US pairs died long before discovery and was in such poor condition that its sex could not be determined. Prospects of estimating zygosity and sex-pairing among these cases all depend on extrapolating from mortalities of the corresponding known groups, primarily involving much later death and therefore possibly arising from a different set of causes. Although this 2% of the sample accounts for 10% of the mortality, little that is plausible can be done with them. Fortunately their numbers are small enough that results are not changed substantially by any distribution other than assigning all of them to one subgroup.

We must also take into account the fact that, among twins of known zygosity, sex ratio differs with race and zygosity. The race difference in sex ratio is significant among MZs ($\chi_1^2 = 8.04$) and in total ($\chi_1^2 = 5.95$). The zygosity difference in sex ratio (known MZ vs either known DZ or all-but-known-MZ) is significant only among blacks ($\chi_1^2 = = 6.55$). Since it is the DZ sex-pairing distribution which is to be estimated, and since the majority of estimated-but-unidentified pairs must be DZ, *race-specific sex fractions among all-but-identified-MZs will be used for the estimates to follow*.

As shown by Table 2, estimated SSDZ mortality rates are substantially higher than those of OSDZs, in all subgroups. With the single exception of white males, estimated SSDZ mortalities are higher than those of MZs. In every subgroup the number of estimated deaths is smaller than the estimated membership of the group.

Table 2 - Estimation of sex x zygosity subgroup mortalities among twins – Fetal and neonatal deaths combined

	White		Black		Pooled	
	Male	Female	Male	Female	Male	Female
Estimated DZ sex fractions (total – known MZ)	0.540	0.460	0.507	0.493	0.523	0.477
Estimated SSDZs	106	78	116	108	222	184
Estimated total MZ SS – estimated SSDZ	110	90	74	120	184	212
Observed MZ mortality	24/92 (0.261)	7/76 (0.092)	12/84 (0.143)	15/128 (0.117)	36/176 (0.204)	22/204 (0.108)
Extrapolated total MZ dead	29	8	12	15	38	23
Estimated DZ deaths (total – MZ) dead	35	24	41	35	79	59
Estimated SSDZ dead = (DZ – OSDZ) dead	24	14	30	20	57	34
Estimated SSDZ mortality rate	0.226	0.179	0.258	0.185	0.257	0.185
Observed OS mortality rate	0.121	0.110	0.098	0.134	0.108	0.123

The estimated binomial distribution of DZ sex pairing *at birth* is the primary potential source of error here. For lack of better prospects, we have used the Weinberg difference method, modified only to the extent of using observed sex fractions in place of 0.5, which change has little overall effect given sex fractions within the range 0.4 to 0.6.

James [11-14] has surveyed several large samples of twins of identified zygosity, and concluded that their sex-pairing distribution departs from binomial by way of a 14% greater in some of his estimates) excess of SS pairs. Among pairs in this sample with both members liveborn, 165 are identified as MZ (99 MC), 184 OS, 112 SSDZ and 74 UZ. Assuming comparable efficiency of zygosity identification between this sample and those surveyed by James, we cannot support the existence of an excess of SSDZ.

The liveborn whites (72 MZ (52 MC), 82 OS, 59 SSDZ, 38 UZ) might have an insignificant excess of SSDZs over OS (86:82) if the UZs contain the same proportion (or more) of DZs as among those identified by genotyping.

Liveborn blacks (93 MZ (47 MC), 102 OS, 54 SSDZ, 36 UZ) show a substantial deficit of SSDZ even when credited with all of the UZs.

As pointed out above, the application of the Weinberg method to twins at birth does imply an assumption we find dubious at best. The results of such estimates may easily be importantly wrong if prenatal loss of DZ twins differs significantly as a function of sex-pairing.

Fetal deaths constitute 44% of the mortality in this sample. It must be noted that the *fetal* mortality considered here includes only deaths occurring after growth sufficient for the presence of a fetus to remain detectable at abortion or delivery. Table 3 shows the results of repeating the analysis above, for fetal and neonatal deaths separately.

DZ fetal mortality does not differ with sex pairing among females of either race. For males, and especially black males, estimated SSDZ fetal mortality is substantially higher than that of OS males. Among blacks, in both sexes, estimated SSDZ fetal mortalities are over twice that observed among MZs. Males exceed females in fetal mortality in whites independent of sex pairing, but only in SS blacks. Black MZ males have less than half the fetal mortality seen in white MZ males. Restricted to monochorionics, black male fetal mortality is less than 60% that of white males.

In neonatal mortality, the estimated SSDZ rate is higher than the observed rates for both OS and MZ, in all subgroups. There is no race difference in DZ neonatal mortality, but the black advantage in MZ male survival continues into the neonatal period.

About Race Differences in the Biology of Twinning

Weinberg method estimates have been used for decades to argue that:

1) Black > White > Oriental in twinning rate and
2) the difference is caused by Black > White > Oriental variation in DZ fraction (as determined by Weinberg method),

from which it is concluded that

3) variation in total frequency of twinning is determined almost exclusively by variation in DZ twinning rates.

In this sample, neglecting pairs with unknown sex-pairing, the sex-pairing fractions differ by race in the direction usually expected, but not significantly ($\chi_1^2 = 0.389$). Since these data are not limited to liveborn twins, some part of the difference from those ex-

https://doi.org/10.1017/S0001566000006036 Published online by Cambridge University Press

Table 3 - Estimating twin mortalities in sex (pairing) x zygosity subgroups

	A. Fetal deaths only					
	White		Black		Pooled	
	Male	Female	Male	Female	Male	Female
Total fetal deaths	31	11	26	19	57	30
Observed MZ	13/92	3/76	5/84	4/128	18/176	7/204
fetal mortality	0.141	0.039	0.060	0.031	0.102	0.034
Extrapolated total MZ						
fetal deaths	15	4	5	4	19	7
Estimated DZ fetal deaths						
(Total – MZ)	16	7	21	15	38	23
Estimated SSDZ dead =						
(DZ – OS) dead	10	3	15	7	26	11
Estimated SSDZs	108	78	114	108	222	184
SSDZ fetal mortality	0.092	0.038	0.131	0.064	0.117	0.060
Observed OS	6/91	4/91	6/112	8/112	12/203	12/203
fetal death rate	0.065	0.044	0.054	0.071	0.059	0.059

	B. Neonatal deaths only					
	White		Black		Pooled	
	Male	Female	Male	Female	Male	Female
Total neonatal deaths	33	21	27	31	60	52
Observed MZ	11/92	4/76	7/84	11/128	18/176	15/204
neonatal mortality	0.120	0.053	0.083	0.086	0.102	0.074
Extrapolared total MZ						
neonatal deaths	13	5	6	10	19	16
Estimated DZ neonatal						
deaths (total – MZ)	20	16	21	21	41	36
Estimated SSDZ dead =						
(DZ – OS) dead)	15	10	16	14	31	23
Estimated SSDZs	108	78	114	108	222	184
SSDZ neonatal rate	0.138	0.128	0.140	0.129	0.140	0.125
Observed OS neonatal	5/91	6/91	5/112	7/112	10/203	13/203
mortality rate	0.054	0.066	0.045	0.063	0.049	0.064,

pectations is already explained by the race and sex-pairing differences in prenatal mortality shown above. Using these values

	OS/total	Fraction male, all but known MZ
Black	112/321 (0.3489)	0.507
White	91/283 (0.3216)	0.540

for Weinberg estimation, and comparing the results with the identified memberships of the zygosity groups, shows an excess of MZs in blacks relative to the Weinberg estimate.

https://doi.org/10.1017/S0001566000006036 Published online by Cambridge University Press

	Black		White	
Predicted	224 DZ	97 MZ (0.302)	183 DZ	100 MZ (0.352)
Identified	166 DZ	106 MZ (0.390)	149 DZ	84 MZ (0.361)
Unidentified	58 DZ	− 9 MZ (−0.184)	34 DZ	16 MZ (0.320)

The numbers of identified black MZ pairs exceed Weinberg estimates in both sexes, more so in males (1.105) than females (1.067). Among whites, identified MZs are 85% of Weinberg estimates in both sexes. Restricting these considerations to liveborn twins, the fit for the white twins is improved slightly, while the discrepancies for the black twins are made worse.

Liveborn whites

All-but-known-MZ 0.534 male, 0.466 female

Predicted: 165 total DZ, 47 MMDZ, 36 FFDZ, 43 MMZ, 42 FMZ
Identified: 52 MC, 20 DCMZ, 82 OS, 58 SSDZ, 38 UZ

Male	27	9	32	22
Female	25	11	26	16

Liveborn blacks

All-but-known-MZ 0.495 male, 0.505 female

Predicted: 204 total DZ, 50 MMDZ, 52 FFDZ, 32 MMZ, 49 FMZ
Identified: 47 MC, 46 DCMZ, 102 OS, 54 SSDZ, 36 UZ

Male	20	18	25	19
Female	27	28	29	17

Race Difference in Chorionicity Fractions Among Monozygotic Pairs

Among all pairs identified as MZs in this sample, in whites, 76% are monochorionic; in blacks, only 57% . $\chi_1^2 = 10.13$ males, 3.82 females, 13.20 pooled, 13.95 total (2df), of which 13.23 (1df) is due to shared association, and 0.72 (1df) to heterogeneity due to sex. The sex difference is not significant in itself or in interaction with the race effect.

Sex fraction is substantially lower in black than in white MZs; pooled over chorionicity, $\chi_1^2 = 8.04$. The difference is due primarily to monochorionics ($\chi_1^2 = 7.17$), but the heterogeneity due to chorionicity is not significant ($\chi_1^2 = 1.32$).

Nigerian twins identified as MZ had a much lower frequency of monochorionicity than in various samples of white MZs [18]. Putting the observed group numbers from that sample through the same calculations we have used here shows that 36/90 pairs matching for sex and genetic markers ("identified MZs") were monochorionic, compared with an average of over 70% in the white samples they reviewed, 76% in this white sample, and 57% in this American black sample.

A first response might be that mortality is higher for monochorionic twins and for black babies, therefore reducing the monochorionic fraction among black twins. Unless

such a difference is confined to the very early mortality not observable in either race in this sample, this simply is not true; observed mortality of monochorionic twins is higher in whites, particulary males; $\chi_1^2 = 7.09$.

Another possible interpretation is that heteromorphism, and thus the efficiency of detecting dizygosity, is reduced in blacks, increasing the fraction of SS dichorionic pairs identified as MZ. To account for the observed discrepancy in live black twin births above, at least 12 of the 46 black pairs diagnosed MZ by bloodtyping would have to be DZ, 6/18 male pairs and 6/28 female pairs. It is difficult to imagine the efficiency of genotyping being that low.

In the Nigerian study, OS pairs as well as SS were genotyped, providing a direct estimate of the efficiency of their marker system at detecting dizygosity. Vlietinck [20] did the same, with a nearly identical system of markers, for a sample of 2,589 twin pairs born in East Flanders, apparently all white. The two estimates of that efficiency (84%) differ by less than one standard error. Since both American racial groups are more heterogeneous than their Flemish and Nigerian counterparts, marker efficiency in this sample could reasonably be expected to be greater (a priori estimate 95% , based on American gene frequencies).

For better or for worse, another basic tenet of twin biology is challenged by these results. Given prenatal mortality of black SSDZs estimated to be substantially greater than that of black MZs, with the difference reversed in whites, there might remain little basis for confidence in the prevailing interpretation of race differences in OS fraction among liveborn twins.

Population differences in liveborn twinning frequency, though to be due to variation in DZ frequency vs constant MZ frequency as estimated by the Weinberg method, could be due instead to correlation between total twinning rate and SSDZ/MZ prenatal mortality ratio. Among the liveborn pairs in this sample, the black twins have both a higher OS fraction (0.357/0.328) and a higher fraction identified as MZ (0.326/0.288). In another paper in these proceedings, we will explore a relationship which has some potential of explaining that one [cf 5].

DISCUSSION

The logic underlying the prevailing belief that MZ infant mortality greatly exceeds that of DZs is clearly circular. The results it has produced, to the extent they can be tested in this sample, are impossible. While the present data do not allow a precise statement of zygosity-specific mortalities, three different approaches without obviously tautological assumptions yield mutually consistent estimates which are at least biologically possible, and which differ strikingly from prevailing belief. Our best interpretation at this point is that mortality differs much more as a function of sex pairing among DZs than as a function of zygosity specifically, whether or not the DZs are pooled over sex pairing classes.

In some respects, it might be considered rather astonishing that we were willing to believe otherwise for so long. The prenatal environment of OS twins has reason to be considered unique among humans. That we have so long neglected consideration of the probability that this should affect development can only be attributed to the lack of obvious or simple reasons to doubt what seemed a plausible explanation.

Much of the plausibility of that explanation derived from another prevailing belief of even longer standing, namely that the cellular origins of MZ and DZ twinning are entirely unrelated. We have been equally willing to believe, on the one hand, that those different origins must somehow explain all of the large twin excess of certain malformations and most of their excess mortality, while on the other hand maintaining that those different cellular origins bear no consequences affecting more or less normal development. Perhaps it was necessary to believe that, in order to continue doing genetic twin studies in the manner Galton taught us, greatly expanded in statistical detail but not in fundamental concept. If (the structure of variance in) development differs in twins of either zygosity from that in singletons, fundamental assumption of that methodology fail [9].

We are presently in a position to challenge these beliefs only because our work of the past ten years has steadily accumulated reasons for such doubt.

There is no zygosity difference in the excess of nonrighthandedness (NRH) among parents of twins, nor in the excess of discordant NRH among second twins, but there may be a sex-pairing difference in the second-twin excess [2]. The familial excess which distinguishes first-degree from second-degree relatives of twins has highly significant heritability; there is no sex-pairing difference in that heritable component. The second-twin excess which seems to differentiate SS from OS twins seems highly likely to be due to a subtle environmental insult first documented by Derom and Thiery [6], to which the left brain hemisphere is probably more sensitive, against which the OS twins seem somehow protected [cf 3].

There is no zygosity difference in a large reduction of dental diameter asymmetry among twins compared to unrelated singletons [4]. There is a sex-pairing difference concerning sex differences in dental asymmetries [3]. Sex can be identified from dental diameter asymmetries with over 95% accuracy for singletons or SS twins, but OS twins are more often than not misclassified by the same rules, and the difference is highly significant.

There is no zygosity difference in the twin excess of double occipital hair whorls reported by Sharma in these proceedings.

Twin excess without zygosity difference is usually interpreted to suggest that twinning itself, specifically some consequence of twin gestation, has caused the trait. But the parents and siblings of twins, with their excess NRH, are not in general themselves twins, and the twins have no excess NRH compared to their first-degree relatives.

There is no significant zygosity difference in the excess of certain malformations in the sibs and offspring of twins, who are seldom themselves twins [5]. The zygosity differences so long reported for the twin excess of those same malformations has been based on the same circular logic that is wrong for mortality.

Each of the findings just discussed relates, in various ways, to the developmental elaboration of brain and body symmetry. In another paper in these proceedings we will review associations between brain symmetry development and twinning of both zygosities and their mutual association with malformations affecting structures the normal development of which depends on embryogenic body symmetry operations. When the mechanism of embryogenic body symmetry determination and the mechanism of twinning are known, we are convinced that some of them will be the same.

We believe that all of these findings indicate that MZ and DZ twinning share some of their causes. Derom and her colleagues [6] have offered strong support for that prospect

from quite another direction: twins and triplets conceived via induced ovulation include a clear excess of MZ twinning events.

SUMMARY AND CONCLUSIONS

Most of twin biology has depended on the belief that MZ and DZ twinning events are of entirely different cellular origins, with only MZs representing significant developmental pathology. The results presented here are strongly inconsistent with this belief, and add substantially to our previously accumulated doubts.

If, as we have come to suspect, MZ and DZ twinning share some of their causes, it would be no surprise if they share some of its consequences, apparently including excessive mortality. The related prevailing belief that populational variation in total twinning rate is driven by DZs is an integral part of those same older belief, and is also quite inconsistent with these results.

Acknowledgements. We are grateful for support of this work by the Dean of East Carolina University School of Medicine, to Dr. Myrianthopoulos and his staff at NINCDS for their hospitality and help in reviewing the data of the Collaborative Perinatal Project, and to Bill Baker for his statistical support.

REFERENCES

1. Boklage Ce (1981a): On the timing of monozygotic twinning events. Prog Clin Biol Res 69A: 155-165.
2. Boklage CE (1981b): On the distribution of monrighthandedness among twins and their families. Acta Genet Med Gemellol 30:167-187.
3. Boklage CE (1985): Interaction between opposite-sex dizygotic fetuses and the assumptions of Weinberg difference method epidemiology. Am J Hum Genet 37:591-605
4. Boklage CE (1987): Developmental differences between twins and singletons in distributions of dental diameter asymmetries. Am J Phys Anthropol (in press).
5. Boklage CE (1987): Twinning, nonrighthandedness, and fusion malformations: Evidence for heritable causal elements held in common. Am J Med Genet 28:67-84.
6. Derom C, Derom R, Vlietinck R, Van Den Berghe H, Thiery M (1987): Increased monozygotic twinning rate after ovulation induction. Lancet i:1236-1238.
7. Derom C, Bakker E, Vlietinck R, Derom R, Van den Berghe H, Thiery M, Pearson P (1985): Zygosity determination in newborn twins using DNA variants. J Med Genet 22:279-282.
8. Derom R, Thiery M (1976) Intrauterine hypoxia – A phenomenon peculiar to the second twin. Acta Genet Med Gemellol 25:314-316.
9. Elston RC, Boklage CE (1978): An examination of fundamental assumptions of the twin method. Prog Clin Biol Res 24A:189-199.
10. Hill AVS, Jeffreys AJ (1985): Use of minisatellite DNA probes for determination of twin zygosity at birth. Lancet, Dec 21/28:1394-5
11. James WH (1971): Excess of like-sexed pairs of diziygotic twins. Nature 232(5308):277-278.
12. James WH (1975): Sex ratio in twin births. Ann Hum Biol 2:365-378.
13. James WH (1976): The possibility of a flaw underlying Weinberg's Differential Rule. Ann Hum Genet 40:197-199.
14. James WH (1979): Is Weinberg's Differential Rule valid? Acta Genet Med Gemellol 28:69-71
15. Layde PM, Erickson JD, Falek A, McCarthy BJ (1980): Congenital malformations in twins. Am J Hum Genet 32:69-78.

16. Myrianthopoulos NC (1970): An Epidemiological survey of twins in a large prospectively studied population. Am J Hum Genet 22:611-629.
17. Myrianthopoulos NC (1985): Malformations in Children from One to Seven Years: A Report from the Collaborative Perinatal Project. New York: Alan R Liss.
18. Nylander PPS, Corney G (1969): Placentation and zygosity of twins in Ibadan, Nigeria. Ann Hum Genet 33:31-40.
19. Uchida IA, Freeman VCP, Gedeon M, Goldman J (1983): Twinning rate in spontaneous abortions. Am J Hum Genet 35:987-993.
20. Vlietinck RF (1986): Determination of the zygosity of twins. Thesis, Katholieke Universiteit, Leuven.

Correspondence: Dr. Charles E. Boklage, Genetics Program, East Carolina University School of Medicine, Greenville, NC 27858-4534, USA.