Multiple Pregnancy

In a remarkable paper that correlates prenatal events and discordance of twins with postnatal outcome, Price (1950) emphasized the importance of “prenatal biases.” He was much concerned with the influence of placentation upon twin development, an aspect that had not often been considered in twin studies. Similar ideas have more recently been echoed by Phillips (1993), who emphasized the influence of the proximity of twin placentas on their ability to support fetal growth. It was Galton (1875), however, cousin of Charles Darwin, and after whom the Galton Institute of Genetics in London is named, who was probably the first to suggest that twins, if properly studied, would yield information that might allow us to discriminate between the effects of heredity and those of the environment. It was his famous “nature versus nurture” concept. The extensive studies conducted by Friedrich Schatz at about the same time suggested that prenatal influences among twins found reflection in the ultimate outcome of the twins. He was instrumental in clarifying that placental study is essential for this understanding. His extensive work is annotated in a bibliographic oddity (Schatz, 1900) that summarizes all his papers and citations therein. His numerous contributions were partially translated for the book on twin placentaion by Strong and Corney (1967). A concise review of the biological aspects of the human twinning process was published by Benirschke and Kim (1973), and a volume by MacGillivray and his colleagues (1988), Twinning and Twins, summarizes most relevant aspects of this interesting phenomenon of nature. Baldwin (1994) has produced a remarkable volume that contains all relevant aspects of placentation of multiple pregnancy and is well illustrated. Finally, Gall (1996) has summarized all practical aspects of multiple gestation, especially the clinical manifestations and therapy.

No doubt, the complexity of human twinning cannot be understood without knowledge of the placentation of twins. Moreover, despite all the benefits reaped from animal studies, the placentation of most relevant species is often very dissimilar from that of humans. Thus, conclusions drawn from nonhuman multiple pregnancies must be interpreted with great care. In recent years, ultrasonographic studies have added materially to our knowledge of prenatal events in twinning and its placentation. Of interest, for instance, are the remarkable observations by Arabin et al. (1996) of extensive intertwin physical interactions. These processes were observed to occur earlier in monochorionic twins than in those with a dichorionic twin placentas.

ZYGOSITY

There are “fraternal” (better named dizygotic, DZ) and “identical” (monozygotic, MZ) twins. In higher multiple births, these may be admixed. That such different classes of twins exist derives from several observations. Fraternal twins may be of different or like sex. An hypothesis is herein helpful: if all twins were DZ, then one would expect a similar sex ratio to be found as that for singletons, that is, approximately 50% M: F, 25%, M: M, and 25% F: F. This is not the case. When large statistics of infant sex at birth are examined, it is found that there is an excess of like-sex twins. This excess is presumed to result from the number of MZ (identical) twins. By subtracting these from the total number of twins, an estimate is obtained for the distribution of MZ and DZ twins in a population. This is the so-called Weinberg rule (1901). Hrubec and Robinette (1984) pointed out that the basis for this rule had previously been published by Bertillon (1874). Weinberg’s “differential method” can be stated with the following formula:

\[ \text{MZ twins} = \text{all twins} - \frac{\text{unlike-sex twins}}{2pq} \]

where \( p \) = frequency of male births and \( q \) = frequency of female births in a population.

Weinberg’s formula should be taken as providing estimates, with considerable errors if it were taken literally. This limitation is particularly understandable when one considers the frequencies of different classes of twins as they exist at the time of conception, for there is much evidence that MZ twins have a higher prenatal death rate than do DZ twins. The Weinberg method cannot correct for losses of only one twin in a gestation, information that is also usually not recorded in birth statistics. It is therefore not surprising that the method has often been criticized (e.g., Renkonen, 1967; and reply by Cannings, 1969; James, 1971, 1984; Keith, 1974). Nevertheless, it is a unique and valuable tool to assess the approximate frequency of DZ versus MZ twins in a
population. Moreover, a recent prospective study that attempted to validate the method has found that the results agree well with findings from placental and known zygosity of twins (Vliegen et al., 1988). An interesting observation of James (1971) is that there is an excess of like-sex pairs among DZ twins. This observation is based on all samples of twins whose zygosity was ascertained by blood grouping. It has so far remained unexplained, but some additional data (James, 1977a,b) show an even more marked excess of females among monoamniotic, monochorionic (MoMo) twins and perhaps in acardiacs. More light has been shed on this phenomenon by the large study of Derom et al. (1988). These authors provided data from the Belgian prospective twin study that included zygosity diagnosis and placental assessment. Not only was the proportion of males reduced among MZ twins (irrespective of chorion status) but there also was a marked reduction of male MoMo twins from what might be expected if this form of twinning occurred at random. The sex proportion of all MZ twins was 0.487, that of MoMo was 0.231, and DZ twins had a proportion of 0.518. Although no large sets of data are yet available, Derom et al. cited evidence that conjoined twins at term are more commonly females, whereas those of abortuses may be more often males. Of the two possibilities to explain this unexpected observation—the higher frequency of late twinning in female conceptuses and the greater abortion rate of male MoMo conceptuses—they favored the former. They referred to the suggestion made by Price (1950) that "unequalization" of X chromosomes may be a cause of late twinning and that this feature is unique to females. The apparent excess of female acardiacs is possibly further confirmation (James, 1977b).

Considerations of the MZ twinning rates come from Allen and Hrubec (1987), who proposed that a slight suggestion exists of a relationship of the MZ twinning rate to maternal age, as is certain for DZ twins. These "constants" of DZ to MZ twin frequencies identified from various statistical considerations, however, are still considered to be arbitrary, and they also appear to differ among various populations.

Other reasons for considering that a proportion of twins is identical, or monozygotic, come from the numerous reports on genetic "identity," let alone the physical similarity exhibited by some twins. Twin research has traditionally involved ascertainment of zygosity by assessment of likeness. Dermatoglyphics (Newman, 1931a; Brismar, 1968; Allen, 1968; Hertling et al., 1970; Reed et al., 1975), and blood grouping (Robertson, 1969; Selvin, 1970) are some of many parameters that have been employed. These methods have not always been decisive in assigning the zygosity for an individual set of twins, although their general reliability is high. For that reason, methods such as mixed leukocyte reaction (Jarvik et al., 1969), skin exchange graft survival (Stanc, 1966), and repeated blood group study (Osborne, 1958) have been advocated. Analysis of banded chromosomes, C bands and Q bands, has been used to ascertain monozygosity (Neurath et al., 1972; McCracken et al., 1978; Morton et al., 1981; Pedrosa et al., 1983). Until recently, this methodology has been the most reliable. These methods established mostly probabilities of twin zygosity, and they were often somewhat imprecise. The mathematical aspects of phenotypic likeness studies have been treated by Meulepas et al. (1988). New methods have now been developed that are more decisive. How important they can be is manifest from the report by St. Clair et al. (1991). Neuman and her colleagues (1990) have proved with RFLPs the dizygosity of aborted tubal twins and suggested that the alleged common monozygosity of ectopic twins may be in error. Norton et al. (1997) advocated the use of DNA diagnostic study of discordant multiple gestational products when chorionic status is indeterminate or not helpful.

The least decisive method for the identification of the zygosity of twins, the likeness assessment, is however also the most widely practiced. It is the easiest method to execute and it correctly asserts that physical characteristics are more alike in MZ twins than they are in DZ twins (e.g., tooth morphology: Lundstroem, 1963; skin color: Collins et al., 1966; cardiac findings: Preis & Srubarova, 1966; immunoglobulin levels: Sowards & Monif, 1972; cholesterol levels: Corey et al., 1974). Fairly reliable accuracy of zygosity diagnosis is said to be achieved with other simple tests, including the use of questionnaires (Cederliöf et al., 1961; Nichols & Bilbro, 1966). These oversimplifications have, however, also led to much misconception. Moreover, they have confused the neonatologist who cares for often markedly discordant twins in the neonatal period. They must have better guidelines for the care of neonatal twins than are generally available to clinicians.

It is now certain that MZ twins are frequently discordant in development; some of this discordance can be secondary to unique placental vascular relations between twins (Schatz, 1886; Verschuer, 1927; Price, 1950) and some has its cause in abnormal placental. Discordance for congenital anomalies is higher in MZ than in DZ twins, a feature that has been critically analyzed by Boklage (1987a), by Mastroiacovo and Botto (1994), and most recently by Hall (1996). Triplets share this feature, according to Suslak et al. (1987). Melnick and Myrianthopoulos (1979) found that the twofold increase of anomalies in MZ twins cannot be validly ascribed to the monochorial placental status that is so prevalent in MZ twins. An exception to this, of course, are acardiacs and the destructive results from prenatal disseminated intravascular coagulation (DIC), which may depend on the origin on the monochorial status of the twin placenta. These authors suggested, as a possible explanation, that the "impetus" for MZ twinning may be similar to that which is the cause of the developmental anomalies. The relative frequency of anomalies in MZ twins (6–9%; with 80% discordance) has important implications with respect to prenatal diagnosis of early embryos (Jarmulowicz, 1989). It has been suggested that, with the new methodology of PCR of DNA amplification, single blastomeres might be sexed and genetically defined in the future. It is unknown at this time whether such loss of blastomeres could result in anomalies, and whether the discordant anomalies of MZ twins are perhaps caused by an unequal splitting of the morula. These are important questions for future research.

Twin studies, as advocated by Galton (1875), endeavor to discern between genetic and environmental influences on fetal development. To be successful, they require accurate zygosity determination of the probands. Walker (1957) stated this requirement emphatically. Moreover, it has been found that if the linkage of genetic traits is not taken into consideration in the zygosity assignment, the probability of monozygosity is overestimated (Sorensen & Fenger, 1974). Kemphorne and Osborne (1961) and Allen and Hrubec (1979) have developed models to undertake such analyses. Allen (1965) proposed excellent guidelines for the design of twin studies, which should be consulted.