By the beginning of the 1980s, perinatal mortality in pregnancies complicated by diabetes at Northwestern University Medical Center (Freinkel 1980) and several other clinics (Coustan et al. 1980; Jovanovic et al. 1980) had declined to levels that were beginning to approximate those of non-diabetic gravida. Thus, an incidence of about 2% was being observed. However, the increased frequency of birth defects associated with diabetic pregnancies had not changed (Soler et al. 1976; Pedersen 1977; Freinkel 1980; Mills 1982) and approximated the values that had persisted worldwide throughout the two to three decades in which the enhanced teratogenicity of diabetic pregnancies had been well documented (Mølsted-Pedersen et al. 1964; Kucera 1971). Indeed, during 1977 to 1981, birth defects were encountered in 4.9% of the pregnancies in our patients with “carbohydrate intolerance . . . with onset or first recognition during pregnancy” (I.e. gestational diabetes mellitus) and in 10.9% of those with known pregestational diabetes, whereas malformations were present in only 2.4% of the offspring of our concurrently enrolled gravida with normal glucoregulation (Simpson et al. 1983). Thus, in our Center, as elsewhere, congenital anomalies had become the leading cause of perinatal death in pregnancies complicated by diabetes, and represented the most compelling unresolved problem in this condition.

The basis for the diabetic embryopathy remained unexplained. However, certain important clues were beginning to emerge. Recognition that organogenesis takes place before the seventh week of gestation in the structures that are susceptible to diabetic embryopathy was becoming more widespread and highlighted the fact that the teratogenic event took place before there was awareness of pregnancy (Mills et al. 1979). As reviewed in detail elsewhere (Freinkel 1988), renewed efforts with animal models using streptozotocin (Deuchar 1977; Baker et al. 1981; Eriksson et al. 1982) rather than alloxan (Watanabe and Ingalls 1963; Endo 1966; Hori et al. 1966) as the diabetogenic agent clearly demonstrated that increased dysmorphogenesis could be replicated with experimentally induced diabetes and did not necessitate “diabetic genes”. However, the animal experiments also underscored that genetic factors could influence the intrinsic suscepti-
bility to the embryotoxic properties of "the diabetic state" (Goldman et al. 1985; Eriksson et al. 1986).

Estimates of glycosylated haemoglobin provided direct support for some relationship between the birth defects and metabolic events during early pregnancy. Thus, an association between markedly elevated values for glycosylated haemoglobin during the first trimester and malformations at birth were being reported in Europe (Leslie et al. 1978) as in the United States (Miller et al. 1981). However, the invocation of faulty maternal metabolism in the pathogenesis of diabetic embryopathy still failed to identify the specific teratogenic factor(s). For example, alterations in maternal haemodynamics, hydration, neurohumoral activity, hormonal counter-regulation and other components of poor regulation could, of themselves, affect uterine perfusion and thereby limit development of the conceptus during the tenuous early postimplantation period. Thus, more precise assessments of underlying events necessitated in vitro studies.

**Studies with Rat Embryo Culture**

Efforts in our laboratory were initiated in 1980 to test the proposition that aberrant fuels of maternal origin might be implicated directly, i.e. Freinkel's hypothesis that fuels and fuel-related products pharmacologically may modify phenotypic gene expression in the newly forming and differentiating cells of the conceptus and thereby modify programmed development ("fuel-mediated organ teratogenesis") (Freinkel 1980, 1981). We used outbred Charles River rats (Crl:CD®[SD]BR) and the whole embryo culture technique of New (1978) for our endeavours. This method enabled us to monitor the progression of embryogenesis in the postimplantation rat embryo from day 9.5 to day 11.5 of development (midnight of the night of mating is designated as day 0 of embryo development; the subsequent 24 h are considered the first day of gestation).

The culture precisely replicates the developmental events that occur during the same 48-h interval in the rat embryo in vivo (New 1978), i.e. the progression of the embryo from the presomite, headfold stage, and a total protein content of approximately 5 μg to the 26–29 somite stage, full neurulation and a protein content of about 150 μg. Attempts to explant the rat embryo for culture at earlier stages of development result in an increased incidence of spontaneous malformations, presumably due to increased fragility and excisional trauma (Cockroft 1984); embryo growth no longer replicates in vivo patterns when culture is extended beyond 11.5 d, presumably because the larger size of the conceptus compromises adequate diffusion and nutrient delivery (New 1978). The 48-h period from day 9.5 to day 11.5 of development in the rat embryo roughly corresponds to days 18 to 28 of human pregnancy and encompasses the interval during which such devastating consequences of faulty neural tube closure as anencephaly, meningomyelocele or spina bifida may be engendered.

For our initial experiments, we examined the dysmorphic potential of individual components of the "diabetic state" by appropriately modifying the culture medium. In our hands (Freinkel et al. 1983a, b, 1984, 1986) as in the prior pioneer studies of Cockroft and Coppola (1977) and Sadler (1980a), supplementation of