Multiple Pregnancies

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 were echoed by Phillips (1993), who emphasized the influence of the proximity of twin placentas on their ability to support fetal growth. But it was Galton (1875), 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—his famous “nature vs. 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 the 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). It summarized all of his papers and citations therein. Some of his numerous contributions were partially translated for the book on twin placenta by Strong and Corney (1967). A concise review of the biologic aspects of the human twinning process was published by Benirschke and Kim (1973), and Twinning and Twins, by MacGillivray and his colleagues (1988), summarizes most relevant aspects of placentation of multiple pregnancies and it is well illustrated. Finally, Gall (1996) summarized all practical aspects of multiple gestations, especially the clinical manifestations and therapy. Several cases of hydatidiform mole in twin gestations have been reported (e.g., Chu et al., 2004). These are discussed in more detail in Chapter 22.

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 animal 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 inter-twin physical interactions. They were observed to occur earlier in monochorionic twins than in those with a dichorionic twin placenta.

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. A 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% MF, 25% MM, and 25% FF. This is not the case. When large statistics of infants’ 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, we have an estimate for the distribution of MZ and DZ twins in a population. This is the so-called Weinberg rule (1901). Weinberg’s “differential method” can be stated with the following formula:

$$MZ\text{twins} = \text{All twins} - \frac{\text{Unlike-sex twins}}{2pq}$$

where $p$ is the frequency of male births, and $q$ is the 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 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 vs. MZ twins in a population. Moreover, a prospective study that attempted to validate the method found that the results agree well with findings from placentation and known zygosity of twins (Vlieetink et al., 1988). An interesting observation by James (1971) is that, among DZ twins, there is an excess of like-sex pairs. This observation is based on small 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 twins with acardiacs. More light has been shed on this phenomenon by the large study of Derom et al. (1988), who provided data from the Belgian prospective twin study that included zygosity diagnosis and placental assessment. Not only was the proportion of males reduced in MZ twins (irrespective of chorion status), but also there 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, and that of MoMo was 0.231, whereas the DZ twins had a proportion of 0.518. Although no large data sets are yet available, the authors cited evidence that conjoined twins at term are more commonly female, whereas those of abortuses may be more often males. Of the two possibilities to explain this unexpected observation—greater frequency of late twinning in female conceptuses and greater abortion rate of male MoMo conceptuses—the authors favored the former. They referred to the suggestion made by Burn et al. (1986) that “unequal lyonization” of X chromosomes may be a cause of late twinning, and that feature is unique to females. But Goodship et al. (1996) found no support for this hypothesis when they examined X-inactivation patterns of umbilical cords of various types of twins. Monteiro et al. (1998b) studied this a little further by comparing X-inactivation patterns of lymphocytes and buccal cells of monochorionic (MC), MZ, and dichorionic (DC) MZ twins. They assumed from earlier studies that lyonization occurs when there are 10 to 20 cells in the embryo and deduced from modeling that “MC-MZ twinning occurs three or four rounds of replication after X inactivation, whereas DC-MZ twinning event occurs earlier, before or around the time of X inactivation.” The apparent excess of female acardiacs is possibly further confirmation (James, 1977) of this phenomenon. The largest prospective survey of twins comes from Belgium (Loos et al., 1999), which encompassed 5089 twins, 158 triplets, and 14 quadruplets or higher multiples. In this survey, zygosity was established by sex, placentation, and genetic markers in over 95%. Dizygotic twins had the same sex proportions as singletons, whereas MZ twins had an excess of females.

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 very 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 are identical, or monozygotic, come from the numerous reports on genetic identity, as well as the physical similarity exhibited by some twins. Twin research has traditionally involved ascertainment of zygosity by assessment of likeness. Dermatoglyphics (Newman, 1931a; Allen, 1968; Brismar, 1968; Herrlin et al., 1970; Reed et al., 1975), and blood grouping (Robertson, 1969; Selvin, 1970) are some of many parameters that have been employed. But 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 stimulation (Jarvik et al., 1969), skin exchange graft survival (Stranc, 1966), and repeated blood group study (Osborne, 1958) have been advocated. Analysis of banded chromosomes, C-bands and Q-bands, has also been used to ascertain monozygosity (Neurath et al., 1972; McCracken et al., 1978; Morton et al., 1981; Pedrosa et al., 1983). Until recently, this has been the most reliable methodology. These methods established mostly probabilities of twins’ zygosity, and they were often somewhat imprecise. The mathematical aspects of phenotypic likeness studies have been treated by Meulapay 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. (1998). Unlike-appearing twins, thought to be dizygotic, had successful inter-twin renal transplant because of mushroom poisoning to one twin. DNA fingerprinting found them later to be monozygotic, whereupon the immunosuppressive therapy was successfully discontinued.

The techniques for the direct comparison of DNA variants have much to be recommended as primary tools. They determine the restriction fragment length polymorphism (RFLP) of twins, and cogent reasons for routinely employing this methodology have been advanced by Machin (1994). These methods of comparing fragments of DNA are quick and decisive, and can be executed on placental tissue, blood, and other tissues (Derom et al., 1985; Hill & Jeffreys, 1985). Importantly, the quantities of tissue needed for this study can be quite small when combined with polymerase chain reaction (PCR). Microsatellites have also been proven to be useful and rapid in the definitive differential diagnosis of MZ vs. DZ twins (Erdmann et al., 1993; Becker et al., 1997). Thus, antenatal samples may readily be processed by this and related modern techniques for accurate determination of twin zygosity (Kovacs et al., 1988) and they have been decisively used to identify the genetic relationships between twins and triplets (Motomura et al., 1987; Azuma et al., 1989). It is of further interest that this method can also be used to identify DNA patterns of macerated stillborn fetuses (Derom 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, however, is 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; Lundström, 1963; skin color; Collins et al., 1966; cardiac findings: Preis & Srubarova, 1966; immunoglobulin levels: Sowards & Monif, 1972; cholesterol levels: Corey et al., 1975, etc.). A fairly reliable accuracy of zygosity diagnosis is said to be achieved with other simple tests, including the use of questionnaires (Cederlöf et al., 1961; Nichols & Bilbro, 1966). These oversimplifications, however, have also led to many misconceptions. Moreover, they have confused neonatologists who care for often markedly discordant twins in the neonatal period and who need better guidelines for care than are generally available.

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), others have their cause in abnormal placentation. Discordance for congenital anomalies is higher in MZ twins than in DZ twins, a feature that has been critically analyzed by Boklage (1987a), Mastroiacovo and Botto (1994), and Hall (1996). There are also other fascinating problems to be resolved; for instance, Zaw and Stone (2002) reported caudal regression syndrome in a set of monzygotic, but dichorionic twins. Is that due to growth disparity of the twins or to the possibility that only one of the twins was exposed to the abnormalities of glucose imbalance of the MZ twins at the time of putative exposure? Some such questions can potentially be resolved by the twin methodology, and Hobbs et al. (2002) drew attention to this in