Pregnancy Metabolism, Diabetes and the Fetus
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Introduction

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The Ciba Foundation has always had a scientific tradition of bringing to the forefront essential issues for mankind. In recent years several Ciba Foundation symposia have been devoted to elucidating the fundamental causes of, and ways of reducing, perinatal wastage and morbidity – in 1960 Congenital Malformations, 1965 Preimplantation Stages of Pregnancy, 1969 Foetal Autonomy, 1973 Intrauterine Infections, 1974 Size at Birth, 1977 The Fetus and Birth, 1978 Major Mental Handicap, and now this symposium on Pregnancy Metabolism, Diabetes and the Fetus.

The past 20 years have seen a steady fall in perinatal mortality throughout the western world. The precise reasons for this are still being debated but it seems likely to be due to better health, following a rise in standards of living, combined with improved medical care. Technological advances have provided the obstetrician and paediatrician with much greater insight into the development and day-to-day health of the fetus, and early detection of conditions such as hypoxia has increased the possibility of reducing morbidity as well as mortality at the time of delivery. The unchanging incidence of congenital malformations in our society, however, still constitutes a major moral and social problem. Early detection of abnormalities, with subsequent termination of pregnancy, is no solution to this problem. More often than not, malformations are unsuspected during pregnancy and many malformed babies survive into later life with varying degrees of serious handicap; in addition, many parents regard legal abortion as unacceptable. It is therefore essential for the origins of malformations to be better defined. The cause may well be multifactorial, and the actual conditions leading to a particular malformation are known in only a small percentage of cases.

Diabetes is a well-defined metabolic disorder, complicating pregnancy, in which the incidence of malformations is up to three times higher than the
normal rate. Thus it is reasonable to suggest that it is the abnormal metabolism of the mother at the time of implantation and organogenesis that may, in some way, be responsible for many of these anomalies. The fetus is to a certain extent autonomous, but its selective metabolic requirements may not be met by the abnormal metabolism of a diabetic mother. Diabetes may therefore serve as a useful, naturally occurring model which can be used to study the evolution of congenital abnormalities. If it can be demonstrated that the metabolic milieu may be responsible for the development of fetal anomalies, then the wider possibility must exist that other forms of metabolic disturbance, for example nutritional deprivation, may increase the possibility of fetal abnormality.

Little is known of the influence of the mother's metabolism on her fetus, and the main aim of this symposium is to lay the foundation for investigations of a possible link between metabolic disturbance in the mother and fetal anomaly. A first step towards achieving this objective is to characterize metabolic interrelationships within the mother, and between the mother and the fetus, in normal pregnancy, particularly in the early, possibly critical, months. From this information it should then be possible to determine the selective metabolic needs of the embryo and fetus. The implications of possessing such knowledge are considerable. Not only may it give us insight into aberrations of fetal development but it could also provide a basis for determining the nutritional requirements of pregnancy that may be of extreme importance to women in the Third World.

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Late pregnancy is attended by significant changes in maternal intermediary metabolism. We have summarized these in extenso elsewhere (Freinkel et al. 1979). They include an enhanced maternal transfer to the metabolism of fat whenever food is withheld ('accelerated starvation' [Freinkel 1965]) and mechanisms for conserving ingested nutrients for delivery to the conceptus when the mother eats again ('facilitated anabolism' [Freinkel et al. 1974]). By sampling circulating fuels in normal gravid and non-gravid volunteers during regular alimentation (with three liquid formula feedings per day), we have been able to demonstrate that the disposition of every major class of nutrient is altered in late gestation (Freinkel et al. 1979). We have emphasized that each of these major fuels is responsive to the action of insulin and that late pregnancy poses significant challenges to maternal insulinogenic reserve.

However, none of these alterations in the mother's metabolism would be nearly as interesting if it were not for the role that maternal fuels play in a unique tissue culture experience. During pregnancy, the conceptus develops from a fertilized egg into a multicellular structure with complex functions. The building blocks are derived entirely from the mother and ultimately determined by what she eats, how she handles these nutrients, and how they are stored and recalled. In essence, then, in the tissue culture system that constitutes pregnancy, the composition of the incubation medium for all newly developing cells is determined in large measure by the vagaries of maternal metabolism.

Four rate-limiting factors can be identified in establishing the conditions for tissue culture. Firstly, the absolute concentration of individual metabolites in the maternal circulation determines the quantitative availability of individual substrates. Secondly, access of these substrates to the conceptus depends upon placental blood flow. As yet, the precise factors which regulate
placental perfusion have not been well delineated; the possibility that ambient fuels and their intraplacental disposition may modulate placental haemodynamics remains an area for potentially fruitful inquiry. Thirdly, the fate of individual fuels can be modified by the concurrent availability of other fuels ('substrate interactions' [Freinkel 1978]). For example, increases in ambient free fatty acids (FFA) can promote steatosis in the placenta (Freinkel 1965; Herrera & Freinkel 1975) and enhance intraplacental formation of lactic acid from glucose (Freinkel 1965). Similarly, ketones, when present, can supplant glucose or lactic acid as placental oxidative fuels (Shambaugh et al. 1977a,b). Thus, structure and function in the conceptus may be altered by the qualitative as well as quantitative characteristics of the prevailing substrate mixture. Finally, transplacental transfer mechanisms (Cornblath & Schwartz 1976; Pedersen 1977a) are the ultimate arbiters of what is available to the fetus. The relationships are summarized in Fig. 1. Maternal glucose crosses the placenta freely by facilitated diffusion and is abstracted continuously in direct proportion to maternal blood sugar levels. Amino acids cross the placenta by active transport systems and differential concentration gradients within the fetal circulation may be influenced by transport competitions as well as substrate interactions. Data concerning FFA are controversial and vary in different species; some transplacental flux appears to be operative in all. Ketones traverse the placenta freely so that the fetus is presented with abundant ketones once an adequate ketonaemia has been established in the mother (Scow et al. 1958; Girard et al. 1973, 1977; Shambaugh et al. 1977a,b).
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1977a). Some fuels such as lactic acid may originate directly within the placenta (Freinkel 1965; Burd et al. 1975) and thus do not depend upon concentrations in the maternal circulation. Moreover, it is becoming increasingly apparent that the fetus is not wholly passive in these exchanges and that the fetus may autoregulate its metabolic mixture by influencing transplacental transfer, or by forming some new fuels from stored fetal precursors (Goodner & Thompson 1967; Jost & Picon 1970; Girard et al. 1977; Battaglia & Meschia 1978).

However, despite the many limitations of existing knowledge, it may not be inappropriate to view circulating metabolites in the mother as an index of the substrates that are available to the developing fetus. In this special relationship, certain additional features should also be underscored. For example, the conceptus per se can modify the maternal environment through its endocrine functions. The placenta elaborates hormones (e.g. placental lactogen [HPL], and sex steroids) into the maternal circulation in ever-increasing amounts which parallel placental growth and development (as reviewed elsewhere [Freinkel et al. 1979]). These hormones do not cross the placenta (Fig. 1); they do, however, exert lipolytic and contrainsulin actions in the mother and thereby provide a permissive setting for such special maternal metabolic adaptations of late pregnancy as 'accelerated starvation' (Freinkel et al. 1979).

The placenta also constitutes an impermeable barrier to insulin. The early observations that maternal insulin does not cross the placenta although it may be sequestered (i.e. bound) within the placenta (Goodner & Freinkel 1961) and actively degraded there (Freinkel & Goodner 1960) have now been confirmed in many laboratories (Cornblath & Schwartz 1976; Pedersen 1977~). Since the disposition of most maternal fuels is intimately linked to the adequacy of insulin secretion in the mother (Freinkel et al. 1979), this means that maternal insulinization may be the prepotent arbiter of the incubation medium in which the conceptus develops. Excursions in maternal fuels should exhibit the greatest swings when maternal insulin is most deficient – and the presentation of substrates to the conceptus should display the greatest lability under such circumstances (Freinkel et al. 1979). In this context, diabetes in pregnancy constitutes a fascinating experiment in cell biology, with ramifications extending far beyond that disease entity per se. It may well provide the best insights into the factors that regulate the tissue culture aspects of gestation.

GESTATIONAL DIABETES

To assess whether even the most minor limitations of maternal insulinization can unmask the dependencies of the conceptus upon maternal fuels, we
have been conducting studies in women with gestational diabetes; that is, asymptomatic gravida discovered to have mild glucose intolerance by routine screening during pregnancy, i.e. White Class A (White 1949).

We feel that definitions must be extremely rigorous for such clinical subgroups. We diagnose gestational diabetes according to the glucose tolerance criteria of O'Sullivan & Mahan (1964). However, we further subdivide gestational diabetics on the basis of the manifest severity of their disturbance in carbohydrate metabolism. Those with fasting plasma glucose below 105 mg/dl (5.8 mmol/l) are designated Class A₁; those with fasting plasma glucose slightly above normal, that is, between 105 and 130 mg/dl (5.8 and 7.2 mmol/l), are classified as A₂ and those whose fasting plasma glucose exceeds 130 mg/dl (7.2 mmol/l) we categorize as Class B. Since postprandial blood sugar, that is, blood sugar after alimentary challenge, is conditioned by the disposition (i.e. ‘utilization’) of exogenous fuels, whereas fasting blood sugar reflects the regulation of endogenous fuel traffic (i.e. restraints to ‘production’ of glucose from endogenous precursors), the distinctions provide pathophysiological as well as clinical insights. We would suggest that ‘underutilization’ constitutes the principal metabolic disturbance in Class A₁, whereas Classes A₂ and B are experiencing varying degrees of ‘overproduction’ in addition to ‘underutilization’.

What is the basic defect in Class A₁ subjects? By plasma fractionation techniques, we have been able to demonstrate that they do not elaborate abnormal secretory products: the relationships between circulating insulin and proinsulin in Class A₁ gestational diabetics are the same as in pregnant women with normal carbohydrate metabolism (Phelps et al. 1975). Gestational diabetics also do not display abnormal feedback relationships between insulin and glucagon; their values for basal plasma glucagon are not disturbed and we have shown that suppressibility of circulating glucagon by oral glucose is well preserved (Danieletal. 1974; Nitzanetal. 1975). All these characterizations have been corroborated in other laboratories (Luyckx et al. 1975; Kuhl 1976; Kuhl & Holst 1976). Our ongoing studies suggest that faulty insulin secretory kinetics may be implicated in most Class A₁ gestational diabetics (Metzger et al. 1975). We have been finding sluggish responsiveness to secretory stimulation in most of them as judged by subnormal increases in plasma immunoreactive insulin (i.e. above normal fasting levels) during the first 15 min after oral glucose – a time when their increments in plasma glucose are not significantly different from those in pregnant women with normal carbohydrate metabolism (Metzger et al. 1975).

In collaboration with Dr Richard L. Phelps we have initiated ‘around the clock’ studies in Class A₁ gestational diabetics to assess whether their minor...
disturbances in oral glucose tolerance are also attended by detectable disturbances in general fuel traffic during meal-eating. It should be recalled that such gestational diabetics have much smaller disturbances in glucoregulation than the pregnant chemical diabetics in whom diurnal observations have been reported previously (Persson 1974; Gillmer et al. 1975a,b; Persson & Lunell 1975; Lewis et al. 1976). For our studies we confine subjects to a Metabolism Ward, as described elsewhere (Freinkelet et al. 1979), and administer 2110 kcal (8860 kJ) per day (containing 275 g carbohydrate and 76 g protein) in three equal feedings at 8:00 a.m., 1:00 p.m. and 6:00 p.m. Subjects remain recumbent for a 24-hour period while blood is sampled from indwelling venous catheters at hourly intervals between 8:00 a.m. and midnight and at 2:00 a.m., 4:00 a.m. and 6:00 a.m.

To date, six women with normal oral glucose tolerance and seven Class A, gestational diabetics have been studied in this fashion in week 32–39 of pregnancy. In the A, gestational diabetics, the rises in plasma glucose are greater within one hour after every meal than in the control subjects, and persist longer; the concurrent increases in plasma insulin appear to be less at one hour, but later values for insulin tend to exceed control values in association with the prolongation of the postprandial hyperglycaemia. Other fuels also show distinct, albeit more subtle, abnormalities in the A, gestational diabetics. Thus, values for FFA after overnight fast tend to be higher and decrements in response to meal-eating smaller; absolute values for triglycerides tend to be higher at most times although meal-eating does not appear to elicit appreciably greater acute increments. Certain of the individual amino acids also show distinct changes. For example, plasma values for the gluconeogenic amino acid, serine, tend to stabilize at slightly higher levels during overnight fasting, whereas postprandial increments in the plasma values for the branched chain amino acid, isoleucine, seem to persist longer after every meal.

These preliminary data suggest that even those minimal derangements of maternal insulin that characterize Class A, gestational diabetes are already attended by some demonstrable disturbances in the metabolism of every class of foodstuff. Thus, any analysis of maternal insulinization that is confined to carbohydrate metabolism alone is simplistic and inconsistent with the broader dimensions of reality.

What about the consequences? Can one demonstrate any changes in the progeny of mothers who displayed such minimal changes in fuel metabolism during pregnancy? The published criteria for gestational diabetes have varied so widely that critical analysis has been difficult and, at times, controversial. Accordingly, for the purposes of this conference, we have reviewed our
own recent experiences with Class A\textsubscript{1} gestational diabetics. None of these patients have been treated with insulin and we question whether any conventional form of insulin delivery can rectify the faulty acute insulin secretory response of the Class A\textsubscript{1} subjects. For our analysis, we have not included patients with added ‘risk factors’, i.e. patients under the age of 20, over the age of 40, and those in whom obesity was sufficient for antepartum weights to have exceeded 150\% of ideal body weight. Within these rigid criteria, the birth weights of infants from our Class A\textsubscript{1} gestational diabetics, when corrected for age at delivery (Lubchenco et al. 1966), have been minimally but significantly ($P < 0.05$; Table 1) greater than those of offspring from age-matched gravida with normal carbohydrate metabolism whom we have followed at the same time.

The syndrome of ‘large babies’ (Pedersen 1977\textsuperscript{b}) thus already may be present even in the most mild forms of gestational diabetes (see above). Macrosomia has been long recognized as one of the hallmarks of diabetes in pregnancy and explained by the classical ‘Hyperglycaemia–Hyperinsulinism’ hypothesis of Pedersen (1977\textsuperscript{b}) (Fig. 2): herein diminished insulin secretion in the mother leads to maternal hyperglycaemia. The glucose freely crosses the placenta, causing hyperglycaemia in the fetus, stimulation of fetal insulin secretion, increased deposition of fat and glycogen (which carcass analyses have corroborated), and the formation of large babies (Fig. 2).

To what extent does birth weight in the offspring of our Class A\textsubscript{1} gestational diabetics conform to the Pedersen theory? We have been examining the relationships between weights of the newborn and antepartum circulating maternal fuels as judged by blood samples secured after 14-hour overnight fast

| TABLE 1 |

The effect of mild diabetes on birth weight

<table>
<thead>
<tr>
<th>Population: age: 20–40 Prepregnancy weight: less than 150% of ideal body weight</th>
<th>Infant weight</th>
<th>Observed (g)</th>
<th>Corrected\textsuperscript{a} (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal carbohydrate metabolism</td>
<td>($n = 77$)</td>
<td>3221 ± 59</td>
<td>1.051 ± 0.19</td>
</tr>
<tr>
<td>Gestational diabetes class A\textsubscript{1}</td>
<td>($n = 38$)</td>
<td>3480 ± 98</td>
<td>1.122 ± .028</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Weights have been adjusted according to the Colorado Scale (Lubchenco et al. 1966) by the ratio: Weight at birth/Expected weight for gestational age
during weeks 32 to 36 of gestation and during tests of oral glucose tolerance. Our preliminary analyses have disclosed that the birth weights (corrected for gestational age [Lubchenco et al. 1966]) of infants of Class A1 gestational diabetics appear to correlate with fasting maternal plasma glucose (P < 0.05), plasma triglyceride (P < 0.05), and plasma alanine (P < 0.05), serine (P < 0.05), valine (P < 0.10), isoleucine (P < 0.05), and glycine (P < 0.05).

It is not completely understood how all these fuels could contribute to macrosomia. The role of glucose is readily encompassed by the Pedersen hypothesis. Similarly, concentration-dependent increased transfer of amino acids across the placenta (Young & McFadyen 1973) could provide the fetus with more building blocks for protein anabolism, gluconeogenesis, or even direct insulinogenic stimulus (especially in the case of the branched-chain amino acid [Fajans & Floyd 1972]). The possible relationships between birth weight and triglycerides are somewhat more tenuous since direct transplacental transfer of triglycerides has been questioned (Dawes 1968). However, we have found that fasting levels for FFA (Freinkel & Metzger 1975) as well as triglycerides (B.E. Metzger & N. Freinkel, unpublished observations, 1978) correlate directly with the total integrated increase in plasma glucose during oral glucose tolerance in weeks 32 to 36 of pregnancy. Therefore, if FFA and/or triglycerides served as alternative oxidative fuels in the mother, they could retard the disposition of ingested glucose and prolong its availability for transplacental delivery to the fetus. On the other hand, slow hydrolysis of esterified lipids in the maternal circulation or within the placenta could effect a sustaining infusion of fatty acids and glycerol for direct utilization in the fetus (Koren & Shafrir 1964).

In any event, our demonstration that even the mildest of gestational diabetes can disturb the traffic in every insulin-dependent fuel in the mother, and
our statistical correlations between the birth weights of the infants of such mothers and the antepartum levels of many circulating fuels besides glucose, prompt the following expansion of the Pedersen hypothesis (Fig. 3): inadequate acute insulin release in response to alimentation may cause 'underutilization' of many ingested nutrients so that postprandial increments for lipids, and selected amino acids as well as glucose, can be greater and more prolonged in the maternal circulation. The fetus thereby would be provided with potential access to more abundant mixed nutrients. Conceivably, placental structure and/or function might also be modified via the increased availability of certain maternal fuels (Freinkel 1965).

The expanded theory could find fuller expression in more severe chemical diabetes (Fig. 4). In such mothers, the limitations in basal insulin output as well as in their acute secretory response to meal-eating would result in maternal 'overproduction' as well as 'underutilization'. Thus, circulating glucose, lipids, and selected amino acids would be raised (and more available to the conceptus) in the fasted state as well as after eating.

Moreover, heightened ketonaemia could be another expression of the 'overproduction' so that the conceptus might be presented with marked qualitative as well as quantitative changes in the substrate mixture (Fig. 4). Allusion has already been made to the potential implications of altered substrate mixtures for the placenta (Freinkel et al. 1979); as will be discussed later, similar considerations may obtain for the fetus.

**FIG. 3.** Fetal development in mild gestational diabetes (maternal 'underutilization' only). The Pedersen hypothesis expanded as described in text.
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THE ROLE OF FETAL INSULIN

In each of the above formulations for macrosomia (Figs. 2-4), more fetal growth should be possible, even without hormonal facilitation, as a simple consequence of the increased provision of substrate(s)*. However, as shown in Figs. 2-4, all the formulations presuppose that fetal anabolism is enhanced further by fetal insulin. The premise of insulin participation is supported by the finding that visceromegaly of all the insulin-sensitive structures contributes to the macrosomia (Fee & Weil 1963; Naeye 1965). Nonetheless, the precise manner in which more fetal insulin becomes available has not been clarified fully. Although fetal islets appear to be capable of adequate insulin synthesis in response to secretory stimulation (Asplund 1973; Heinze et al. 1975), their capacity to release insulin acutely may be developed less fully. Despite conflicting data (Kervran & Girard 1976), most evidence

* Intrinsic to all the 'increased substrate' theories of macrosomia is the assumption that the higher concentrations of nutrients in the maternal circulation can gain access to the conceptus. This need not always be the case. Clinicians have long appreciated that the offspring of long-standing diabetics may be 'small for gestational age' (SGA). The SGA offspring are particularly common in diabetics with calcific changes in pelvic vasculature. Clearly, substrates can only exert meaningful impact when delivery is not compromised by anatomical or functional restraints to placental blood flow and/or permeability.
suggests that nutrient secretagogues elicit limited acute increases in insulin release from fetal islets even during the period of greatest fetal growth, that is late in gestation (see Milner et al. 1975 for detailed review). This implied lack of maturation of stimulus-secretion coupling in fetal islets has been documented by a number of other metabolic parameters besides insulin release. For example, we found that the poorly responsive islets of the 21.5-day-old rat fetus also display a diminished capacity to accumulate and retain orthophosphate and an attenuated phosphate efflux and nucleotide turnover during acute stimulation with glucose (Asplund & Freinkel 1978).

For humans, much of our knowledge concerning intrauterine islet secretory performance has been based on inferences derived from experiences with neonates. Thus, using analysis for plasma C-peptide, we have been able to confirm (Phelps et al. 1978) the earlier observations based on assays for immunoreactive insulin (Isles et al. 1968; Falorni et al. 1972; Mølsted-Pedersen & Jørgensen 1972) that the newborn of mothers with normal carbohydrate metabolism secrete insulin sluggishly when challenged with intravenous glucose during the first few hours of life (Fig. 5). However, some increment

![Graph](image-url)  
Fig. 5. Beta cell response to intravenous glucose in normal neonates. The heavy arrow denotes the 2-min interval during which glucose was infused into normal infants 2–4 h after birth. Mean ± S.E.M. values for IRI and C-peptide are presented. Each point is based on observations secured in nine infants. (Reprinted from Phelps et al. 1978.) IRI: immunoreactive insulin.
does occur (Isles et al. 1968; Falorni et al. 1972; Mølsted-Pedersen & Jørgensen 1972; Phelps et al. 1978) so that, presumably, the levels of circulating insulin in the fetus before delivery should also be increased coincident with the augmented delivery of nutrient secretagogues from the mother. Much evidence suggests that additional mechanisms become operative in the fetus when maternal insulinization is deficient. The islets of infants from diabetic mothers display clear-cut histological evidence of hyperplasia (Dubreuil & Anderodias 1920; Cardell 1953; Driscoll et al. 1960; Naeye 1965). We have found that basal plasma levels of C-peptide at two to three hours after birth in the offspring of diabetic mothers are generally not outside the range of values seen in infants of mothers with normal carbohydrate metabolism (Phelps et al. 1978) (Fig. 6). However, the concomitant basal values for plasma glucose are lower (Fig. 6) so that prevailing 'steady-state' ratios for plasma C-peptide/glucose are increased (Phelps et al. 1978). We feel that these seemingly altered feedback relationships between basal insulin secretion and ambient glucose in the offspring of diabetic mothers may, perhaps, reflect an increase in the absolute number of islets – and, hence, an increased matrix of secretory tissue already available during intrauterine life. The ratios need not connote full secretory maturation of the individual islets. The latter can only be evaluated by acute secretory challenge. As shown in Fig. 7, we have encountered an adult-type of acute insulin release, that is, a maximal outpour-

![Graph showing relationships between basal plasma glucose and basal plasma C-peptide. Blood samples were secured at 2-4 h of age in nine newborn infants of diabetic mothers (IDM) and nine infants from mothers with normal carbohydrate metabolism and similar socioeconomic backgrounds. (Adapted from Phelps et al. 198. )](image)
ing of insulin already evinced in the earliest sample of blood during intra-
venous tests of glucose tolerance two hours after birth in only about half of
the infants of diabetic mothers (Phelps et al. 1978). These have also been
the infants in whom subsequent rates of disposition of intravenous glucose ($K_t$)
were most rapid (Fig. 7).

From all the foregoing, it follows that fetal insulin deserves inclusion in any
analysis of fetal contributions to the tissue culture aspects of gestation. How-
ever, it may be helpful to differentiate between several possible levels of
impact. Firstly, a sluggish release of insulin from a normal complement of
fetal islets could abet macrosomia without any mechanisms beyond height-
ened substrate delivery having to be invoked. (Although data are sparse, an
adequate number of insulin receptors appear to be present in the peripheral
tissues of the human fetus in late gestation [Thorsson & Hintz 1977] so that
responsiveness of fetal tissues to insulin action need not be restricted by lack of
receptors.) Secondly, in most infants of diabetic mothers, the absolute com-
plement of islets appears to be increased so that the total elaboration of insulin
at any level of substrate may be augmented disproportionately. Finally, in a
finite proportion of the infants, an adult-type pattern of acute insulin release

\[ \text{FIG. 7. Pattern of } \beta \text{ cell secretory response in infants of diabetic mothers. Subjects have been}
\text{subdivided on the basis of fractional rates of glucose disposition which exceed values in normal}
\text{neonates } (K_t > 2.0) \text{ or fall within the normal range } (K_t < 2.0). \text{ Mothers of all infants except}
\text{infant 6 had received insulin treatment during pregnancy. Plasma C-peptide measurements}
\text{were secured after removing potentially cross-reacting, antibody-bound proinsulin. (Reprinted}
\text{from Phelps et al. 1978.)} \]
can be elicited. Presumably, this type of secretory responsiveness in utero could enable the normal adult 'fed state' (Freinkel 1978) to be replicated in the fetus. In other words, such infants should be able to respond to an acute influx of nutrients from the mother with an acute outpouring of insulin and thereby effect maximal fetal anabolism. In so far as somatomedin-type growth factors are responsive to nutrient and insulin interactions (Phillips et al. 1978), one may even hypothesize that such relationships could favour the formation of additional 'growth factors' in the fetus (Figs. 3 and 4). Somatomedin bioactivity has already been demonstrated in cord serum and found to correlate with birth weight and gestational age in normal human pregnancy (Gluckman & Brinsmead 1976).

It remains to be established which of the components of the fetal fuel mixture (e.g. glucose; selected amino acids; other nutrients) acting alone, additively or synergistically are responsible for the induction of extra islets and the secretory maturation of some of them when delivery of substrate is heightened. Ongoing studies with tissue culture of fetal islets (Hellerstrom et al. 1978) should provide clarification.

IMPLICATIONS FOR CELL BIOLOGY

Our formulation has implications far beyond the 'large baby' syndrome. In essence, we have postulated that the formation (and perhaps maturation) of certain cells during fetal life can be influenced by altered nutrient flux. Certain ramifications of this postulate must be recognized: although all cells are formed in utero, not all cells have the same life cycle or replicative potential (Fig. 8). Some are 'terminal' cells which persist through most of the life of the host and can be established only during finite periods in human development. For example, it has been suggested that much of the total complement of adipocytes is laid down during late intrauterine and early neonatal life, and during a brief interval in adolescence (Knittle & Hirsch 1968; Hirsch & Knittle 1970). Similarly, although some regenerative capacity may be operative (Hay 1971), it is felt that differentiation and replication of muscle cells may occur chiefly during intrauterine life, late childhood and adolescence (Cheek 1968). In contrast, the cells of tissues such as the gastrointestinal tract, liver, kidney, skin or blood, etc. retain replicative potential throughout life and may be renewed continuously (Fig. 8).

Within this framework, what have we achieved with the 'large babies'? In accordance with current concepts of cell biology, supranormal endowment may have been effected for certain 'terminal' cells during their intrauterine
phase of differentiation and formation. Adipocytes must be included in this category. Although data on the number of fat cells in large babies from diabetic mothers are sparse, carcass analyses would indicate that adipose tissue is indeed increased (Fee & Weil 1963). We do not know whether this relative plethora persists or whether compensating cut-backs during the other periods of cell formation eventually result in a normal complement of adipocytes. However, pro tem., one may ask whether recipients of intrauterine surfeit are more vulnerable to obesity in adult life? In other words, has nurture conspired with nature to make these offspring more susceptible to maturity-onset diabetes in later life by influencing the number of adipocytes that are formed in utero? Prospective data are limited and inconclusive. However, some suggestive evidence has accrued with regard to the opposite side of the coin: significantly less obesity has been found in 19-year-old men subjected to food shortage during the last trimester of intrauterine life and the first few neonatal months in the Dutch famine of 1944-45 (Ravelli et al. 1976). (In the same series, significantly higher rates of obesity were encountered when famine had occurred during the first half of pregnancy [Ravelli et al. 1976]. It has been postulated that this may have coincided with a critical period in intrauterine life during which hypothalamic centres regulating food intake are differentiating.)

What does this mean with regard to the development of pancreatic islets? Studies increasingly suggest that β cells may also constitute ‘terminal’
or 'near-terminal' cells with finite and limited replicative capacity (Logothetopoulos 1972). If so, increased formation of β cells, premature maturation of some of them, and attendant reduction in the secretory potential of other islet components, such as the α cells, could have meaningful consequences. It could result in a form of premature β cell ageing and contribute to diminished insulinogenic reserve in later life. Data concerning the incidence of maturity-onset diabetes in the offspring of diabetic mothers vis-à-vis diabetic fathers would be particularly interesting with regard to this example of nurture influencing nature during a critical period of cell differentiation and replication.

The possible implications are perhaps most intriguing for brain. Although not considered to be responsive to insulin action, brain cells represent classical 'terminal', non-replicative structures. As reviewed recently (Nowak & Munro 1977; Winnick & Morgan 1979), brain development is completed during the perinatal period in most laboratory animals and structural as well as functional changes have been elicited by manipulating maternal nutrition during this interval. Even neurohumoral components within the brain may be affected: for example, long-range deficits in hypothalamic thyrotropin-releasing hormone have been reported after neonatal caloric deprivation in rats (Shambaugh & Wilber 1974). It is commonly believed that the full complement of brain cells in humans is formed during intrauterine life and the first few neonatal months (Fig. 8). Whether ambient fuels can influence the development of the human brain remains controversial. Some suggestive data warrant citation. Brain weights below the average figure for more normal infants of similar body weights have been found in infants of diabetic mothers (Cardell 1953), even when corrected for dry weight (Fee & Weil 1963). Although macrosomia affecting all viscera except the brain could account for some of these differences (Naeye 1965), Gruenwald has reported that brains from infants of diabetic mothers are smaller on an absolute as well as a relative basis (Gruenwald 1966). Moreover, survey data from largely urban populations in the United States (Churchill & Berendes 1969; Churchill et al. 1969) and a recent confirmatory report dealing with American rural subjects (Stehbens et al. 1977) have indicated that ketonuria in the mother (and the implied increased delivery of ketones to the developing fetus) may be followed by documentable reductions in I.Q. when offspring are tested during early childhood. By contrast, the offspring of mothers deprived of food during the Dutch famine of 1944–1945 have not demonstrated significant reductions in I.Q. when tested at the time of induction into the military (Stein et al. 1972).
The qualitative aspects of the fetal fuel mixture may be particularly relevant to brain cells. Many laboratories (as reviewed in Shambaugh et al. 1977a) have shown that fetal brain can oxidize ketones. We are currently trying to assess the implications of this for the disposition of other fuels (Shambaugh et al. 1977b). As summarized in Fig. 9, Shambaugh has established that added ketones can depress oxidation of fuels such as glucose, lactic acid and amino acids (not shown) when surviving fetal brain slices are incubated with substrate mixtures that simulate the relationships that obtain in vivo (Shambaugh et al. 1977b). Ongoing efforts are directed at trying to determine whether ketones also modify the disposition of such fuels along other biosynthetic pathways. The question assumes particular importance during fetal life. Whereas the oxidative potential of ketones can effectively sustain function in adult brain (Owen et al. 1967), the metabolic desiderata may be quite different when new non-replicating brain cells are being formed.

The concept of 'terminal' cells, responsive to the composition and disposition of ambient nutrients during their period of induction, is one that has not received sufficient attention in the past. We have conjectured about a few possibilities above; the list could be expanded to include many other, perhaps transcendent, possibilities.

Each possibility can only be evaluated by carefully designed prospective studies in which correlations are sought between appropriate antepartum and perinatal events, and the subsequent performance of 'terminal' cells in the progeny. Such longitudinal efforts are currently under way in our laboratory.

FIG. 9. Effect of β-hydroxybutyrate (β-OH B) on glucose and lactate oxidation by fetal brain. Fetal rat brains were derived from fed mothers on day 20 of gestation. Brain slices were incubated with glucose, lactate and 0 (open bars) or 5.4 mM (cross-hatched bars) D.L-β-hydroxybutyrate, as shown above. Heights of the bars denote the evolution of 14CO2 from [U-14C]glucose or [2-14C] lactate. (Adapted from Shambaugh et al. 1977b.)
SUMMARY AND CONCLUSIONS

Maternal insulin may be the prepotent arbiter of fuel metabolism during late pregnancy. Diurnal observations have disclosed that even the most minor disturbances in maternal insulinization are attended by demonstrable changes in the disposition of every major class of nutrients. However, the implications extend far beyond the fuel economy of the mother. Pregnancy represents a type of tissue culture in which the conceptus develops de novo and the composition of the tissue culture medium is determined by the maternal fuels which gain access to the conceptus. As in all tissue culture exercises, the developing cells may be greatly influenced by the nature of the incubation medium. For many of the developing fetal cells, this carries few long-range implications since they will be undergoing continuous renewal throughout the lifetime of the offspring. Other cells, however, are more ‘terminal’ structures. They have limited replicative capacity and some of the total endowment and function of these cells in the offspring may be influenced by intrauterine and perinatal events. These include brain cells, fat cells, muscle cells and perhaps the $\beta$ cells of the pancreatic islets. Thus, pregnancy constitutes a unique opportunity for nutritional and biochemical engineering. It is the arena par excellence in which nurture, as exemplified by the character of maternal fuels, may influence nature, as represented by the intrinsic genetic endowment of the fetus. No single period in human development provides a greater potential for long-range ‘pay-off’ via a relatively short-range period of enlightened metabolic manipulation.

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