Maternal Diabetes in Rats

II. Effects on Fetal Growth and Protein Turnover

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The developmental growth of the rat fetus was studied between days 14 and 21 of pregnancy in normal control, established-diabetic, gestational-diabetic, and insulin-maintained–diabetic mothers. Measurements of fetal body weights and protein mass revealed a suppression of growth in the diabetic pregnancies, probably arising from reduced hyperplasia. Growth of the liver and skin appeared to be suppressed in proportion to the whole fetus, whereas the lung, brain, and particularly the heart were relatively well protected from growth retardation. Fetal growth during development, and its retardation in association with the hyperglycemic state, was explained by measuring the rates of fetal protein turnover in vivo. Both the protein synthetic and degradative rates gradually declined during normal development. However, in the diabetic pregnancies, fetal protein synthesis was consistently lower than control rates, whereas protein degradation increased sharply toward the end of gestation. These changes in protein synthesis and breakdown probably combine to yield a smaller fetus in the absence of normoglycemia.
***Diabetes* 37:1671–77, 1988**

Macrosomia has long been a feature of established-diabetic and gestational-diabetic pregnancies in humans (1,2). However, more recently, with the aid of ultrasound techniques, fetal growth retardation has been observed during the first trimester followed by macrosomia in the 3rd trimester (1,3,4). The extent of the early growth retardation appears to correlate with the severity of the maternal diabetes (4,5) and with the frequency of congenital malformations in the fetus (6,7). The laboratory rat, rendered diabetic by streptozocin (STZ), has been widely used as a model for studying the pathophysiological effects of maternal diabetes. Fetal growth retardation is a consistent feature of these diabetic pregnancies. As such, this rat model probably more closely reflects the early events in human diabetic pregnancies and may be useful for studying the etiology of delayed somatic growth.

Normal intrauterine growth is characterized by a large and rapid accumulation of protein (8). However, relatively few studies in either humans or animals have examined the normal developmental changes in fetal protein turnover that control this accretion of protein. To our knowledge, no previous studies have considered how these developmental changes are affected by the imposition of hyperglycemia during gestation. Consequently, we set out to establish the normal developmental patterns of fetal growth in the rat and to define any aberrations associated with diabetic pregnancies. During the third and final week of gestation, the rates of protein turnover were measured in vivo in all fetuses to explain any differences in the growth patterns between normal, gestational-diabetic, and established-diabetic pregnancies. The effectiveness of insulin therapy in preventing aberrations in fetal growth was also investigated.

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Received for publication 21 October 1987 and accepted in revised form 29 June 1988.

MATERIALS AND METHODS

Fetal growth was studied in the same pregnant female rats used in the preceding study (see Robinson et al., this issue). Diabetes was induced by 40 mg of STZ/kg body wt, and insulin-replacement therapy was administered by 4 U s.c. insulin every 24 h (9). Blood glucose concentrations were measured as described and illustrated in the companion article (Robinson et al., this issue).

Fetal growth was measured between 14 and 21 days of gestation in all four groups of mothers, i.e., in normal control, established-diabetic, gestational-diabetic, and in established-diabetic rats maintained on insulin throughout the pregnancy (insulin-maintained–diabetic rats) (Robinson et al., this issue). Average rates of protein synthesis in the whole

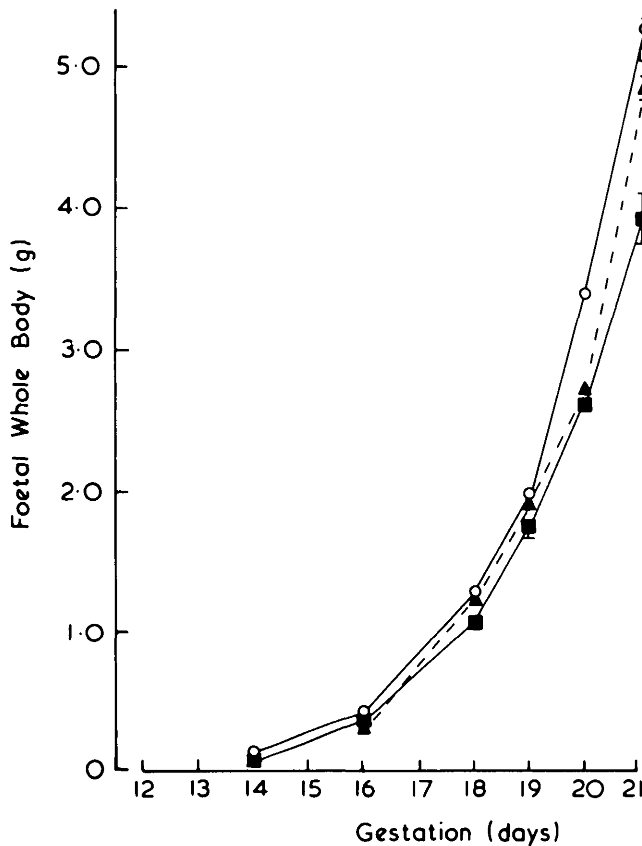

FIG. 1. Fetal body weights from control and diabetic pregnancies. Pregnancy was interrupted at intervals between days 14 and 21 of gestation and fetuses removed and weighed from control (○), gestational-diabetic (▲), and established-diabetic (■) mothers. Fetuses were removed from established insulin-maintained–diabetic rats (□) on day 21 only. Each *point* represents mean ± SE of ≥15 fetuses from ≥3 mothers. All values within established-diabetic ($P < .01$) and gestational-diabetic ($P < .01$) groups are statistically significant between days 18 and 21.

fetus were also measured in vivo over the same period of gestation after administration of 150 μmol of phenylalanine to each mother via a lateral tail vein (8). This injection included 65 μCi of L-[4-^{3}H]phenylalanine (sp act 24 Ci/mmol; Radiochemical Centre, Amersham, Bucks, UK) and was delivered in 1 ml 0.9% NaCl/100 g body wt. The suitability of this method for measuring synthetic rates in the fetus and its individual tissues has previously been described in detail (8,10). Exactly 10 min after administration of phenylalanine, the injected mothers were decapitated and exsanguinated for 10 s. The abdominal cavity was rapidly opened in each case and the uterus and its contents immersed in ice-cold water to chill the fetuses. Ten fetuses per mother were then isolated at random. Five were immediately frozen in liquid N_2, weighed, and stored at −20°C, while the major visceral organs were dissected from the remaining 5 fetuses.

Frozen whole bodies were homogenized in ice-cold 0.3 M perchloric acid (PCA) (1:50 wt/vol) by use of a Waring blender and then a glass homogenizer. The specific radioactivities of [^{3}H]phenylalanine in both the free pool (S_A, PCA-soluble fraction) and covalently bound in protein (S_B) were measured as described previously (8; Robinson et al., this issue). The fractional rate of synthesis of fetal proteins (K_s), defined as the percentage of the protein mass synthesized per day, was calculated from

$$K_s = \frac{S_B}{S_A \times t} \times 100$$

where t is the time in days. Fetal growth rates (K_g, the percentage of change in protein mass per day) were determined as the net accumulation of protein measured over 2 days spanning the time at which the synthesis rates were measured. Because growth arises from an imbalance between the rates of protein synthesis (K_s) and breakdown (K_b), the latter rate was calculated by subtracting the two measured parameters; i.e., $K_b = K_s - K_g$. No standard errors or statistical analyses can be presented with these indirect rates because they are calculated from mean values.

The protein content of whole-body fetuses was measured by the method of Lowry et al. (12), with bovine serum albumin (Sigma) as standard. Whole-body RNA and DNA were also extracted and measured as described elsewhere (14). Student's two-tailed t test was employed for all statistical analyses.

RESULTS

Fetal growth was monitored over the last week of gestation in four groups of mothers and three litters, i.e., in normal control, established-diabetic, gestational-diabetic, and insulin-maintained–diabetic rats. In all situations, fetal body weights increased exponentially (Fig. 1). However, in the diabetic pregnancies, the fetuses were consistently lighter. The growth retardation of the established-diabetic rats; was significant ($P < .01$) from day 18 on and was consistently more pronounced than in the gestational group. In contrast, the fetuses of insulin-maintained–diabetic mothers were not significantly different from controls at day 21 of gestation (Fig. 1). Because fetal growth retardation was accompanied by placental enlargement, the fetal-to-placental weight ratios

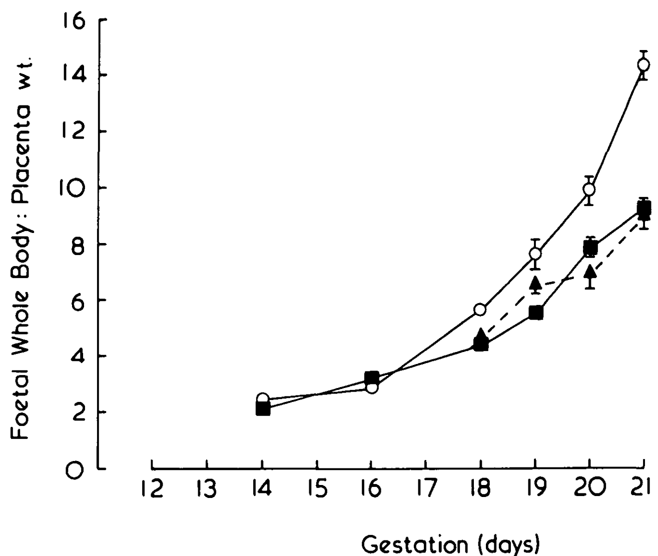

FIG. 2. Changes in fetal-to-placental weights in control and diabetic pregnancies calculated from normal control (○), gestational-diabetic (▲), established-diabetic (■), and insulin-maintained–diabetic (□) mothers for ≥12 fetuses (Fig. 1) and placentas (Robinson et al., this issue) at each age. From day 18, all values in the 2 diabetic groups are statistically different ($P < .01$) from controls.

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were shifted to the right in the diabetic pregnancies (Fig. 2; Robinson et al., this issue). That is, the ratios for the diabetic rats decreased ($P < .01$) relative to the controls, reflecting the opposing movements of the two measured parameters.

Diabetes can sometimes be accompanied by dehydration. Hence we measured the water and protein contents of all fetuses to check whether dehydration alone could explain the differences in fetal weights (Fig. 1). No significant differences were found in the water contents of the fetuses from all four types of pregnancies; e.g., water constituted 88.8 ± 0.4% of fetal weights at day 21. Genuine growth retardation of the diabetic fetuses was further confirmed by their protein contents (Fig. 3). Although the protein composition of all fetuses (ranging with age from 5 to 9% of the wet wt) was unchanged by the status of the maternal environment, the protein mass was consistently decreased (Fig. 3), reflecting the differences in body weight (Fig. 1). The RNA and DNA contents but not the protein-to-DNA ratio were decreased between days 18 and 20 relative to controls (Figs. 4 and 5). These findings suggest that less nuclear proliferation and hence fewer cells are probably found in the diabetic fetuses (i.e., DNA; Fig. 5*a*). However, although fewer in number, the cells present are probably of a similar size to those in the age-matched control fetuses (i.e., protein/DNA; Fig. 5*b*). Hence, the suppression of growth in the diabetic fetuses is

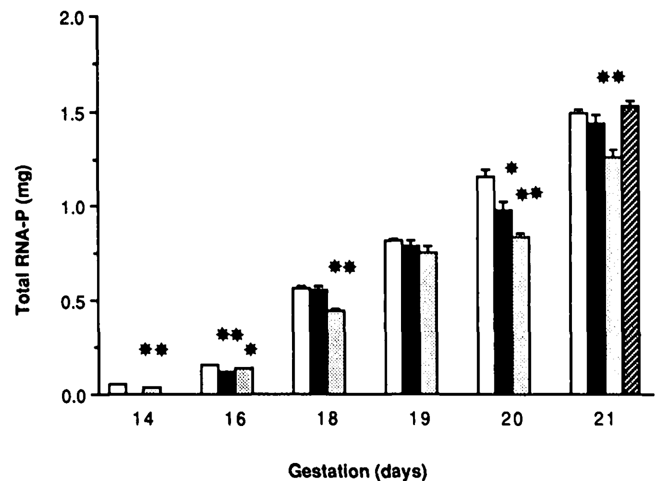

FIG. 4. Fetal RNA content during normal and diabetic pregnancies. Whole-body fetal RNA was extracted and assayed as described by Goldberg and Goldspink (14) on fetuses isolated between days 14 and 21 from control (*open bars*), gestational-diabetic (*solid bars*), and established-diabetic (*stippled bars*) rats. At 21 days, values for fetuses from insulin-maintained–diabetic (*hatched bar*) mothers were also measured. Each *point* represents mean ± SE for ≥12 fetuses removed from 3 mothers. *$P < .01$ and **$P < .001$ compared with controls and determined with Student's *t* test.

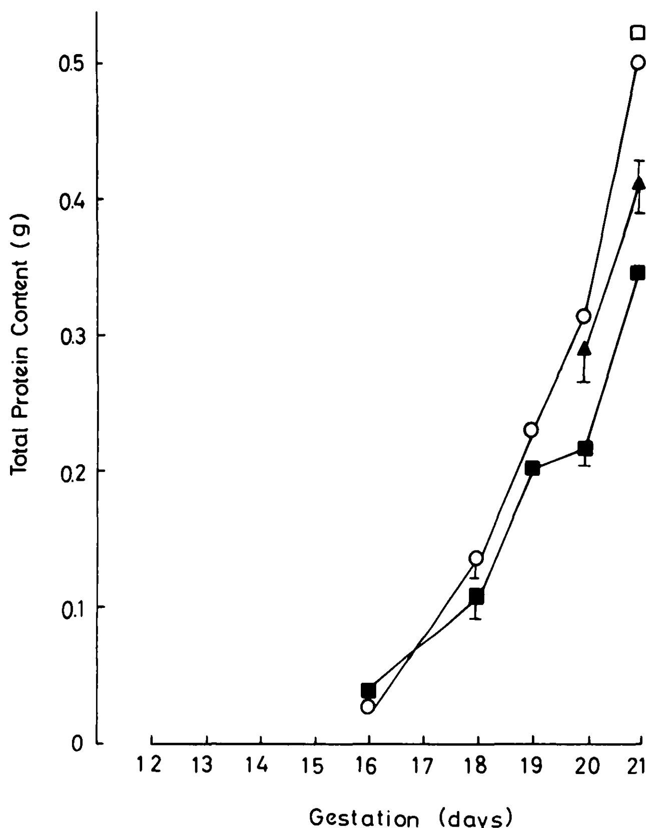

FIG. 3. Protein content of normal and diabetic fetuses measured by method of Lowry et al. (12) in whole-body homogenates. Each *point* represents mean ± SE of ≥10 fetuses from control (○), gestational-diabetic (▲), established-diabetic (■), and insulin-maintained–diabetic (□) mothers. Protein mass of established-diabetic ($P < .25$ at days 18 and 19, $P < .001$ at days 20 and 21) and gestational-diabetic ($P < .05$ at day 20, $P < .01$ at day 21) fetuses are significantly lower than in control animals.

probably related more to a reduction in hyperplasia than changes in cellular hypertrophy.

To explain both the normal developmental growth of the fetus and the growth suppression associated with hyperglycemia, fetal rates of protein turnover were measured in vivo. Between days 14 and 21, the total rate of protein synthesis in the enlarging control fetuses increased ~32-fold, i.e., from 7.5 ± 0.3 to 241 ± 31 mg/day. In comparison, both groups of diabetic fetuses consistently displayed lower developmental increases in their total synthetic rates. By 21 days of gestation, the fetuses from the established-diabetic and gestational-diabetic pregnancies were synthesizing 41 and 33%, respectively, less protein per day than their equivalent controls. However, when differences in fetal size were eliminated by the use of fractional rates, it became evident that synthesis in the control fetuses actually remains constant between days 14 and 18, progressively declining thereafter (Fig. 6*a*). At days 16–19 and 21, the fractional rates of synthesis in fetuses from diabetic mothers were significantly ($P < .01$) lower than those of normal controls, thus providing a possible explanation for the suppression of growth. At the one gestational age where insulin replacement was investigated (i.e., 21 days), the fall in protein synthesis in the diabetic rats was prevented. Indeed, in the presence of insulin, the rate was elevated above the control value (25%; $P < .01$).

Insight into how the alterations in the rate of protein synthesis may have been effected can be gained by examining the changes in fetal RNA. For example, at full term the suppression of synthesis in the fetuses of established-diabetic mothers correlated with a 29% decrease ($P < .001$) in the ribosomal activity compared with age-matched control values of 155 ± 7 g of protein synthesized per g RNA-P. In contrast, near the end of gestation, the ribosomal capacity was changed little with respect to the control value of 3.0 ± 0.06 mg RNA-P/g protein. On the basis of these limited data,

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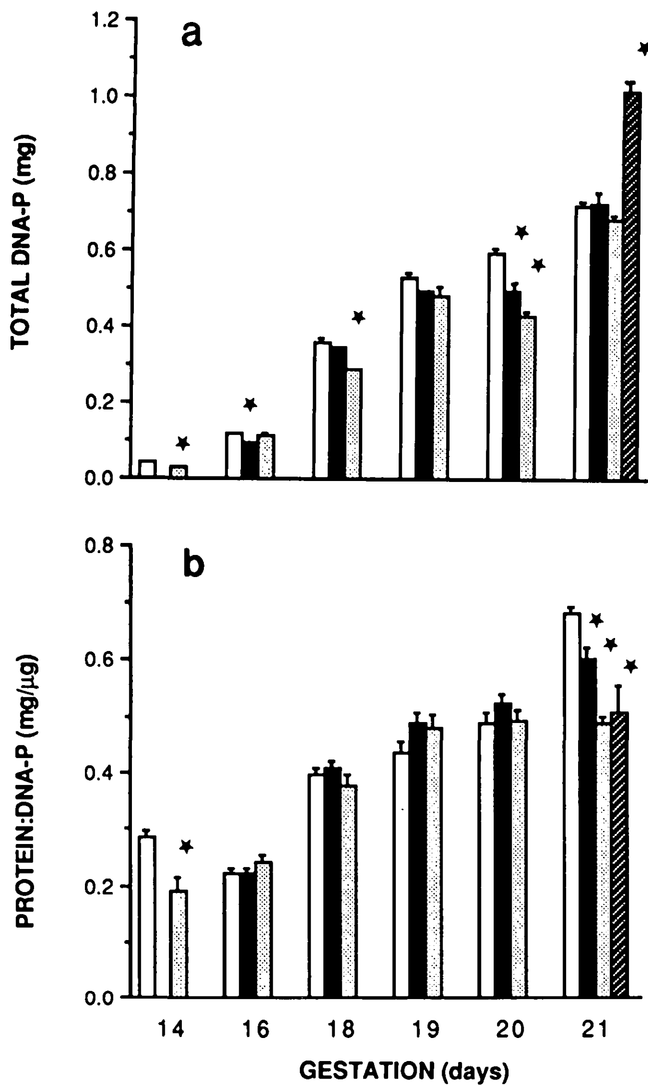

FIG. 5. Total DNA and protein-to-DNA values in fetuses from control and diabetic mothers. Whole-body fetal DNA (*a*) was extracted and assayed, as described by Goldberg and Goldspink (14). Protein-to-DNA-P ratios (*b*) were calculated from data in Figs. 3 and 5*a*. Each value represents mean ± SE of ≥10 fetuses from control (*open bars*), gestational-diabetic (*solid bars*), and established-diabetic (*stippled bars*) mothers. At day 21, fetuses from insulin-maintained–diabetic mothers (*hatched bars*) were also measured. *$P < .01$ compared with control values and determined with Student's *t* test.

the inhibition of synthesis rates in response to hyperglycemia may possibly be attributed to a decrease in the ribosomal activity.

Although the suppression of protein synthesis provides one plausible explanation for the growth retardation in the diabetic fetuses, protein accretion is also controlled by the rate of protein degradation (Fig. 3; 15). Unfortunately, meaningful rates of protein degradation are technically very difficult to measure directly in vivo (16). Hence, the degradative rates in the fetuses were determined indirectly from the measured rates of synthesis and growth (see MATERIALS AND METHODS). This procedure provides degradative rates that are as good as, if not better than, other methods currently available (17). From these data, it appears that the fetal rates of protein breakdown initially increase and then slowly decline during normal development (Fig. 6*b*). However, in both types of diabetic fetuses, protein degradation appears to be appreciably elevated above control values toward the end of gestation. This late increase, combined with the lower rate of synthesis in the diabetic rats, probably accounts for the divergence of growth from that of the normal control fetus (Figs. 1, 3, and 6*a*).

Finally, five tissues were removed from the fetuses of normal and diabetic pregnancies at full term, and their wet weights were measured relative to those of fetal whole bodies (Table 1). This enabled us to determine whether proportionate or disproportionate growth retardation had occurred in the individual tissues of diabetic fetuses. The absolute weights of the liver, lung, brain, and skin from diabetic fetuses were less (6–26%) than those of controls, the effect being more marked in the established-diabetic pregnancies. Indeed, most of these differences were statistically significant ($P < .01$) in the established-diabetic group, but only the skin was in the gestational-diabetic animals ($P < .001$). Unlike these four organs, the fetal hearts from both diabetic

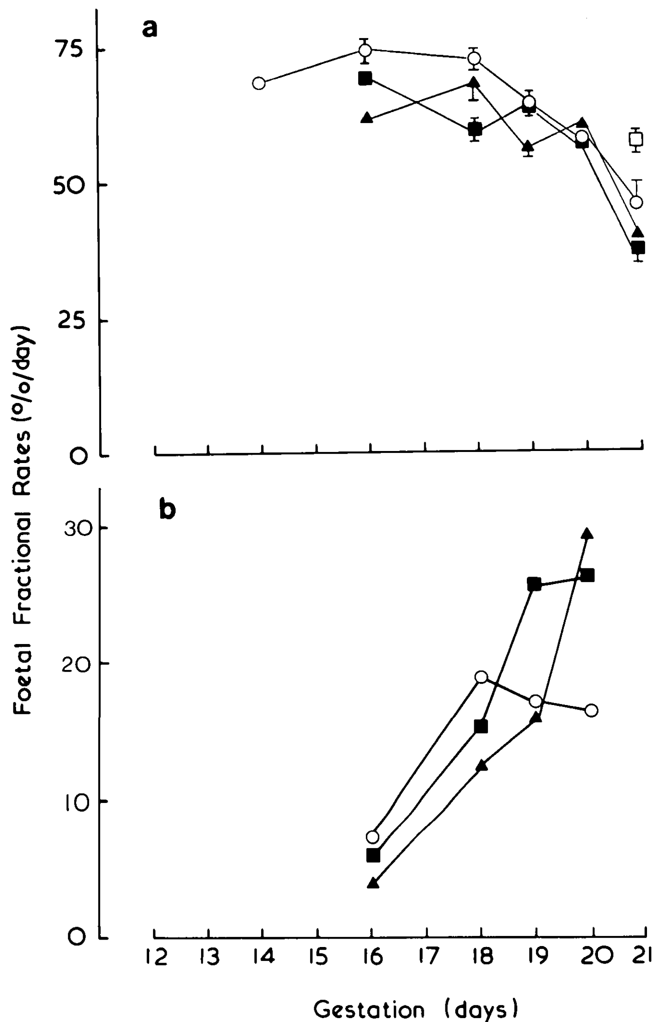

FIG. 6. Fractional rates of protein synthesis and protein breakdown in fetuses from control and diabetic mothers. Protein synthesis (*a*) was measured in fetuses after injection of [^{3}H]phenylalanine into mothers' tail vein (11). Indirect rates of protein breakdown (*b*) were calculated as differences between measured rates of synthesis and growth (see MATERIALS AND METHODS). At least 12 fetuses at each age were studied for normal control (○), established-diabetic (■), gestational-diabetic (▲), and insulin-maintained–diabetic (□) mothers.

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TABLE 1
Normal and diabetic fetal organ weights at day 21 of gestation

	Control	Gestational diabetic	Established diabetic	Insulin-maintained diabetic
Liver weight (mg)	385 ± 8.6	362 ± 18	294 ± 12†	362 ± 24
Change vs. controls (%)		−6.0	−23.6	−6.0
Proportion of whole body (%)	7.3	7.4	7.4	7.1
Lung weight (mg)	170 ± 5.3	155 ± 6.1	152 ± 6.1	167 ± 11
Change vs. controls (%)		−8.8	−10.6	−1.8
Proportion of whole body (%)	3.2	3.2	3.8	3.3
Heart weight (mg)	33.9 ± 1.6	38.4 ± 2.2	35.0 ± 0.9	36.0 ± 1.9
Change vs. controls (%)		+13.3	+3.2	+6.2
Proportion of whole body (%)	0.64	0.79	0.88	0.70
Brain weight (mg)	164 ± 4.4	148 ± 5.6	144 ± 4.4*	163 ± 4.9
Change vs. controls (%)		−9.8	−12.1	−0.6
Proportion of whole body (%)	3.1	3.1	3.6	3.2
Skin weight (mg)	1020 ± 24	917 ± 25*	756 ± 27†	961 ± 32
Change vs. controls (%)		−10.1	−25.9	−5.8
Proportion of whole body (%)	19.3	18.8	19.0	18.8

Wet-weight values represent means ± SE from ≥12 fetuses taken from ≥3 mothers in each experimental group. Percentage of increase or decrease in absolute organ weight relative to value in control fetuses and percentage of contribution of organ to fetal whole-body weight are shown.
*$P < .01$ and †$P < .001$ vs. control values by Student's *t* test.

pregnancies were either unchanged or slightly heavier than the equivalent control tissues. It is, therefore, obvious that not all of the fetal tissues are affected to the same extent within the overall growth retardation of the diabetic fetuses. For example, at this stage of pregnancy, the fetuses from established-diabetic mothers were 25% lighter than age-matched controls (Fig. 1). However, only the liver and skin experienced the same magnitude of growth suppression (Table 1). The lung, brain, and especially the heart were relatively well protected, and as such their contributions toward fetal body weights became greater than those of the control organs. In general, the insulin replacement successfully prevented most of these changes from occurring.

DISCUSSION

In our experimental design, we attempted to avoid a number of potential problems that previous investigators have not always considered. Variations in fetal size can arise simply from differences in the number of fetuses per litter. Litter size was therefore standardized by using only females destined for their second pregnancies. Having taken this precaution, we were satisfied that diabetes per se did not affect litter size (average 12.3 ± 0.5 fetuses). Unlike other studies, we found no evidence of malformed fetuses and little (4%) evidence of fetal resorptions in the diabetic pregnancies (18,19). To provide accurate gestational ages, matings were restricted to 12 h. Although diabetes was successfully induced by 40 mg STZ/kg (Robinson et al., this issue), this dose was sublethal and left the animals in a healthy state for ≥6 wk, an essential prerequisite of our experimental design (20). Higher doses of this diabetogenic agent are known to be toxic, leading to systolic hypotension and bradycardia presumably due to impairment of the baroreceptor reflex (21). No significant problems of sterility (~10%) were encountered with the diabetic animals 2 wk after treatment with STZ. Hence, we did not need to consider injecting pregnant animals with diabetogenic drugs, a highly undesirable procedure that has been employed in some earlier investigations (22,23). This practice runs the risk of affecting placental growth and exposing the conceptus to teratogenic influences (24).

Despite enormous advances in the management of pregnant women with diabetes, this condition continues to present a challenge to obstetricians and pediatricians. Poorly controlled diabetic pregnancies can lead to an increased incidence of perinatal mortalities, congenital malformations, macrosomia, and a variety of neonatal complications (1). In contrast to the human situation, the diabetic rat, which is often more severely hyperglycemic, usually gives rise to smaller offspring than normal controls (Fig. 1; 18,25). This was true in this study, with fetal growth retardation being a consistent feature of the last week of gestation (Figs. 1 and 3). Hence, unlike in humans, there was no suggestion of fetal growth retardation early in gestation (3) followed by accelerated growth and macrosomia in the 3rd trimester (1,4). The overall growth retardation in the fetal rat was disproportionate with respect to its individual tissues; the skin and liver were the most severely affected, with the brain and lungs retarded to a lesser extent (Table 1). The preferential protection of some tissues, e.g., the brain, is a feature also found in other situations where the maternal environment is disturbed, e.g., maternal malnutrition or starvation (26). A slight absolute enlargement of the heart was found in the diabetic rat fetuses, which may correspond with the cardiomegaly found in human diabetic fetuses (Table 1; 27). Except for the effects on growth, which were more pronounced in the established diabetic rats, there were few discernable differences in either the fetuses or placentas (Robinson et al., this issue) when comparing the two types of diabetic pregnancies. Appropriate insulin replacement, capable of restoring normoglycemia (Robinson et al., this issue), largely prevented these changes in the individual tissues and whole fetus (Table 1; Fig. 1).

It has been suggested that in mildly hyperglycemic humans the transmission of high plasma levels of glucose, amino acids, and lipids to the fetal circulation induces early maturation of the β-cells in the fetal pancreas with consequent hyperinsulinemia (28,29). Because insulin's action is

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anabolic (30–32), accelerated growth and hence macrosomia ensues. In the diabetic rat, blood glucose concentrations well above normal were found in both the maternal and fetal circulations (33; Robinson et al., this issue). The actual values were ~4 mM less on the fetal side of the placenta than that on the mother's side (Robinson et al., this issue). However, the rat at birth is developmentally immature compared with most other mammals (34). Hence, it may not simply be a case of condensing the 9 mo of human gestation into 3 wk for the rat. Of particular importance is the maturation of the β-cells in the fetal rat pancreas, which is thought to occur proportionately later, i.e., at days 20 and 21 of gestation (35). So even if hyperinsulinemia were induced, there would be little time for growth to be accelerated before parturition.

In certain circumstances, reduced placental size and function can directly result in fetal growth retardation. In this study, the fetal-placental unit was briefly examined, but only with respect to its size and not its function. Relative to controls, the diabetic fetal-to-placental weight ratios decreased as gestation proceeded (Fig. 2). These changes are somewhat unusual but not unique. For example, pregnancies at high altitude (36) or where the intake of alcohol is high (37) yield similar disparities between fetal and placental growth. Disturbances in the maternal environment (e.g., starvation) more usually retard placental growth to a greater extent than fetal growth, thereby yielding higher fetal-to-placental weight ratios (38). Despite the placentomegaly in the diabetic rats, severe reductions in blood flow (19) and thickening of the trophoblastic barrier (39) have been reported and may impair the transfer of metabolites between the maternal and fetal circulations, hence restricting fetal growth.

Fetal growth increased exponentially with a rapid accretion of protein during the last week of gestation (Figs. 1 and 3). Correlating with these normal developmental changes was a gradual but progressive decrease in protein turnover (Fig. 6). These developmental changes in the whole animal then continue throughout postnatal life, with both a progressive decrease and convergence of the absolute rates of synthesis and degradation (8,16). In the diabetic pregnancies, the decrease in synthesis and the increase in breakdown act in a complementary fashion to explain the observed growth suppression (Fig. 6). As such, these changes are more suggestive of hypoinsulinemia than hyperinsulinemia. Other hormonal imbalances associated with diabetes could also be influencial in inducing these changes in protein turnover. Direct regulation of protein turnover via changes in the concentrations of blood glucose seems unlikely (32). Nonetheless, restoration of normoglycemia does maintain normal growth patterns (Figs. 1, 3, and 4; Table 1).

ACKNOWLEDGMENTS

This work was made possible by the financial support of the British Heart Foundation and the Eastern Health and Social Services Board in Northern Ireland.

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