Relationship Between Maternal and Fetal Fuels and Placental Glucose Transfer in Rats with Maternal Diabetes of Varying Severity

EMILIO HERRERA, MANUEL PALACIN, ANTONIA MARTIN, AND MIGUEL ANGEL LASUNCION

SUMMARY

Gestational diabetes mellitus (GDM) is a nonhomogenous entity known to affect fetal development in different ways in both rats and human beings. The degree of severity of diabetes could affect the maternal-fetal transfer of metabolic fuels and consequently influence fetal development. To study this hypothesis, pregnant rats were made diabetic by streptozocin (STZ) treatment (45 mg/kg) at day 7 of gestation and were treated with different daily doses of insulin until the 20th day of gestation, when they were killed and examined. Differences in plasma glucose levels in the groups studied were not accompanied by differences in plasma glycerol, β-hydroxybutyrate (β-OHB), or total amino acid levels in mothers or their fetuses. Fetal/maternal ratios of these circulating fuels were not modified by maternal diabetes, whereas the glucose level was enhanced in diabetic rats not treated with insulin. Placental glucose transfer was studied directly with a recently reported in situ experimental design and was found to increase linearly with maternal glycemia, independently of whether this was modified by insulin treatment or by acute intravenous (i.v.) infusion of glucose in normal animals. Lactate production by the fetal/placental unit decreased in proportion to the glucose level in the maternal circulation.

The present data indicate that the diabetic condition of the mother rat does not modify the mechanisms of placental transfer of metabolic fuels to the fetus, and that the actual transfer is mainly dependent on the concentrations of these fuels in the maternal circulation. The limited capacity of the fetus to handle the great influx of glucose through the placenta of a highly hyperglycemic mother may aggravate the diabetic condition of the fetus, affecting its subsequent development.

DIABETES 1985; 34 (Suppl. 2):42–46.

From the Departamento de Investigación, Centro Ramón y Cajal, and Departamento de Bioquimica, Facultad de Medicina, Universidad de Alcalá de Henares, Madrid, Spain.

Address reprint requests to Professor Emilio Herrera, Ph.D., Departamento de Investigación, Centro Ramón y Cajal, Crta. de Colmenar Km 9, 28034-Madrid, Spain.

Gestational diabetes mellitus (GDM) affects a nonhomogenous group of patients, including offspring as well as mothers.[1] Although excessive body weight is a striking feature in infants of diabetic women,[1,2] mothers with abnormal glucose tolerance may deliver babies of normal body weight.[3] The rat, receiving injections of alloxan or streptozocin (STZ) before[4–6] or during pregnancy,[7–9] has been widely used as an experimental model for the study of GDM, and depending on its severity, fetal weight may be increased,[10] unmodified,[4] or reduced.[5–8] Changes in fetal growth in GDM have been attributed to parallel changes in fetal circulating insulin levels, which, among other factors, are modulated by the metabolic fuels crossing the placenta.

Placental composition and metabolism are reportedly modified in diabetic pregnancy in humans[11–13] as well as in rats,[10,13,14] and these findings may indicate changes in placental metabolite transfer. To investigate this possibility, in the present study we determined the circulating levels of different metabolic fuels as well as placental glucose transfer by using an in situ placental preparation[15] in STZ-treated, pregnant rats. To learn how the severity of diabetes affects these parameters, STZ-diabetic rats receiving different insulin treatments were also studied.

MATERIALS AND METHODS

Animals. At day 7 of gestation (estimated by the appearance of spermatozoids in vaginal smears), Wistar rats (196–215 g body wt) were made diabetic by a single i.v. injection of streptozocin (STZ) (45 mg/kg body wt) dissolved in 50 mM citrate buffer, pH 4.5. Other rats were injected with only the buffer at the same gestation time and served as normal controls. Groups of STZ-treated rats received a daily s.c. injection of 0.5, 1.5, or 3 IU of porcine insulin (Insulin Novo Ultralente, Novo Industri A/S, Copenhagen, Denmark) per 100 g body wt from the 8th day of gestation. All rats were killed and examined at day 20 of gestation.

Steady-state concentration of metabolites and RIA of insulin. Animals were killed by decapitation without anesthesia and blood was collected from the neck wound into chilled, heparinized receptacles. After being weighed, the conceptus was dissected and fetuses were decapitated for blood collection. Aliquots of plasma were deproteinized[16] for the analysis of glucose,[17] β-hydroxybutyrate (β-OHB),[18] and glycerol.[19] Other plasma aliquots were deproteinized with 10% sulfosalicylic acid in 0.1 N HCl for the analysis of amino acids[20] using a Beckman 121 MB autoanalyzer. Insulin was assayed[21] in other plasma aliquots by using a radioimmunoassay kit specific for the rat that was generously provided by Novo.

Placental glucose transfer. Rats were studied under nembutal anesthesia (33 mg/kg body wt) with our previously described surgical procedure[15] and 10 μCi of either (U-^{14}C)-D-glucose or (U-^{14}C)-L-glucose (The Radiochemical Center, Amersham, United Kingdom, sp. act. 257 and 58 mCi/nmol, respectively) dissolved in 250 μl of 0.9% NaCl (infusion rate of 12.5 μl/min) were infused for 20 min through the left uterine artery. After collection of maternal blood from the aorta into heparinized syringes, fetuses from the left and right uterine horns were immediately excised and decapitated for blood collection into heparinized receptacles. The fetal blood from all left and right uterine horns were pooled separately. Maternal and fetal plasma aliquots were used for counting total radioactivity and deproteinization.[16] Aliquots of protein-free supernatants were used for glucose assay[17] and for ^{14}C-glucose and ^{14}C-lactate purification by ion-exchange chromatography[22] using microcolumns made with AG 1-X8 200–400 chloride and AG 50W-X8 200–400 H^+ (Bio-Rad Laboratories, Richmond, California), rinsed with distilled water and with 0.5 N formic acid for ^{14}C-glucose and ^{14}C-lactate elutions, respectively. Recovery experiments showed that 96.6% of ^{14}C-glucose and 0.2% of ^{14}C-lactate initially added to plasma were eluted in the "glucose fraction," whereas 0.4% of the ^{14}C-glucose and 88.2% of ^{14}C-lactate were recovered in the "lactate fraction."

Expression of results. Radioactive values were corrected by considering 1 × 10^6 dpm as the total radioactivity infused per rat. Results were expressed as means ± SEM and statistical comparison between the groups was performed with the Student's *t*-test.

RESULTS

At the 20th day of gestation, the maternal body free of conceptus structures, conceptus, fetus, and placenta weights as well as maternal and fetal plasma insulin levels were significantly reduced in STZ-diabetic rats as compared with normal controls (Table 1). All of these differences disappeared in diabetic animals treated daily with either 0.5 or 1.5 IU insulin/100 g body wt (Table 1). Litter size was not modified in diabetic mothers with or without insulin treatment as compared with normals (Table 1). Maternal plasma glucose concentration was approximately 6.5 times higher in diabetics than in normals, and although 0.5 IU of insulin produced a striking reduction in this parameter, values were still significantly higher than in normals (Table 1). In diabetic rats treated with 1.5 IU of insulin, maternal plasma glucose values decreased to levels significantly lower than in normals. Plasma levels of glucose in fetuses were below values in their respective mothers. Glycemia in fetuses from diabetic mothers was about 15 times greater than in those of normals, whereas plasma glucose levels were quite normalized in fetuses from diabetic mothers treated with 0.5 or 1.5 IU insulin, and did not differ from control values (Table 1). Consequently, the

TABLE 1
Effect of STZ diabetes and daily treatment with different doses of porcine insulin on maternal fetal and placental weights and circulating metabolic fuels in the 20-day pregnant rat

	Normal	Diabetic	Diabetic + 0.5 IU insulin	Diabetic + 1.5 IU insulin
Maternal free-of-conceptus body wt (g)	256 ± 7	223 ± 10*	243 ± 8	241 ± 8
Conceptus wt (g)	61.4 ± 3.0	49.8 ± 3.4*	57.1 ± 1.0	49.3 ± 5.3
Litter size (no. fetuses/mother)	10.4 ± 0.6	11.3 ± 0.9	11.6 ± 0.5	9.8 ± 1.0
Fetus body wt (g)	3.89 ± 0.16	3.07 ± 0.13**	3.65 ± 0.10	3.63 ± 0.07
Placenta wt (g)	0.51 ± 0.02	0.45 ± 0.01*	0.47 ± 0.01	0.46 ± 0.03
Maternal RIA-insulin (μU/ml)	100 ± 4	35 ± 5***	134 ± 27	172 ± 36
Fetal RIA-insulin (μU/ml)	135 ± 24	67 ± 9††*	94 ± 10	117 ± 13
Maternal plasma glucose (mM)	5.3 ± 0.1	34.5 ± 1.8***	5.9 ± 0.3*	3.5 ± 0.6***
Fetal plasma glucose (mM)	1.6 ± 0.1†††	23.7 ± 2.2††***	1.7 ± 0.3†††	1.2 ± 0.2††
Fetal/maternal plasma glucose ratio	0.30 ± 0.02	0.69 ± 0.06***	0.28 ± 0.04	0.40 ± 0.08
Maternal plasma glycerol (μM)	193 ± 29	220 ± 32	189 ± 29	234 ± 36
Fetal plasma glycerol (μM)	101 ± 34	158 ± 37	88 ± 12	63 ± 7†
Fetal/maternal plasma glycerol ratio	0.48 ± 0.09	0.59 ± 0.09	0.49 ± 0.15	0.34 ± 0.09
Maternal plasma β-OHB (μM)	398 ± 69	518 ± 71	304 ± 65	272 ± 53
Fetal plasma β-OHB (μM)	426 ± 51	425 ± 60	279 ± 48	272 ± 35*
Fetal/maternal plasma β-OHB ratio	1.02 ± 0.12	0.97 ± 0.12	0.92 ± 0.10	1.35 ± 0.28
Maternal plasma total amino acids (mM)	4.04 ± 0.18	3.56 ± 0.35	3.84 ± 0.21	3.79 ± 0.23
Fetal plasma total amino acids (mM)	8.16 ± 0.89†††	7.36 ± 0.80†††	6.77 ± 0.42†††	6.62 ± 0.55†††
Fetal/maternal plasma total amino acid ratio	2.1 ± 0.1	2.1 ± 0.4	1.9 ± 0.2	1.8 ± 0.2

Means ± SEM of 5–12 rats/group. Statistical comparisons versus normals are shown by * and those between fetuses and their respective mothers by †: * or †, $P < 0.05$; ** or ††, $P < 0.01$; and *** or †††, $P < 0.001$.

fetal/maternal plasma glucose ratio was significantly enhanced in diabetic rats, whereas it did not differ in the insulin-treated diabetic groups as compared with controls. Circulating levels of other metabolic fuels such as β-OHB, glycerol, and total amino acids were similar in untreated diabetic, insulin-treated diabetic, and normal mothers and their fetuses. Fetal/maternal ratios of these parameters were as expected, being below 1 for glycerol, around 1 for β-OHB, and above 1 for total amino acids, and none of these ratios differed in diabetic and normal animals (Table 1).

The enhanced fetal/maternal plasma glucose ratio in diabetic rats may be a consequence of augmented placental glucose transfer and/or reduced fetal glucose utilization. To study these points, our first efforts were focused on determining whether the nonspecific passage of glucose through the placenta differed in diabetic and normal rats. Since L-glucose is not recognized by the placental glucose carrier,[23] the nonspecific leak was estimated by the different percentage of radioactivity found in plasma of fetuses from the left versus right uterine horns after infusion through the left uterine artery of (U-^{14}C)-L-glucose or (U-^{14}C)-D-glucose. This value was 5.73 ± 1.48 in untreated diabetic rats and 5.14 ± 1.06% in normals (N = 5/group, difference not significant), indicating that nonspecific glucose leak through the placenta was similar and almost negligible in diabetic and normal animals. The transfer of (U-^{14}C)-D-glucose was studied as previously described[15] and, as shown in Figure 1, this parameter increased greatly in diabetic rats compared with normals. This difference was smaller but still significant ($P < 0.05$) in diabetic rats treated with 0.5 IU insulin, and it disappeared completely in those treated with 1.5 IU insulin. The dependence of this parameter on the level of circulating maternal glucose concentration was studied in diabetic rats treated with 3 IU insulin, which had much lower plasma glucose levels (0.56 ± 0.16 mM) than the other groups (Table 1), and also in normal rats infused with glucose (15 mg/min/rat) through the jugular vein for 60 min before and then during the 20 min of the experiment, which attained plasma glucose levels of 15.9 ± 3.1 mM.* As is also shown in Figure 1, placental (U-^{14}C)-D-glucose transfer was significantly reduced in the diabetic rats treated with 3 IU insulin and enhanced in normals infused with glucose as compared with normal, untreated animals (Figure 1). A positive and highly significant ($P < 0.001$) correlation was found when individual values of (U-^{14}C)-D-glucose transfer to the fetuses from all the groups studied were plotted against their respective maternal plasma glucose levels (Figure 2), indicating the direct relation between these two parameters.

After 20-min infusion of (U-^{14}C)-D-glucose through the maternal left uterine artery, a certain proportion of the total fetal plasma radioactivity appeared as ^{14}C-lactate. Since infused (U-^{14}C)-D-glucose is isotopically diluted with maternal glucose, the percentual amount of lactate formed from infused (U-^{14}C)-D-glucose by the fetal/placental unit was estimated

$$\text{GLUCOSE TRANSFER } \left(\text{nmols/min/ml}\ \frac{1}{\varphi}\right) = \frac{\text{(DPM L.F.)} - \text{(DPM R.F.)}}{20\ \text{min}} \times \frac{1}{^{14}\text{C-glucose S.A. in uterine artery plasma}} \times \frac{1}{\varphi}$$

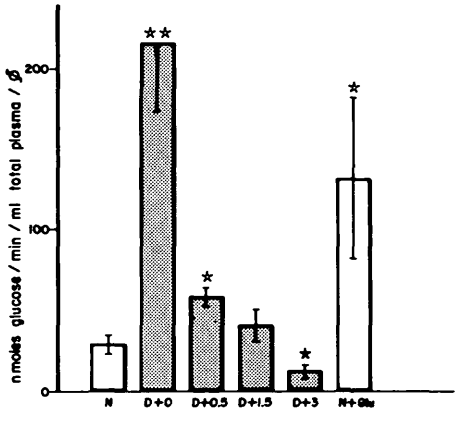

FIGURE 1. Placental transfer of glucose in diabetic rats (D) treated with different daily doses of porcine insulin or of normal animals acutely infused or not with glucose (1.2 g/rat) through the jugular vein. Means ± SEM of 4–8 rats. Statistical comparisons versus untreated normal animals: *P < 0.05 and **P < 0.01. Placental transfer was estimated as the difference in total plasma radioactivity between the left (L.F.) and right fetuses (R.F.) divided by the time of infusion with (U-^{14}C)-D-glucose through the maternal left uterine artery (20 min) and by the ^{14}C-glucose specific activity (S.A.) in the maternal artery. The data are expressed as a function of the uterine blood flux (φ), which is unknown.

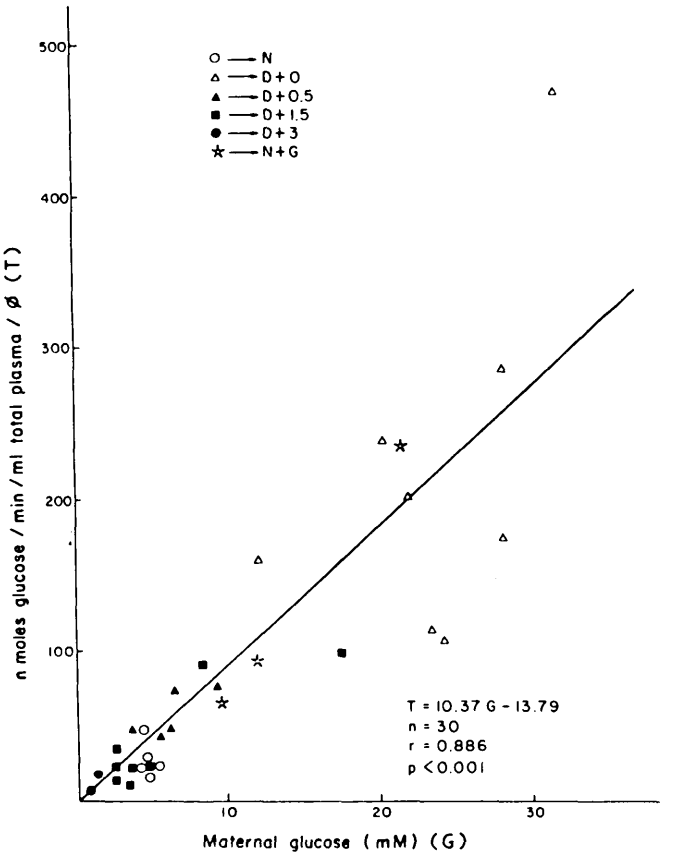

FIGURE 2. Linear relationship between placental glucose transfer and plasma maternal glucose concentration in rats with diabetes of varying severity. Placental transfer estimated as indicated for Figure 1.

*In fetuses from mothers used in placental transfer studies, plasma insulin levels were 61 ± 9 μU/ml in those from untreated diabetic mothers and 102 ± 7, 108 ± 11, or 92 ± 15 μU/ml in those from diabetic mothers treated with 0.5, 1.5, or 3 IU insulin/100 g, respectively, the difference between the insulin-treated and the untreated diabetics being statistically significant ($P < 0.05$ or less). In fetuses from normal mothers, plasma insulin levels were 120 ± 13, whereas those from normal mothers infused with glucose through the jugular vein were 150 ± 11 μU/ml, the difference between these two groups being not significant ($P > 0.05$).

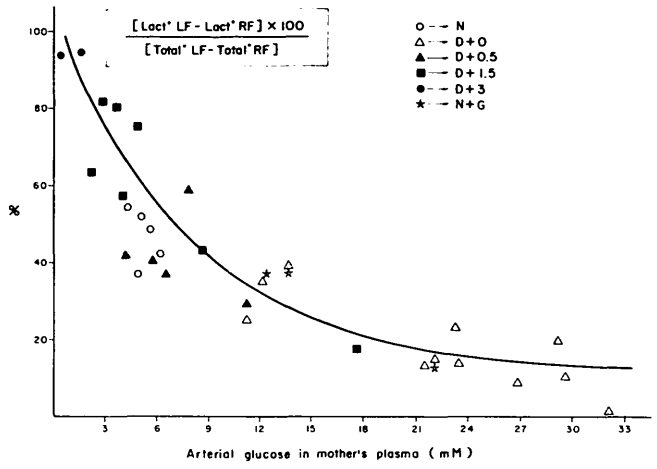

FIGURE 3. Relationship between the percentage of lactate production by the fetal/placental unit from infused (U-^{14}C)-D-glucose through the maternal left uterine artery and actual maternal circulating glucose concentration in rats of varying diabetic severity. The percentage of lactate production was estimated as the ratio between the difference between plasma ^{14}C-lactate values (Lact*) in the left (LF) and the right fetuses (RF) and the difference in plasma total radioactivities (Total*), times 100.

by the difference in ^{14}C-lactate present in plasma of fetuses from the left and right uterine horns. Data shown in Figure 3 express this lactate production as the percentage of maternal/fetal transfer plotted against maternal plasma glucose concentration in individual animals from all experimental groups studied. There is evidently an inverse, although not linear, relation between lactate production by the fetal/placental unit and maternal glycemia. This relation seems to depend more on the level of maternal plasma glucose than on pancreatic damage, because in normal rats (not treated with STZ but infused with glucose) it is maintained in the same range as in the actual diabetic animals (Figure 3).

DISCUSSION

The present results showing that STZ diabetes in pregnant rats reduces fetal body weight are in agreement with previous findings.[5,7-9,24] This effect coincides with a reduction in fetal plasma insulin levels, and the overall picture contrasts with the macrosomia and hyperinsulinemia normally found in infants of diabetic women.[25] We have recently discussed the differences in diabetogenic tendencies during pregnancy in rats and human beings,[26] and the present findings clearly show the dissimilarities between gestational diabetes in man and STZ diabetes in the pregnant rat. These differences may result from the fact that a milder form of maternal diabetes is more usual in humans, since some control of glucose homeostasis is always attempted. In our diabetic rats treated with insulin doses of 0.5 IU/100 g, a mild hyperglycemia persisted, presenting a condition more comparable to the human one because fetal body weight and circulating insulin were normalized. Prevention of fetal macrosomia and hyperinsulinism in diabetic rats may be a consequence of the incapacity of rat fetuses to store fat before birth even after insulin treatment,[27] as well as of the shorter period of gestation as compared with humans.

With the exception of glucose, the fetal/maternal ratios of metabolites normally crossing the placenta were not modified in our experimental groups, suggesting that transfer of metabolites to the fetus is independent of the severity of maternal diabetes, being modulated by their respective concentrations in the mother. To our knowledge, this is the first report of the determination of placental glucose transfer in the diabetic rat and it was found to be directly related to maternal glucose concentration, indicating that its quantitative value also varies according to the severity of diabetes. Previous studies in normal sheep have also revealed a direct correlation between maternal fetal glucose transfer and maternal glycemia.[28] The present results demonstrate that nonspecific placental leak is unmodified in the diabetic rat, indicating that the specific placental glucose transfer is not saturated at maternal plasma glucose levels as high as 30 mM. Fetal accumulation of glucose during severe maternal glycemia coincides with its reduced conversion to lactate, suggesting that, unlike the case of placental glucose transfer, the fetus has a limited capacity to handle the glucose load coming from a highly hyperglycemic mother. This limitation seems to be independent of fetal β-cell function, because it occurred in fetuses from diabetic mothers with low insulin levels and also in those from normal mothers infused acutely with glucose, which have much higher insulin levels. While extrapolation of these findings to human beings should be made with caution, they do suggest that in the diabetic mother, placental transfer of different fuels is unaffected and depends on their concentrations in maternal circulation. The fetus of the diabetic mother is unable to handle the great influx of glucose at the rate received, increasing its diabetic condition, which together with its relative insulin deficiency, affects its development.

ACKNOWLEDGMENTS

The authors wish to thank Caroline S. Delgado for her editorial help and Professor Norbert Freinkel for his advice in the preparation of the study.

This study was supported by a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social, Spain.

REFERENCES

[1] Freinkel, N.: Banting lecture 1980. Of pregnancy and progeny. Diabetes 1980; 29:1023–35.

[2] Farquhar, J. W.: The infant of the diabetic mother. Postgrad. Med. J. 1969; 45:806–11.

[3] Pezzarossa, A., and Coppola, F.: Relative effects of gestational diabetes, obesity and excessive weight gain on foetal growth. IRCS Med. Sci. 1983; 11:83–84.

[4] Kervran, A., Guillaume, M., and Jost, A.: The endocrine pancreas of the fetus from diabetic pregnant rat. Diabetologia 1978; 15:387–93.

[5] Pitkin, R. M., and Van Orden, D. E.: Fetal effects of maternal streptozotocin-diabetes. Endocrinology 1974; 94:1247–53.

[6] Eriksson, U., and Swenne, I.: Diabetes in pregnancy: growth of the fetal pancreatic B-cells in the rat. Biol. Neonate 1982; 42:239–48.

[7] Golob, E. K., Rishi, S., Becker, K. L., Moore, C., and Shah, N.: The effect of streptozotocin-induced diabetes on pancreatic insulin content of fetuses. Diabetes 1970; 9:610–13.

[8] Aerts, L., and Van Assche, A.: Rat foetal endocrine pancreas in experimental diabetes. J. Endocrinol. 1977; 73:339–46.

[9] Cuezva, J. M., Burkett, E. S., Kerr, D. S., Rodman, H. M., and Patel, M. S.: The newborn of diabetic rat. I. Hormonal and metabolic changes in the postnatal period. Pediatr. Res. 1982; 16:632–37.

[10] Pitkin, R. M., Plank, C. J., and Filer, L. J., Jr.: Fetal and placental composition in experimental maternal diabetes. Proc. Soc. Exp. Biol. Med. 1971; 138:163–66.

[11] Heijkenskjold, F., and Gemzell, C. A.: Glycogen content in the placenta of diabetic mothers. Acta Paediatr. Scand. 1957; 46:74–80.

[12] Ginsburg, T., and Jeacock, M. K.: Same aspects of placental carbohydrate metabolism in human diabetes. J. Obstet. Gynaecol. Br. Commonw. 1966; 73:452–59.

[13] Diamant, Y. Z., Metzger, B. E., Freinkel, N., and Shafrir, E.: Placental

lipid and glycogen content in human and experimental diabetes mellitus. Am. J. Obstet. Gynecol. 1982; 144:5–11.

[14] Hagerman, D. D.: Metabolism of tissues from pregnant diabetic rats in vitro. Endocrinology 1962; 70:88–95.

[15] Lasunción, M. A., Testar, X., Palacín, M., Chieri, R., and Herrera, E.: Method for the study of metabolite transfer from rat mother to fetus. Biol. Neonate 1983; 44:85–92.

[16] Somogyi, M.: Determination of blood sugar. J. Biol. Chem. 1945; 160:69–73.

[17] Hugget, A. S. G., and Nixon, D. A.: Use of glucose oxidase, peroxidase, and O-dianisidine in determination of blood and urinary glucose. Lancet 1957; 1:368–70.

[18] Williamson, D. H., Mellanby, T., and Krebs, H. A.: Enzymatic determination of D(−)-B-hydroxybutiric acid and acetonic acid in blood. Biochem. J. 1962; 82:90–96.

[19] Garland, P. B., and Randle, P. J.: A rapid enzymatic assay for glycerol. Nature 1962; 196:987–88.

[20] Martín del Río, R., and Latorre-Caballero, A.: Presence of γ-aminobutyric acid in rat ovary. J. Neurochem. 1980; 34:1584–87.

[21] Heding, L. G.: Determination of total serum insulin (IRI) in insulin diabetic patients. Diabetologia 1972; 8:260–66.

[22] Zorzano, A., and Herrera, E.: Liver and kidney cortex gluconeogenesis from L-alanine in fed and starved rats. Int. J. Biochem. 1984; 16:263–67.

[23] Yudilevich, D. L., Eaton, B. M., Short, A. H., and Leichtweiss, H. P.: Glucose carriers at maternal and fetal sides of the trophoblast in guinea-pig placentas. Am. J. Physiol. 1979; 237:C205–12.

[24] Eriksson, U., Dahlström, E., Larsson, K. S., and Hellerström, C.: Increased incidence of congenital malformations in the offspring of diabetic rats and their prevention by maternal therapy. Diabetes 1982; 31:1–6.

[25] Pedersen, J.: The Pregnant Diabetic and Her Newborn. Copenhagen, Munksgaard 1977:1–180.

[26] Herrera, E., and Zorzano, A.: Is the rat a proper model for studying human diabetogenic tendencies in pregnancy? *In* Lessons from Animal Diabetes. Shafrir, E., and Renold, A. E., Eds. London, John Libbey, 1984:699–704.

[27] Stangenberg, M., Eklöf, A.-Ch., Dahlquist, G., and Persson, B.: Lack of effect on body weight and content of nitrogen and fat after insulin administration to fetal rats. Biol. Neonate 1981; 40:240–45.

[28] Hay, W. W., Jr., Sparks, J. W., Wilkening, R. B., Battaglia, F. C., and Meschia, G.: Fetal glucose uptake and utilization as functions of maternal glucose concentration. Am. J. Physiol. 1984; 246:E237–42.