Neonatal Macrosomia in Maternal Diabetes

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Summary. In order to determine the effect of maternal diabetes on the somatic growth of the rat fetus and to elucidate mechanisms underlying the control of fetal growth, concentrations of DNA and proteins and DNA polymerase- α activities in neonates were examined. The maternal status was classified as normal (no urinary glucose excretion), mildly diabetic (0.01–0.99 g/day urinary glucose), and severely diabetic (1.00 g/day or more urinary glucose). The total DNA contents in mg/neonate were 26.8 ± 2.2 (mean $\pm$ SEM), 31.3 ± 2.5 , and 29.4 ± 2.7 for neonates from normal, mildly diabetic and severely diabetic mothers, respectively. The DNA polymerase activities in (cpm/g neonate) $\times 10^{-3}$ for the same groups of neonates were 432 ± 58 , $1,008 \pm 74$, and 888 ± 118 , respectively. These results indicate that the neonatal macrosomia disappears as the severity of maternal diabetes increases. Furthermore, DNA polymerase is one of possible biochemical sites through which macrosomia is manifested in diabetic pregnancies.

Key words: Streptozotocin diabetes in rat, maternal diabetes, neonatal macrosomia, protein, DNA, DNA polymerase- α .

Fetal and neonatal macrosomia induced by maternal diabetes have long been recognized in human pregnancy [1–6]. However, biochemical mechanisms underlying the macrosomia are not clearly understood and the exact relationship between macrosomia and fetal mortality is not known. In animal model systems, the results concerning the body weights of fetuses and neonates are controversial [7–14]. Although there is evidence to indicate that macrosomia in experimental animals is frequently

associated with mild diabetes and that the macrosomia is a consequence of true somatic growth [11–14], there is a lack of direct evidence showing that severity of the maternal diabetes modulates the body weight of fetuses. Moreover, biochemical mechanisms responsible for the changes in the rate of somatic growth have yet to be elucidated, if indeed the fetal growth can be modulated by the severity of maternal diabetes. We have, therefore, examined the effect of maternal diabetes of different severities on the neonatal weight, DNA and protein contents and DNA polymerase activities.

Material and Methods

Sprague-Dawley rats were obtained from ARS Co. (Madison, Wis.). Animals were given Purina Rat Chow (Purina Co., St. Louis, Mo.) and water ad libitum. All biochemicals including streptozotocin (Lot No. 67-04251), deoxyadenosine 5'-triphosphate (dATP), deoxyguanosine 5'-triphosphate (dGTP), deoxycytosine 5'-triphosphate (dCTP), and thymidine 5'-triphosphate (TTP) were obtained from Sigma Chemicals Co. (St. Louis, Mo.). (Methyl- ^3H)-thymidine 5'-triphosphate (^3H -TTP, 50 Ci/mmol) was purchased from New England Nuclear Co. (Boston, Mass.). All other chemicals were obtained from Fisher Scientific Co. (St. Louis, Mo.).

Streptozotocin Administration

Streptozotocin was dissolved in a 0.01 mol/l citrate buffer solution (pH 4.0), and was given IP to female rats which had been starved for the preceding 18 h period. Each animal was given one injection of the streptozotocin solution containing the desired amount. The dosage per animal ranged from 0 to 39 mg/kg. Feeding of animals was resumed 8 h after the streptozotocin administration.

Urinary Glucose Assay

On the 10th day after the administration of streptozotocin, the total amount of urine excreted by an individual animal in a 24 h period was collected in a metabolic cage. The glucose concentra-

tion in the urine sample was assayed by the hexokinase-glucose-6-phosphate dehydrogenase coupled method [15], and the total amount of glucose excreted by an animal in a 24 h period was estimated.

We have previously studied the pharmacodynamics of streptozotocin [16] and observed that the maximum urinary glucose level was reached on the 10th day after one IP administration of streptozotocin and the urinary glucose excretion remained at that level for the subsequent 20 days. On the basis of these observations the present methods for the induction and evaluation of maternal diabetes were adopted.

Mating and Collection of Neonates

A group of 10 female rats weighing 200–250 g was housed in one cage and one male rat was placed in the cage from 1700 h to 0800 h. The animals were weighed and inspected daily. The presence of sperm on vaginal smears indicated the mating, and conception was assumed to have taken place between 0200 h and 0800 h [17]. For the purpose of estimating the length of gestation period, the time of conception was considered as 0500 h. In order to eliminate possible causes of variations in neonatal sizes, the following precautionary measures were implemented: One male rat was used throughout the experiments, and female rats were discarded if they failed to mate before their body weight reached 250 g. Only those litters containing 10–12 neonates which were delivered in the presence of laboratory personnel were included in the study. The time of onset of parturition of each litter was used for estimating the length of gestation period. Individual neonatal weight was measured immediately after birth before nursing or cannibalism could take place. The neonates were frozen quickly in liquid nitrogen and stored in an airtight plastic bag at -20°C until used.

Measurement of DNA and Protein Concentrations of Neonates

The neonate was thawed, rinsed thoroughly in a cold buffer solution containing the following in mmol/l: Tris-HCl, 37.0 (pH 7.3); KCl, 50.0; MgCl_2 , 3.3; EDTA, 0.5; 2-mercaptoethanol, 6.0. After blotting to remove the excess buffer solution, the neonate was weighed and homogenized in a Polytron homogenizer (Brinkmann Instruments Co.) for 1 min. The final concentration was adjusted with the buffer solution to 1 g of neonate per 10 ml of the total homogenate. The concentrations of DNA and proteins in the homogenate were estimated by the methods of Schneider [18] and Lowry et al. [19], respectively. From these results the content of DNA (mg/neonate) and concentration of proteins (mg protein/mg DNA) were calculated.

DNA Polymerase- α Activity Assay

The remainder of the total homogenate was centrifuged at $10,000 \times g$ for 20 min and the supernatant fraction was centrifuged again at $105,000 \times g$ for 60 min. The $105,000 \times g$ supernatant fluid was diluted with the Tris buffer solution so that the final protein concentration would be in the range of 1–2 mg/ml. The diluted sample was used for the polymerase activity assay. The protein concentration of the enzyme sample was estimated by the method of Lowry et al. [19].

The polymerase activity was determined by measuring the polymerization of radioactive TTP into acid insoluble fraction in the presence of activated calf thymus DNA as template [20, 21]. The assay medium contained 150 mmol/l each of dATP, dGTP, and dCTP, 75 mmol/l ^3H -TTP (20 Ci/mmol), and 0.18 mg/ml activated DNA in the buffer solution described above. A 50 μl

sample of the $105,000 \times g$ supernatant fluid (after appropriate dilution) was placed in an aliquot of 200 μl of the assay medium, and this reaction mixture was incubated at 37°C for 60 min. At zero-time and at the end of 60 min incubation period, 25 μl aliquots of the reaction mixture were withdrawn in triplicate, applied to filter paper discs, which were washed and dried according to the procedures described by Bollum [20]. The treated discs were placed in individual scintillation counting vials containing 10 ml of scintillation fluid and the radioactivity measured in a Beckman CPM 100 Scintillation Counter. The amount of radioactive TTP incorporated into the acid insoluble material (cpm/mg of $105,000 \times g$ supernatant fluid) is a measure of DNA polymerase- α activity. The radioisotope counting methods have been described previously [22–24].

Statistical Methods

Throughout this report data were expressed in mean $\pm$ SEM whenever applicable, and significance of the differences between mean values being compared were examined by Student's t-test. The difference was considered significant when $P < 0.05$. For the graphic representation of the relationship between the severity of diabetes and neonatal weight (Fig. 1), the regression line was obtained by the equation, $Y = \bar{y} + b(X - \bar{x})$, where Y is the estimated value of neonatal weight, $\bar{y}$ is the mean neonatal weight, b is the regression coefficient, X is a given amount of 24 h urinary glucose, and $\bar{x}$ is the mean value of 24 h urinary glucose for the population studied. The correlation coefficient (R) for these two variables and the significance of the correlation coefficient (P) were determined also. All of these statistical analyses were performed according to the methods described by Woolf [25].

Results

Figure 1 shows the mean neonatal weight of litter mates from mothers with diabetes of different severities. The severity of diabetes was expressed in terms of 24 h urinary glucose excretion. The body weight of the neonate decreased as the amount of maternal urinary glucose increased. The regression line differed significantly from the horizontal line ($R = -0.733$; $P < 0.01$).

Table 1 compares the body weights and gestation periods of neonates from mothers in the different categories of diabetes. The female rats that excreted 0.01–0.99 g/day urinary glucose were designated as mildly diabetic and those which excreted 1.00 g/day or more were termed as severely diabetic. Some animals which had received 33 mg/kg streptozotocin excreted less than 1.00 g/day urinary glucose, while others which had received 32 mg/kg streptozotocin excreted urinary glucose in excess of 1.00 g/day.

The body weight of neonates from mildly diabetic mothers was significantly greater than the body weight of neonates from either normal or severely diabetic mothers. The gestation period for severely diabetic mothers was significantly longer than that for normal mothers. The difference between the ges-

Table 1. Effects of maternal diabetes on neonatal weight and gestation period

Status	Maternal data		Neonatal data	
	Streptozotocin (mg/kg body weight)	Range of urinary glucose (g/day)	Mean body weight (g)	Mean gestation period (day)
Normal (n = 8)	0	0	6.37 ± 0.11 (n = 88)	21.7 ± 0.3
Mildly diabetic (n = 8)	29–33	0.01–0.99	7.26 ± 0.10 ^a (n = 92)	22.4 ± 0.1
Severely diabetic (n = 8)	32–36	1.00 ≤	6.74 ± 0.22 ^b (n = 86)	23.5 ± 0.5 ^a

^a P<0.05 when compared with normal^b P<0.05 when compared with mildly diabetic **Fig. 1.** Body weight of new-born rats in relation to the severity of maternal diabetes. Female rats weighing 200 g in body weight were given different dosages of streptozotocin, and on the 10th day thereafter the amount of urinary glucose excreted by each animal in a 24 hour period was measured. These animals were mated subsequently, and the neonates were obtained for the study. Each point in the figure represents the mean neonatal weight of litter mates. The regression equation and the method for determining correlation coefficient (R) and significance of correlation coefficient (p) are described in the text

tation periods for normal and mildly diabetic mothers was insignificant.

The effect of maternal diabetes on the DNA content and DNA polymerase activities of neonates is shown in Table 2. The DNA content was the highest in the neonates from mildly diabetic mothers, which was followed by the DNA content of neonates from severely diabetic mothers, and the value for normal neonates was the lowest. The DNA content of neo-

Table 2. DNA content and DNA polymerase activity of neonate

Maternal status	DNA (mg/neonate)	DNA polymerase (cpm/g neonate) × 10 ⁻³
Normal	26.8 ± 2.2 (n = 88)	432 ± 58
Mildly diabetic	31.3 ± 2.5 ^a (n = 92)	1,008 ± 74 ^a
Severely diabetic	29.4 ± 2.7 ^{a,b} (n = 86)	888 ± 118 ^{a,b}

^a and ^b as in Table 1

nates from severely diabetic mothers was significantly greater than the DNA content of normal neonates in the population studied here. The protein concentration (mg protein/mg DNA) remained approximately the same for the neonates in all of the categories (minimum of 17.6 ± 1.7 and maximum of 18.2 ± 1.6).

The DNA polymerase activity in the neonates from mildly diabetic mothers was the highest, which was followed by the activity in the neonates from severely diabetic mothers. The polymerase activity in the neonates from severely diabetic mothers was significantly lower than the activity in the neonates from mildly diabetic mothers, but was still significantly higher than the enzyme activity in the normal neonates.

Discussion

Fetal or neonatal macrosomia has been observed in pregnancy of mildly diabetic mothers in human and in animal model systems [2, 4, 6, 12, 13]. However, the possibility that the somatic growth of the fetus is modulated by the severity of maternal diabetes has not been examined. The results presented here show

that neonatal macrosomia is produced only under mildly diabetic conditions of mothers and the macrosomia disappears as the severity of maternal diabetes worsens (Fig. 1 and Table 1). Extrapolation of the data shown in Figure 1 suggests that neonates from mothers with more severe diabetes would be smaller than the neonates from normal mothers. This observation is consonant with some of the previous reports [7, 8, 26, 27] showing the smaller than normal fetus in diabetic mothers. It is interesting to note that the neonatal macrosomia disappears in the pregnancy of the severely diabetic mothers, despite their longer gestation period. The prolongation of the gestation period in diabetic pregnancy has been observed previously [7, 11]. The gestation period of the macrosomic neonates (from mildly diabetic mothers) reported here is not significantly different from the gestation period of normal neonates (Table 1). These evidences indicate that the prolonged gestation period is an unlikely primary cause of macrosomia in diabetic pregnancies.

The DNA content in mammalian tissue has been equated to the cell population in the tissue, and the rate of DNA synthesis in the tissue has been considered to be an accurate measure of the rate of cell proliferation [22, 28–35]. Therefore, the present results are interpreted as meaning that the principal mode of modulation of the somatic growth of fetus in diabetic mothers is by altering the cell proliferation rate. The tissue level of the polymerase activities participates in the control of DNA content and thereby of the somatic growth of fetus in diabetic pregnancies.

The changes in DNA polymerase activities have been attributed to the corresponding changes in DNA synthesis which accompany, invariably, the changes in the rate of cell proliferation in a variety of tissues under different pharmacological and pathophysiological conditions [22, 28–35]. The cause of large discrepancies between the increments of DNA contents and DNA polymerase activities observed in this study (Table 2) is not immediately clear. However, maternal diabetes has been known to cause changes in structural and functional properties of placentas and to affect the transport of metabolites from the maternal side to the fetal side [36–39]. Therefore, the metabolite levels in the fetus may serve as a rate limiting factor for fetal growth even in the presence of a plethora enzyme, particularly in the case of severely diabetic mothers.

Macrosomia has been attributed frequently to the increased incidence of fetal and neonatal deaths in both human and animal model systems [3, 11, 40–43]. On the other hand, increases in fetal and neonatal deaths were observed also with those

mothers which produce smaller than normal fetuses and neonates due probably to the overt diabetic conditions of the mothers [7]. In fact, one statistical study shows that 26.2% of the pregnancies in the diabetic (overt) and only 8.5% in the prediabetic groups ended in stillbirth [2]. The present study shows unequivocally that neonatal macrosomia disappears when the maternal diabetes becomes severe. These observations pose a pertinent question, whether or not macrosomia per se is directly related to the fetal or neonatal mortality especially if the increase in the fetal DNA polymerase activity is indeed the primary cause of the macrosomia.

Acknowledgement. This work was supported by a research grant, HD 11614, from the NICHD, USPHS.

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Received: March 8, 1979
and in revised form: November 28, 1979

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